EVOLUTIONARY NEUROSCIENCE

EDITED BY

JON H KAAS

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Academic Press is an imprint of Elsevier

ACADEMIC
PRESS

Academic Press is an imprint of Elsevier
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK
525 B Street, Suite 1900, San Diego, CA 92101 4495, USA

First edition 2009

Library of Congress Catalog Number: 2006935559

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN 13: 978 0 12 375080 8
ISBN 10: 0 12 392560 6

Printed and bound in Spain.

07 08 09 10 10 9 8 7 6 5 4 3 2 1

DEDICATION

These volumes are dedicated to the memory of Theodore Holmes Bullock, a remarkable scientist and pioneer in the fields of comparative neurobiology and brain evolution. In some 60 years and 400 published titles, Ted truly altered both fields with studies spanning all major metazoan groups. He was that rare neurobiologist, perhaps the only one, who could have made significant contributions to each of the volumes that comprise this work.

Beginning in 1940, Ted's study of the functional organization of the nervous system in the enteropneust acorn worms laid the foundation for his contributions on neural evolution in deuterostomes. This benchmark publication was followed by studies of the giant nerve fibers in earthworms and squid, studies that pioneered the use of giant axons as synaptic models. His interests in the organization and evolution of invertebrate nervous systems culminated in the 1965 publication of *Structure and Function in the Nervous Systems of Invertebrates*, written in collaboration with G. Adrian Horridge. More than 40 years later, it is a testament to Ted and Adrian that these two volumes are still considered the definitive work in the field.

Even as Ted continued working on invertebrate nervous systems, he also turned his attention to the physiology of infrared receptors in pit vipers, the electroreceptors of gymnotid fishes, tectal units in frogs, and the physiological basis of slothfulness. The 1950s and 1960s thus marked a major expansion in the focus of Ted's research, as he began to probe sensory and integrative problems in the nervous systems of so-called 'lower vertebrates' and also began to consider broader topics involving the basic organization of neurons and how they code and process information. Not surprisingly, he was one of the pioneers and founders of the new discipline of neuroethology, and such multifarious endeavors continued to command his attention throughout the 1970s and 1980s.

Given Ted's remarkable grasp of both cellular and integrative neural mechanisms, one might have anticipated that he would develop an added interest in slow wave activity and cognition in animals as diverse as crayfish and humans, and these investigations were the focus of much of his effort during the latter years of his career.

While most scientists slow down as they approach 'old age', Ted's interests and insights continued to amaze all who knew him, and his fascination with nature never waned. In spite of a remarkably full and busy life, Ted always found time to encourage and inspire others. Many of the authors and their chapters in these volumes clearly reflect not only Ted's scientific contributions but also the excitement about nervous systems and their evolution that he brought to personal interactions with anyone who ever sat across his desk or poked in a tide pool with him.

R. GLENN NORTHCUTT

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PART IV: PRIMATE BRAIN EVOLUTION

PREFACE

This volume is for readers who are curious about how complex brains, such as the human brain, evolved from the much simpler nervous systems of ancient non-vertebrate ancestors. The chapters for this volume have been carefully selected from those in a larger, more comprehensive four-volume effort, the *Evolution of Nervous Systems*, published in 2007. To help fill in gaps, two short essays have been added from the 2009 *Encyclopedia of Neuroscience, online*. The chapters reflect the thoughts of the most knowledgeable experts in the field. While this condensation left out many wonderful chapters, both short and long, it allowed publication of a single volume on brain evolution that preserves much of the intent of the original four volumes while bringing a collection of exciting essays to a broader readership. The present chapters are presented in four parts that preserve the broad topics of the original four volumes. The first section of eight chapters includes historical and current theory on brain evolution, observations on brain development, as evolution depends on altered development, and current concepts of how the first nervous systems were organized. The second series of eleven chapters focuses on the nervous systems of primitive vertebrates, fishes, amphibians, reptiles, and birds, with many comparisons with mammals. The chapters provide an understanding of how the nervous system of mammals evolved, as well as how other vertebrates evolve complex, but different nervous systems. The third series of fifteen chapters covers the evolution of mammalian brains. As the use of skull endocasts from fossil mammals offers a direct window into the past, the sequence starts with a discussion of how fossils can help us understand brain evolution. Other chapters discuss the origin and evolution of neocortex, as this homolog of the small, thin dorsal cortex of reptiles became the highly variable, flexible, and often dominant brain structure in mammals. Included chapters also discuss the evolution of other parts of the brain, such as the basal ganglia, cerebellum, dorsal thalamus, and sensory and motor systems. The last series of nine chapters provides a broad view of primate evolution, describes the role of vision in shaping the nervous system of early primates, and outlines the evolution of sensory and motor systems in primates. Other chapters discuss frontal cortex, and how hemispheric specializations and systems for language, gesture, and tool use evolved in humans.

While the selection of specific chapters for this collection was my responsibility, I am deeply indebted to the volume editors of the earlier series, George Striedter, John Rubenstein, Theodore Bullock, Leah Krubitzer, and Todd Preuss, for their wisdom and efforts in selecting outstanding authors for chapters and carefully editing the results. I also thank Johannes Menzel, Publisher, and Elsevier for bringing this present volume and the previous series to life. I hope this volume provokes and guides students of brain evolution, generates further interest, and results in future publications with new and greater contributions to our understandings of brain evolution.

JON H. KAAS

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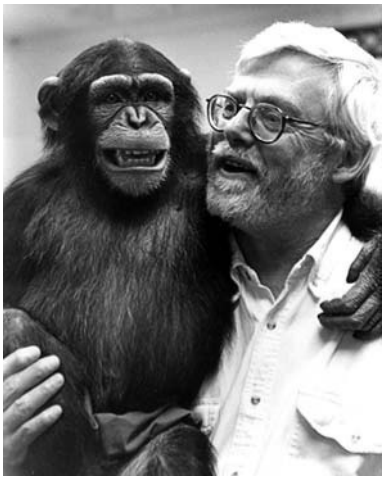
BIOGRAPHY OF EDITOR-IN-CHIEF



Jon H. Kaas is currently Distinguished Centennial Professor of Psychology at Vanderbilt University. He received his PhD training in comparative studies of forebrain organization in mammals in the laboratory of I. T. Diamond at Duke University, and postdoctoral training studying cortical organization in the comparative neurophysiology laboratory of C. N. Woolsey at the University of Wisconsin. His research has focused on determining the organizations of sensory and motor systems in mammals, especially in primates, with an effort to understand the evolution of the forebrain from early mammals to present-day humans. He has published over 275 research papers and 170 reviews. He is an elected member of the National Academy of Sciences, and of the American Academy of Arts and Sciences. He is also a member of the La Jolla Group for Explaining the Origin of Humans.

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INTRODUCTION

Evolutionary neuroscientists have existed since Darwin's days, and they are unlikely to go extinct. As long as neurobiological research is performed on a diversity of species, there will be those who seek to synthesize the disparate data, and for that synthesis the theory of evolution is indispensable. Still, a full 'evolutionary synthesis' in neuroscience is just beginning to take shape. No previous publication surveys the full spectrum of work in evolutionary neuroscience, ranging as it does from genes to behavior (via anatomy, physiology, and embryology) and from minute invertebrates to whales, elephants, and *Homo sapiens*. That is why this series of four volumes is so invaluable; it attempts to cover all aspects of evolutionary neuroscience. Volume 1, which lies before you now, is the most far-ranging of all. Its three most integrative aspects are the following.

First, Volume 1 surveys a vast array of theoretical ideas about nervous system evolution. In the olden days, evolutionary biology was dominated by the idea of a phylogenetic scale, but we now know that evolution is nonmonotonic and nonlinear. We have more accurate phylogenetic trees and well-developed methodologies for reconstructing what evolved from what. We also know how to link evolutionary changes in genes, anatomy, and physiology to evolutionary changes in behavior by means of both correlative and experimental analyses. As a result, we can construct scenarios of how evolution tinkered with nervous systems to help adapt species to their environments. In addition to such evolutionary 'case studies', we have some fairly general theories on how nervous systems evolve. For example, we know a great deal about how nervous systems scale and how conserved sets of genes and processes are used to produce nervous systems that, to previous generations of evolutionary neuroscientists, seemed completely dissimilar. These advances are covered in this book.

Second, the present volume includes a broad compilation of data on nervous system development. Neuroscientists now recognize that evolutionary changes in adult nervous systems are largely caused by changes in neural development. Meanwhile, developmental neuroscientists have made astonishing progress in unraveling the molecular mechanisms of neural development in multiple species, leading to an explosion in evo–devo neuroscience. One major theme emerging from this work is that many aspects of neural development are highly conserved across vast swaths of phylogeny. A second theme is that changes in one part of a developing neural system can cause a slew of generally adaptive and frequently compensatory changes in other parts of the system. Future work must now define how specific modifications in gene expression and function lead to evolutionary diversity of brain structure, connectivity, and plasticity.

Third, this volume is unusual in that it covers both invertebrates and vertebrates. Of the three major textbooks on evolutionary neuroscience that have been published in the last 10 years, none discuss invertebrates at length. Most of them discuss invertebrate nervous systems merely in the context of tracing vertebrate brain origins. Indeed, recent advances in comparative molecular biology have seriously altered how we think about the evolutionary origins of vertebrate brains. However, invertebrate nervous systems are well worth studying in their own right, for their own rich diversity and enigmatic elegance. Moreover, when working with invertebrates, it is frequently possible to combine behavioral, molecular, anatomical, and physiological analyses, and to extend such work across a multitude of species. Such broadly integrative studies have revealed that invertebrate nervous systems vary dramatically in size, complexity, and functionality, but still are built from highly conserved sets of genes. In that respect, they are quite similar to vertebrate nervous systems, though the diversity is more extreme.

Thus, this first volume shows evolutionary neuroscience to be a vast and vibrant field that holds enormous possibilities for new discoveries. Every chapter synthesizes an immense amount of data and integrates experiment and theory, ontogeny and phylogeny, invertebrate and vertebrate neurobiology, genes and behavior, and/or anatomy and physiology. Given this diversity of synthetic efforts, the chapter sequence was difficult to optimize. For example, it was impossible to construct separate sections for developmental and invertebrate neurobiology, because so much of the most interesting developmental work was performed on invertebrates. This may be frustrating for readers who seek a straightforward connecting thread, but actually, in this respect, the chapters merely resemble the evolutionary products they discuss: they weave a tangled web of insights, themes, and approaches that does not form a linear sequence. Still, or perhaps therefore, they ought to serve as fertile soil for further thoughts and work. That, at least, is our hope.

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AN INTRODUCTION TO HISTORY, THEORY, METHODS AND CONCEPTS

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1 History of Ideas on Brain Evolution

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Glossary

allometry	The notion that changes in the size of an object (e.g., the body or the brain) entail predictable changes in the proportional sizes of its compo nents. In contrast, isometric scaling involves no changes in an object's proportions.
convergence	The independent evolution of similar structures or functions from non homologous ancestral precursors.
developmental constraint	The notion that the mechanisms of development bias the production of phenotypic variants that natural selec tion can act on.
encephalization	Brain size relative to what one would expect in an organism of the same type (i.e., species or other taxonomic group) and body size. Synonym: rela tive brain size.
heterochrony	Phylogenetic changes in the relative timing of developmental events or in the relative rates of developmental processes.
homology	The relationship between two or more characters that were continuously pre sent since their origin in a shared ancestor. For a more detailed defini tion, especially for neural characters, see Striedter (1999).
mosaic evolution	The notion that, as brains evolve, individual brain regions may change in size independently of one another. In contrast, concerted evo lution indicates that brain regions must change their size in concert with one another.

The field of evolutionary neuroscience is more than 100 years old, and it has deep pre-evolutionary roots. Because that illustrious history has been reviewed repeatedly (Northcutt, 2001; Striedter, 2005) and is treated piecemeal in several articles of this book, I shall not review it fully. Instead, I will discuss a selection of the field's historically most important ideas and how they fit into the larger context of evolutionary theory. I also emphasize ideas that are, or were, controversial. Specifically, I present the field's central ideas in contrast pairs, such as 'common plan versus diversity' and 'natural selection versus constraints'. This approach scrambles the chronology of theoretical developments but helps to disentangle the diverse strands of thought that currently characterize evolutionary neuroscience. It also helps to clarify which future directions are likely to be most fruitful for the field.

1.1 Common Plan versus Diversity

One of the most famous battles of ideas in comparative biology was that between Etienne Geoffroy St. Hilaire and George Cuvier over the existence, or not, of a common plan of construction (or Bauplan) for animals (Appel, 1987). Geoffroy was of the opinion, previously developed by Buffon (1753), that all animals are built according to a single plan or archetype, but Cuvier, France's most illustrious morphologist, recognized at least four different types. Their disagreement erupted into the public sphere when Geoffroy in 1830 endorsed the view that the ventral nerve cord of invertebrates is directly comparable (today we say 'homologous') to the spinal cord of vertebrates. Cuvier responded that Geoffroy was speculating far beyond the available data, and he reasserted publicly that the major types of animals could not be linked by intermediate forms or topological transformations. This Cuvier–Geoffroy debate was followed closely by comparative biologists all across Europe, who were already flirting with the idea of biological evolution or, as they called it, the transmutation of species. If Cuvier was right, then

evolution was impossible. On the other hand, some of Geoffroy's hypotheses (e.g., his proposal that insect legs correspond to vertebrate ribs) did seem a trifle fanciful. Thus, the Cuvier–Geoffroy debate embodied much of the ambivalence surrounding evolution in the first half of the nineteenth century.

After Darwin offered a plausible mechanism for the transmutation of species, namely, natural selection (Darwin, 1859), the idea of biological evolution took hold and, by extension, Geoffroy's ideas gained currency. Innumerable homologies were sought and, frequently, revealed (Russel, 1916). Most impressive was the discovery of extensive molecular homologies between species that span the metazoan family tree (Schmidt-Rhaesa, 2003). It was striking, for example, to discover that many of the genes critical for early brain development are homologous between insects and vertebrates (Sprecher and Reichert, 2003). Indeed, the invertebrate and vertebrate genes are sometimes functionally interchangeable (Halder *et al.*, 1995; deRobertis and Sasai, 1996). Those discoveries supported Geoffroy's view that all animals were built according to a common plan, which could now be understood to be a common genetic blueprint or 'program' (Gehring, 1996). Indeed, many biologists proceeded to search for molecular genetic homologies that could reveal previously unimagined morphological homologies (Janies and DeSalle, 1999). Geoffroy would have been thrilled. There are, however, problems with the view that animals are all alike.

The most serious problem, in my view, is that homologous genes may sometimes be involved in the development of adult structures that are clearly not homologous (Striedter and Northcutt, 1991). For example, insect wings and vertebrate nervous systems both depend on *hedgehog* function for normal development, but this does not make neural tubes and insect wings homologous (Baguña and Garcia-Fernandez, 2003). Instead, findings such as this suggest that evolution tends to work with highly conserved 'master genes' (Gehring, 1996) or, more accurately, tightly knit assemblies of crucial genes (Nilsson, 2004), which it occasionally reshuffles by altering their upstream regulatory elements and/or downstream targets. Evolution is a terrific tinkerer that manages to create novelty from conserved elements. This conclusion echoes Geoffroy's arguments insofar as it acknowledges that "Nature works constantly with the same materials" (Geoffroy, 1807), but it does not mesh with the view that evolution built all animals according to a single plan. What we have, then, is at least a partial rapprochement of the positions held by Cuvier and Geoffroy: adult organisms do conform to several different body plans, but they are built by shuffling repeatedly a highly conserved set of genes (Raff, 1996). Therefore, a crucial question for research is how evolutionary changes in networks of developmentally important genes influence adult structure and function.

Implicit in the preceding discussion has been the idea that adult species differences arise because of evolutionary changes in development (Garstang, 1922). This idea is commonly accepted now, but, back in the nineteenth century, Haeckel (1889) used to promote its polar opposite, namely, the notion that phylogeny creates ontogeny (see Gould, 1977). Haeckel also promoted the idea that all vertebrates pass through a highly conserved phylotypic stage of embryonic development (Slack *et al.*, 1993). Studies have, however, challenged the phylotypic stage idea by showing that the major groups of vertebrates can be distinguished at all stages of embryogenesis (Richardson *et al.*, 1997). An intriguing aspect of that early embryonic variability is that it consists mainly of differences in the timing of developmental processes (Richardson, 1999). Little is known about the genes that generate those changes in developmental timing (also known as heterochrony), but some of them, at least, are likely to be fairly well conserved across species (Pasquinelli and Ruvkun, 2002). More importantly, the notion that adult diversity is based on evolution changing the temporal relationships of conserved processes represents another reconciliation of Cuvier's insistence on adult diversity with Geoffroy's belief in a common plan. Thus, the field of evolutionary developmental biology (evo-devo for short) has overcome the once so prominent dichotomy between conservation and diversity. Its major challenge now is to discover the mechanistic details of how conserved genes and processes are able to produce such diverse adult animals.

Evo-devo thinking has also invaded neuroscience, but evo-devo neurobiology still emphasizes conservation over diversity. For example, we now have extensive evidence that all vertebrate brains are amazingly similar at very early stages of development (Puelles *et al.*, 2000; Puelles and Rubenstein, 2003). However, we still know very little about how and why brain development diverges in the various vertebrate groups after that early, highly conserved stage or period. Looking beyond vertebrates, we find that insect brain development involves at least some genes that are homologous to genes with similar functions in vertebrates (Sprecher and Reichert, 2003). This is remarkable but does not prove that insects and vertebrates are built according to a common plan – if by that we mean that the various parts of adult insect brains all have vertebrate homologues. For example, the finding that several conserved genes, notably

Pax6, are critical to eye development in both invertebrates and vertebrates, does not indicate that all those eyes are built according to a common plan. The crucial question, which we are just beginning to explore, is how the conserved genes are tinkered with (reshuffled, co-opted, or redeployed) to produce very different adult eyes (Zuber *et al*., 2003; Nilsson, 2004). This, then, seems to be the future of evo-devo neurobiology: to discover how highly conserved developmental genes and processes are used to different ends in different species. As I have discussed, this research program has ancient roots, but it is just now becoming clear.

1.2 *Scala Naturae* versus Phylogenetic Bush

The idea of evolution proceeding along some kind of scale from simple to complex also has pre-evolutionary roots. Aristotle, for example, ordered animals according to the degree of perfection of their eggs (see Gould, 1977). Later religious thinkers then described an elaborate scale of nature, or *scala naturae*, with inanimate materials on its bottom rung and archangels and God at the other extreme. The early evolutionists, such as Lamarck, transformed this static concept of a *scala naturae* into a dynamic phylogenetic scale that organisms ascended as they evolved. Darwin himself had doubts about arranging species on a scale, but most of his followers had no such qualms (Bowler, 1988). Even today, the phylogenetic scale is taught in many schools and it persists in medicine and academia. For example, the National Institutes of Health's (NIH) guide for institutional animal care and use still recommends that researchers, whenever possible, should work with "species lower on the phylogenetic scale" (Pitts, 2002, p. 97). On the other hand, most contemporary evolutionists have pronounced as dead both the *scala naturae* and its postevolutionary cousin, the phylogenetic scale (Hodos and Campbell, 1969). What do those modern evolutionists cite as the scales' cause of death?

One fatal flaw in the idea that species evolve along a single scale is that, as we now know, evolution made at least some species simpler than their ancestors. Salamanders, for example, are much simpler, especially in brain anatomy (Roth *et al*., 1993), than one would expect from their phylogenetic position. Even more dramatically, the simplest of all animals, the placozoans, are now thought to have evolved from far more complicated ancestors (Collins, 1998). As more and more molecular data are used to reconstruct phylogenies, it is becoming apparent that such secondary simplification of entire animals has occurred far more frequently than scientists had previously believed (Jenner, 2004) – perhaps because they were so enamored of the phylogenetic scale. A second major problem with *scala naturae* thinking is that the order of species within the scale depends on which organismal features we consider. For example, many fishes would rank higher than mammals if we based our scale on skull complexity, which was reduced dramatically as early mammals evolved (Sidor, 2001). Similarly, dolphins rank high if we look only at brain size, but relatively low if we consider neocortical complexity, which was reduced as the toothed whales evolved (Morgane and Jacobs, 1972). Most people tacitly agree that 'higher animals' are warm-blooded, social, curious, and generally like us, but once we try to be more objective, the single 'chain of being' (Lovejoy, 1936) fractionates into a multitude of different chains, none of which has any special claim to being true.

This multiple-chains idea becomes self-evident once we have grasped that species phylogenies are just like human family trees; they are neither ladders, nor trees with just a single trunk, but bushes or tumbleweeds (Striedter, 2004) with branches growing in divergent directions. Within a given branch, or lineage, complexity may have increased at some points in time and decreased at others, but even if complexity increased more frequently than it decreased, the overall phylogeny would fail to yield a single scale, because complexity tends to increase divergently in different lineages. For example, bats, honeybees, and hummingbirds are all incredibly complex, compared to their last common ancestor, but they are each complex in different ways. Of course, we can pick one parameter and build a scale for that – we can, for instance, compare the ability of bats, honeybees, and hummingbirds to see ultraviolet (UV) radiation – but different parameters might well yield different scales. Simply put, changes that occurred divergently in different lineages will not, in general, produce a single overarching scale. This insight is old hat to evolutionary biologists, but news to many neuroscientists (Hodos and Campbell, 1969). In part, therefore, the persistence of *scala naturae* thinking in the neurosciences reflects a lack of proper training in contemporary evolutionary theory. In addition, I suspect that human minds possess a natural tendency for ordering disparate items linearly. Such a bias would be useful in many contexts, but it would make it difficult to comprehend (without training) the divergent nature of phylogeny.

Although *scala naturae* thinking persists in neuroscience generally, evolutionary neuroscientists have labored to expunge its ghost. For example, a consortium of 28 comparative neurobiologists

revised the nomenclature of avian brains to replace the terms *neo*striatum, *archi*striatum, and *paleo*striatum – which suggested that brains evolved by the sequential addition of new brain regions – with terms devoid of *scala naturae* overtones (Reiner *et al.*, 2004a, 2004b; Jarvis *et al.*, 2005). Some of the replacement names are terms that were already used for brain regions in other vertebrates; they reflect our current understanding of homologies. However, some of the new terms – e.g., nidipallium and arcopallium – are novel and intended to apply exclusively to birds. These novel names were coined because bird brains, particularly bird forebrains, have diverged so much from those of other vertebrates (including reptiles) that strict one-to-one homologies are difficult, if not impossible, to draw for several regions (Striedter, 1998, 1999). Thus, the revised terminology reflects a new consensus view that avian brains did not evolve by the sequential addition of new brain areas, yet also reminds us that bird brains are full of features that evolved quite independently of those that feature in mammalian phylogeny. In other words, the new terminology avoids *scala naturae* overtones and, instead, combines the notion of a common plan with that of divergent complexity.

As comparative neurobiologists reject the notion of a *scala naturae*, they stand to lose a central part of their traditional justification for working on nonhuman brains. No longer can they argue that research on other brains must be useful because nonhuman brains are always simpler, and therefore easier to comprehend, than human brains. Instead, they must admit that some nonhuman brains are stunningly complex and, more importantly, that their phylogenetic paths toward complexity diverged from the primate trajectory. That is, complex bird, fish, or insect brains are not mere steps along the path to human brains, but the outcome of divergent phylogenies (see Evolution of the Nervous System in Fishes, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?). Does this suggest that research on nonhuman brains should cease to be funded? I do not think so, but the justification for working on nonhuman brains ought to be tweaked.

One obvious alternative justification is that all brains are likely to share some features, especially if they come from close relatives. Another good justification for research on nonhuman brains is that, compared to human brains, the former are much more amenable to physiological and anatomical research. This line of justification assumes that the model differs from the target system only in those respects that make the model easier to study, and not in the respects that are modeled – an assumption that sometimes fails. It now appears, for example, that the auditory system of owls, which was generally regarded as an ideal model for sound localization in vertebrates, exhibits some highly specialized features (McAlpine and Grothe, 2003). This finding, at first glance, suggests that research on bird brains is wasteful, but this is a simplistic view. Research on the owl's auditory system has taught us much about how neurons compute behaviorally relevant information and it serves as an invaluable reference against which we can compare sound processing in other species, including humans. Furthermore, some differences between a model and its target can lead to surprising discoveries. Much might be gained, for example, from studying why some nonhuman brains are far more capable than primate brains of repairing themselves (Kirsche and Kirsche, 1964). Thus, model systems research can be useful even if the model is imprecise. A third, less frequently discussed, justification for examining the brains of diverse species is that comparative research can bring to light convergent similarities, which in turn might reveal some principles of brain design. For example, the discovery that olfactory systems in both vertebrates and many different invertebrates exhibit distinctive glomeruli strongly suggests that those glomeruli are needed for some critical aspects of odorant detection and analysis (Strausfeld and Hildebrand, 1999).

Therefore, research on nonhuman brains need not be justified in terms of a presumed phylogenetic scale. Instead, comparative neurobiology is valuable because (1) all brains are likely to share some features, (2) nonhuman brains are more amenable to some types of research, and (3) the study of diverse nonhuman brains can lead to the discovery of design rules for brains. Historically, only the first of these alternatives has been widely discussed, but all are logically sound, and none depend on the existence of a *scala naturae*.

1.3 Relative Size versus Absolute Size

The most obvious difference between species is that they differ enormously in size. Because life began with tiny organisms, evolutionary increases in body size must have outnumbered or outpaced the decreases. This is true of organisms generally, but it also holds for several individual lineages, including mammals and, within mammals, primates (Stanley, 1973; Alroy, 1998). The most fascinating aspect of those changes in body size is that they involved much more than the isometric scaling up or down of the ancestral condition; they involved allometric changes in the proportions of body parts and physiologic processes. For example, skeletal mass increases disproportionately with increasing body size, whereas

heart rate decreases. Countless studies – on both vertebrates and invertebrates – have documented these allometries and explored their functional implications (Calder, 1984; Schmidt-Nielsen, 1984).

Much less is known about the causes of allometry. Studies on allometry in insects showed that some scaling relationships are readily modifiable by natural or artificial selection (see Emlen and Nijhout, 2000; Frankino *et al.*, 2005). This finding suggests that even tight scaling laws are not immutable, which would explain why many traits scale differently (e.g., with different exponents) in different taxonomic groups (Pagel and Harvey, 1989). A very different, more theoretical line of research has shown that numerous allometries, specifically those with power law exponents that are multiples of 1/4, may have evolved because the optimal means of delivering metabolic energy to cells is through an hierarchically branching, fractal network of vessels whose termini (e.g., capillaries) are body size-invariant (West *et al.*, 1997; Savage *et al.*, 2004; West and Brown, 2005). This theory is mathematically complex and still controversial (Kozlowski and Konarzewski, 2004; Brown *et al.*, 2005; Hoppeler and Weibel, 2005), but it is elegant. Furthermore, because the theory of West *et al.* is based in part on the assumption that natural selection optimizes phenotypes, it is consistent with the aforementioned finding that allometries are modifiable by selection. However, West *et al.*'s (1997) theory cannot explain (or does not yet explain) why some organs, such as the brain, scale with exponents that are not multiples of 1/4. Nor can it easily explain taxonomic differences in scaling exponents. Thus, the causal – physiological and/or developmental – bases of allometry are coming into focus but remain, for now, mysterious.

Brain scaling, in particular, remains quite poorly understood (see Principles of Brain Scaling). The discovery that brains become proportionately smaller with increasing body size dates back to the late eighteenth century (Haller, 1762; Cuvier, 1805–1845). Since then, numerous studies have documented brain allometry in all the major groups of vertebrates (Deacon, 1990a; van Dongen, 1998) and even some invertebrates (Julian and Gronenberg, 2002; Mares *et al.*, 2005). Generally speaking, those studies confirmed that in double logarithmic plots of brain size versus body size, the data points for different species within a given lineage tend to form a reasonably straight line, indicating the existence of a simple power law. The slope of those best-fit lines are almost always less than 1, which reflects the aforementioned fact that brains generally become proportionately smaller with increasing body size. The large body of work on brain–body scaling further revealed that data points for different taxonomic groups often form lines with similar slopes but different y intercepts. These differences in y intercepts are known as differences in relative brain size or encephalization. They seriously complicate efforts to draw a single allometric line for any large taxonomic group (Pagel and Harvey, 1989), but they allow us to identify evolutionary changes in relative brain size among some smaller taxonomic groups. For example, they allow us to determine that relative brain size increased with the origin of mammals, with the origin of primates, several times within primates, with the origin of the genus *Homo*, and, last but not least, with the emergence of *Homo sapiens* (see Primate Brain Evolution). Overall, such phylogenetic analyses suggest that, among vertebrates, relative brain size increased more frequently than it decreased (Striedter, 2005).

Enormous effort has gone into determining the functional significance of evolutionary changes in brain–body scaling. Darwin, for example, had argued that relative brain size is related to "higher cognitive powers" (Darwin, 1871), but defining those powers and comparing them across species has proven difficult (Macphail, 1982). Consequently, most subsequent investigators shied away from the notion of general intelligence, or 'biological intelligence' (Jerison, 1973), and focused instead on more specific forms of higher cognition. Parker and Gibson (1977), for example, proposed that a species' degree of encephalization is related to its capacity for extracting nutritious fruits and nuts from their protective shells. Several authors have stressed correlations between brain size and 'social intelligence' (Byrne and Whiten, 1988; Dunbar, 1998; Reader and Laland, 2002). Collectively, these studies reinforced the sense that relative brain size is, somehow, related to some forms of intelligence. However, relative brain size also correlates with several other attributes, such as longevity, home-range size, diet, and metabolic rate (for a review, see van Dongen, 1998). The latter correlations, with diet and metabolism, have received particularly lavish attention (Martin, 1981; McNab, 1989; Aiello and Wheeler, 1995). Paradoxically, the discovery of so many correlations has led some evolutionary neuroscientists to despair: there are too many correlates of relative brain size, and many of them come and go, depending on which taxonomic group is being examined and which statistical methods are used for the analyses (e.g., Bennet and Harvey, 1985; Iwaniuk *et al.*, 1999; Deaner *et al.*, 2000; Beauchamp and Fernández-Juricic, 2004; Jones and MacLarnon, 2004; Martin *et al.*, 2005). Too many contested hypotheses, too little certitude.

There is not much clarity on why brains scale so predictably with body size. Early workers argued that

brains generally scale against body size with a power law exponent close to 2/3 because the brain's sensory and motor functions were related to the body's surface area, which presumably scales with that same exponent (Snell, 1891; Jerison, 1973). According to this view, brain sizes in excess of that predicted by the 2/3 power law are due to increases in the brain's nonsomatic, cognitive regions. This would explain the correlations between relative brain size and some forms of intelligence. Unfortunately, there are two major problems with this view. First, brain–body scaling exponents often differ substantially from 2/3 (van Dongen, 1998; Nealen and Ricklefs, 2001). The second problem is that the brain's more cognitive regions also scale predictably with body size (Fox and Wilczynski, 1986), undermining the assumption that brains are divisible into regions that scale with body size and regions that do not. Therefore, the excess neuron hypothesis (Striedter, 2005) is dead. In searching for an alternative, some have suggested that brain–body allometry is linked to the scaling of metabolic rates. This hypothesis is based on the observation that, in at least some taxonomic groups, brain size and basal metabolic rate scale against body size with similar exponents (Martin, 1981; Mink *et al*., 1981). However, other studies have shown that the correlation between brain size and metabolism is not tight, once the mutual correlation with body size is factored out (McNab, 1989). This correlational slack presumably arises because species differ in how much of the body's total energy supply they deliver to the brain (Aiello and Wheeler, 1995; Kaufman, 2003), but this just underscores that relative brain size is not so tightly linked to metabolic rate.

Overall, the lack of clarity on what causes brains to scale predictably with body size, and how to interpret deviations from the scaling trends, has caused interest in relative brain size to fade. Increasingly, evolutionary neuroscientists have turned away from relative brain size and asked, instead, how the size of individual brain regions correlates with various behavioral parameters (Harvey and Krebs, 1990). This shift in research strategy makes sense, because, after all, the brain is functionally heterogeneous. However, even studies that focus on correlations between single brain areas and specific behaviors – some refer to them as neuroecological studies – are controversial because: (1) the behavioral parameters are difficult to quantify and/or define (Bolhuis and Macphail, 2001), (2) neuronal structure–function relationships are complex and often poorly understood, (3) it is difficult to decide *a priori* whether one should correlate behavioral parameters against a region's absolute size, its proportional size, or its size relative to expectations (Striedter, 2005), and (4) the methods for establishing statistically significant correlations in phylogenetic data remain debatable (Felsenstein, 1985; Garland *et al*., 1992; Smith, 1994; Martin *et al*., 2005). Brave neuroscientists are continuing to tackle those problems, but the larger problem of how to deal with relative brain size – how to find its causes and its functional significance – is fading from view. Perhaps we need a new approach to understanding relative brain size – perhaps one that is linked more directly to the physiological and geometric properties of brains (West and Brown, 2005) – but this novel direction is not yet apparent.

As interest in relative brain size waned, interest in absolute brain size waxed, mainly because many of the brain's internal structural and functional features turn out to scale predictably with absolute brain size. Best studied is the phenomenon of size-related shifts in brain region proportions (Sacher, 1970; Finlay and Darlington, 1995). In mammals, for example, the neocortex becomes disproportionately large as absolute brain size increases, whereas most other regions become disproportionately small. A second interesting scaling law is that a brain's degree of structural complexity tends to increase with absolute brain size. Within the neocortex, for example, the number of distinct areas increases predictably with neocortex size (Changizi and Shimojo, 2005). A third fascinating aspect of brain scaling is that the amount of white matter within mammalian brains scales allometrically with absolute brain size (Ringo, 1991; Zhang and Sejnowski, 2000). This connectional allometry, taken together with the fact that synapse size and density are relatively size-invariant, indicates that brains become less densely interconnected, on average, as they increase in size (Stevens, 1989; Deacon, 1990a, 1990b; Striedter, 2005). All of this signifies that brains change structurally in many ways as they vary in absolute size. Many of those changes have clear functional implications. For example, it has been suggested that, as hominid brains increased in size, the axons interconnecting the two cerebral hemispheres became so sparse and long that the hemispheres became less capable of interacting functionally, which led to an increase in functional asymmetry (Ringo *et al*., 1994; see The Evolution of Hemispheric Specializations of the Human Brain). Considerations such as these suggest that absolute brain size is a much better predictor of brain function than relative brain size, at least among close relatives (Striedter, 2005).

In retrospect, we can say that evolutionary neuroscientists historically have overemphasized relative brain size. As Dunbar (2006) put it, comparative neurobiologists have too long been "dragooned into worrying about relativizing brain size by a very peculiar view that body size must be the default

determinant of brain volume." Can we explain this undue emphasis? Partly, evolutionary neuroscientists may have worried that focusing on absolute brain size and linking it to higher cognitive powers would force us to conclude that whales and elephants, with their enormous brains, are smarter than humans. This is a valid concern, for few would doubt that humans are – or at least can be – the most intelligent creatures on earth. However, whales and elephants are behaviorally complex, and humans may well be special because they are unique in possessing symbolic language (Macphail, 1982). Furthermore, it seems to me that large whales, with large brains, are more intelligent (both socially and in their hunting strategies) than dolphins or small whales. This hypothesis remains to be tested, but it points to a strategy for reconciling absolute and relative brain size: among close relatives, comparisons of absolute brain size are most informative, but in comparisons of distant relatives (e.g., whales and humans), relative brain size is a more potent variable (Striedter, 2005). This view is consistent with the finding that, among primates, social group size correlates more strongly with absolute brain size than with relative brain size (Kudo and Dunbar, 2001; Striedter, 2005). It also serves as a productive counterweight to the field's traditional, almost exclusive emphasis on relative brain size.

1.4 Natural Selection versus Developmental Constraints

Darwin's theory of natural selection entails two main components, namely, that (1) organisms produce offspring with at least some heritable variation and (2) that organisms generally produce more offspring than their environment is able to sustain. Given those two components, some variants are bound to be fitter than others in the sense that their offspring are more likely to survive and produce offspring. This difference, in turn, will cause the heritable traits of the fitter variants to spread in the population. Given this, Darwin's most "dangerous idea" (Dennett, 1995), one can explain an organism's attributes in terms of the selective pressures that promoted their spread and, hence, their current existence. An enormous number of such adaptational explanations have been proposed. Many stress that natural selection optimized features for specific functions; others emphasize that natural selection tends to produce optimal compromises between competing functions and/or costs (Maynard Smith, 1982). Generally speaking, the explanatory power of these adaptational explanations derives solely from natural selection's second step, the sorting of offspring. Generation of the variants that are sorted is usually assumed to be random and, hence, irrelevant to explanations of the phenotype. This 'adaptationist paradigm' (Gould and Lewontin, 1979) has dominated evolutionary theory for most of its history.

In the 1970s and 1980s, however, the adaptationist paradigm was challenged by authors who stressed that the variants available to natural selection may not really be random (Gould and Lewontin, 1979; Alberch, 1982; Maynard Smith *et al.*, 1985). Central to those challenges was the idea that, even if mutations are random at the genetic level, those random genetic mutations are channeled, or filtered, through mechanisms of development that favor the emergence of some phenotypes. Some structures may be impossible for embryos to develop; others are likely to emerge (Alberch, 1982). If this is true, then natural selection chooses not among a random selection of phenotypes but from a structured set that is determined, or at least biased, by the mechanisms of development. This idea is important, because it suggests that development constrains the power of natural selection to set the course of evolutionary change. It threatens natural selection's widely assumed omnipotence. Some authors carried this threat so far as to exhort biologists to halt their search for adaptive scenarios and to research, instead, the 'generative' mechanisms of development (Goodwin, 1984). Fortunately, most evolutionary biologists today seek a more balanced rapprochement of embryology and evolutionary biology (Gilbert *et al.*, 1996; Wagner and Laubichler, 2004).

Specifically, evo-devo biologists today tend to accept the concept that natural selection is the most prominent determinant of who thrives and who dies, no matter how constrained development might be. They also tend to stress that development itself is subject to descent with modification – i.e., evolution – which means that even fairly tight constraints can change. Therefore, explanations couched in terms of natural selection are not antithetical to those involving developmental constraints, but complementary (Striedter, 2005). Still, the synthesis of natural selection and developmental constraints remains uncertain in one key respect: what if the mechanisms of development were shaped by natural selection to produce variants that are much fitter than one would expect by chance? Then the distinction between the generative and selective components of natural selection (see above) would blur. The developmental production of variants would no longer be random with respect to a species' ecology. This hypothesis, which was pushed furthest by Riedl (1977), is interesting and potentially profound, but not yet supported by much evidence.

Brains were historically considered to be shaped by natural selection, unencumbered by developmental constraints. In general, the size and structure of both entire brains and individual brain regions were thought to be optimized. Jerison (1973, p. 8), made this idea explicit when he wrote that "the importance of a function in the life of each species will be reflected by the absolute amount of neural tissue of that function in each species." How development produced that fine-tuning was never specified. Presumably, the idea was that genetic mutations could vary the size and structure of individual brain regions freely, leading to steady improvements in fitness until an optimum was reached. Little thought was given to the possibility that brains might be constrained in how they could evolve. However, a few authors proposed that trophic dependencies between interconnected brain regions might cause entire circuits or systems to change size in unison rather than piecemeal (Katz and Lasek, 1978). Such 'epigenetic cascades' (Wilczynski, 1984) might channel evolution (Katz *et al*., 1981), but they would not constrain natural selection, because the cascades help to optimize functional brain systems by matching the size of interconnected neuronal populations. That is, epigenetic cascades act not against, but in conjunction with, the optimizing power of natural selection; they are not classical constraints, which may explain why they have rarely been discussed (Finlay *et al*., 1987).

The idea of brains evolving under a restrictive developmental rule was proclaimed forcefully by Finlay and Darlington (1995). Their argument was founded on the observation that the various major brain regions in mammals scale against absolute brain size with different allometric slopes (Sacher, 1970; Gould, 1975; Jerison, 1989). Although this finding was well established at the time, it had not been explained; it was a scaling rule without a cause. Finlay and Darlington's major contribution was to propose that the height of a region's allometric slope was related to the region's date of birth (i.e., the time at which the region's precursor cells cease to divide), with late-born regions tending to become disproportionately large with increasing brain size. Why does this relationship exist? Finlay and Darlington (1995) showed that their late-equals-large rule emerges naturally if neurogenetic schedules (i.e., the schedules of what regions are born when) are stretched as brains increase in size and compressed when they shrink. This insight, in turn, prompted Finlay and Darlington to hypothesize that brain evolution is constrained to stretch or compress neurogenetic schedules and cannot, in general, delay or advance the birth of individual regions. In other words, even if evolution 'wanted' to increase the size of only one brain region, it would be 'forced' to change also the size of many other brain regions. Thus, Finlay and Darlington argued that development constrains brains to evolve concertedly, rather than mosaically.

Finlay and Darlington's developmental constraint hypothesis has been challenged by various authors, who all pointed out that brains do sometimes evolve mosaically (Barton and Harvey, 2000; Clark *et al*., 2001; de Winter and Oxnard, 2001; Iwaniuk *et al*., 2004; Safi and Dechmann, 2005). In addition, Barton (2001) has argued that correlations between region size and absolute brain size are due to functional requirements, rather than developmental constraints. Specifically, Barton (2001, p. 281) reported that the sizes of interconnected brain regions in what he called a functional system exhibited "significantly correlated evolution after taking variation in a range of other structures and overall brain size into account." Finlay *et al*. (2001) countered that such system-specific evolution may indeed occur, particularly for the so-called limbic system (see also Barton *et al*., 2003), but that this does not negate the existence of developmental constraints. In a review of this debate, I concluded that most of it may be resolved by arguing that instances of mosaic (and/or system-specific) evolution occur against a background of concerted, developmentally constrained evolution (Striedter, 2005). Both Finlay and Barton seem open to this kind of rapprochement (Finlay *et al*., 2001; Barton, 2006).

The debate on mosaic versus concerted evolution highlights how little we know about the evolution of neural development or, for that matter, about the role that natural selection played in shaping brains. The developmental data used to support Finlay *et al*.'s (2001) hypothesis came from just 15 species and were collected by several different laboratories, using diverse methodologies. Moreover, the data are limited to dates of neurogenesis. We know virtually nothing about species differences (or similarities) in how large brain regions are prior to neurogenesis, how quickly the regions grow, or how much cell death they endure. Data on these other, relatively neglected aspects of brain development might reveal additional constraints, and they might clarify how regions can evolve mosaically even if neurogenetic schedules are conserved.

Similarly lacking are data on natural selection and the brain. Although several analyses have shown that the size of some brain regions (relative to absolute brain size) correlates with aspects of a species' behavior or ecology (e.g., Clark *et al*., 2001; de Winter and Oxnard, 2001; Iwaniuk *et al*., 2004),

such correlations are only indirect evidence for natural selection. More direct data are difficult to gather, because direct demonstrations of natural selection at work require measurements of heritability and fitness functions. As it is, we know so little about how selection acts on brains that debates on its potency are bound to erupt. Clearly, more studies must be performed before we can reach firm conclusions about which aspects of brain development and evolution are tightly constrained and which are subject to specific selective pressures.

1.5 One Law, Many Laws, or None

Is human history explicable in terms of general principles or laws? This question has been debated extensively. Some scholars insist that history is based largely on a few major laws, playing out against a background of far less important noise. Others argue, instead, that history is so full of contingencies (or accidents) that general or universal laws are blown to bits. I am not competent to review this debate but find myself most sympathetic to the intermediate position taken by Hempel (1942) in his call for a nomological–deductive approach to history. Basically, Hempel argued that historical events can be explained only by reference to various general (deterministic or probabilistic) laws that causally link preceding events or conditions to the event being explained. For example, an account of why an automotive radiator cracked during a frost would involve both historical contingencies and general laws relating temperature to pressure (Hempel, 1942). Similarly, events in human history can be explained by "showing that the event in question was not 'a matter of chance', but was to be expected in view of certain antecedent or simultaneous conditions" (Hempel, 1942) and the operation of several, often implicitly assumed, general laws. This nomological–deductive methodology waxes and wanes in popularity (Kincaid, 1996; McIntyre, 1996), but it seems logical in principle. Naturally, one may debate whether human behavior is predictable enough to yield the kind of laws that are needed for nomological–deductive explanations (Beed and Beed, 2000).

Evolutionary biologists have likewise debated the role of general laws in explaining the past, which in their realm is phylogeny. Some have argued that natural selection is a universal law that can be used to explain the emergence of many, if not most, biological features. Others have countered that natural selection is a mathematical truth, rather than an empirically determined law (Sober, 2000). More importantly, many biologists have pointed out that the results of natural selection are not highly predictable. Gould (1989) made this argument when he declared that rewinding the tape of life on earth and playing it again would not lead to a repeat performance. Biological history is full of accidents, of happenstance. Therefore, Gould argued, evolutionary explanations must be crafted one event at a time, without recourse to general laws. On the other hand, Gould did grant that evolution is constrained by diverse physical principles, by rules of construction and good design, and by some scaling rules (Gould, 1986, 1989). In his view, "the question of questions boils down to the placement of the boundary between predictability under invariant law and the multifarious possibilities of historical contingency" (Gould, 1989, p. 290). Gould placed this boundary "so high that almost every interesting event of life's history falls into the realm of contingency" (Gould, 1989, p. 290). This appears to be an extreme position, for many other evolutionary biologists place that same boundary lower. They tend to be far more impressed than Gould by the degree of convergent evolution in the history of life (Carroll, 2001; Willmer, 2003). They look, for example, at the convergent similarities of eyes in vertebrates and octopi and conclude that some design rules for eyes exist. In sum, disagreements persist about the placement of Gould's boundary between predictability and contingency, but most biologists accept that evolutionary explanations must involve at least some causal laws (Bock, 1999).

Given this context, it is not surprising that neuroscientists are conflicted about the importance of general laws for explaining the evolutionary history of brains. Marsh (1886) had proposed that brains consistently increase in size over evolutionary time, but later authors vehemently disagreed (see Jerison, 1973; Buchholtz and Seyfarth, 1999). Personally, I think that Marsh did have a point, for brain and body size have both increased, at least on average, in several vertebrate lineages (see Striedter, 2005). Still, Marsh's laws were merely descriptions of phylogenetic trends, not causal laws. The first explicitly causal law of brain evolution was Ariëns Kappers' (1921) law of neurobiotaxis, which states that cell groups in evolution tend to move toward their principal inputs. Unfortunately for Ariëns Kappers, later studies showed that cell groups do not move quite so predictably and called into question some of the mechanisms that supposedly produced neurobiotaxis. The next major putative law of brain evolution was Ebbesson's (1980) parcellation principle, which states that brains become more complex by the division of ancestrally uniform

cell groups into daughter aggregates that selectively lose some of their ancestral connections. This principle was strenuously criticized by most comparative neuroanatomists, mainly because its empirical foundation was shaky (see Ebbesson, 1984). Although a weak version of Ebbesson's theory, stating merely that brains become less densely connected as they increase in size, is probably defensible (Deacon, 1990a; Striedter, 2005), the strong version of Ebbesson's original idea has failed the test of time: plenty of data now show that brains evolve not only by the loss of connections, but also by creating novel projections.

Confronted with this abundance of failed brain evolution laws, most evolutionary neuroscientists have emphasized only a single, undisputed regularity of brain evolution, namely, that numerous aspects of brain structure and function are highly conserved across species. Specifically, they focused, á la Geoffroy St. Hilaire, on the existence of common plans of construction and highlighted molecular homologies between invertebrates and vertebrates (see above). This has been productive. It is important to note, however, that the principle of phylogenetic conservation predicts stability and does not deal explicitly with change. Is brain phylogeny subject to just a single law, which states that brains change little over time? Or are there also laws of evolutionary change in brains? I affirmed the second possibility (Striedter, 2005), but laws of evolutionary change in brains are no doubt difficult to find. C. J. Herrick, a founding father of evolutionary neuroscience, put it well:

> Most scientific research has been directed to the discovery of the uniformities of nature and the codification of these in a system of generalizations. This must be done before the changes can be interpreted. The time has come to devote more attention to the processes and mechanisms of these changes… but it is much more difficult to find and describe the mechanisms of … [the] apparently miraculous production of novelties than it is to discover the mechanical principles of those repetitive processes that yield uniform products (Herrick, 1956, p. 43).

The last few years have seen an uptick in the number of studies that address evolutionary change and novelty in brains (Aboitiz, 1995; Catania *et al*., 1999; Rosa and Tweedale, 2005), and modern research on brain scaling and developmental constraints (see above) has advanced our understanding of the regularities that lurk within brain variability. In addition, a rapidly increasing number of studies is beginning to reveal genomic changes that are probably linked to changes in brain size and/or structure (e.g., Dorus *et al*., 2004; Mekel-Bobrov *et al*., 2005). Therefore, the time Herrick discussed, when evolutionary change becomes a focus of analysis (see also Gans, 1969), is probably at hand.

Thus, I envision a future in which most evolutionary neuroscientists will embrace many different laws, some dealing with constancy and some with change. A few philosophers of science (e.g., Beatty, 1995) might decry such a vision, because they think that any natural law deserving of its name must apply universally, in all contexts and without room for other, countervailing laws. I have no training in philosophy, but think that all scientific laws apply only in specified domains and given assumptions (Striedter, 2005). In the real world, particularly in the complex world of biological systems, most laws or principles are sometimes excepted. This does not make them useless but, instead, prompts us to ask what causes the observed exceptional cases (West and Brown, 2005). If we understand the causal basis of our laws, then the exceptions should, with further work, become explicable. In other words, I think that evolutionary neuroscientists can fruitfully avail themselves of Hempel's nomological–deductive approach to history. To some extent, they always have.

1.6 Conclusions and Prospects

In summary, the history of evolutionary neuroscience features some serious missteps, such as the idea that brains evolved in a phylogenetic series and Ariëns Kappers' law of neurobiotaxis, but it also reveals considerable progress. The *scala naturae* has ceased to guide the research of evolutionary neuroscientists and the idea of neurobiotaxis has quietly disappeared. The once stagnant field of brain allometry is showing signs of revival, largely because of new statistical techniques and a new emphasis on absolute brain size. The debate about concerted versus mosaic evolution persists, but directions for rapprochement are emerging. In general, the field has flirted with a broad variety of theoretical ideas and found some of them wanting and others promising. In terms of theory, the field is still quite young, but it is poised to mature now.

Predicting directions of growth for any science is problematic, but I believe that most future developments in evolutionary neuroscience will parallel developments in other, non-neural domains of evolutionary biology. After all, the history of evolutionary neuroscience is full of ideas that originated in non-neural areas of biology. For example, the methodology of phylogenetic reconstruction or cladistics (which I did not discuss in this article but have treated elsewhere; see Striedter, 2005) was originally developed

by an entomologist (Hennig, 1950; see also Northcutt, 2001). Similarly, evolutionary developmental biology was burgeoning before it turned to brains (Hall, 1999). Therefore, I think it likely that the future of evolutionary neuroscience has already begun in some non-neural field. Maybe molecular genetics, with its new emphasis on evolutionary change (Dorus *et al*., 2004), will soon take center stage. Maybe the excitement about linking physiological allometries to metabolic parameters (West and Brown, 2005) will infect some mathematically inclined evolutionary neuroscientists. Or perhaps the next big thing in evolutionary neuroscience will be microevolutionary studies that integrate across the behavioral, physiological, and molecular levels (Lim *et al*., 2004). Maybe the future lies with computational studies that model *in silico* how changes in neuronal circuitry impact behavior (e.g., Treves, 2003). It is hoped that all of these new directions – and more – will bloom. If so, the field is headed for exciting times.

On the other hand, evolutionary neuroscientists are still struggling to make their findings relevant to other neuroscientists, other biologists, and other taxpayers. It may be interesting to contemplate the evolution of our brains, or even the brains of other animals, but can that knowledge be applied? Does understanding how or why a brain evolved help to decipher how that same brain works or, if it does not work, how it can be repaired? Are advances in evolutionary neuroscience likely to advance some general aspects of evolutionary theory? All of these questions remain underexplored (see Bullock, 1990).

Near the end of the nineteenth century, Jackson (1958) attempted to apply evolutionary ideas to clinical neurology, but his efforts failed. It has been pointed out that some species are far more capable than others at regenerating damaged brain regions (e.g., Kirsche and Kirsche, 1964) and that nonhuman apes tend not to suffer from neurodegenerative diseases such as Alzheimer's (Erwin, 2001). Such species differences in brain vulnerability and healing capacity might well help us elucidate some disease etiologies or lead to novel therapies. Unfortunately, this research strategy has not yet succeeded. Thus far, evolutionary neuroscience's most important contribution has been the discovery that human brains differ substantially from other brains, particularly nonprimate brains, which means that cross-species extrapolations must be conducted cautiously (Preuss, 1995). This is an important message, but it can be construed as negative in tone. Hopefully, the future holds more positive discoveries.

Work on justifying evolutionary science is especially important in the United States, where anti-evolutionary sentiment is on the rise. Many conservative Christians believe that evolution is a dangerous, insidious idea because it makes life meaningless (Dennett, 1995). Add to this fear the notion that our thoughts and feelings are mere products of our brains (e.g., Dennett, 1991) and evolutionary neuroscience seems like a serious threat to God's supremacy. Although this line of argument is well entrenched, Darwin and most of his immediate followers were hardly atheists (Young, 1985). Instead, they either distinguished clearly between God's words and God's works, as Francis Bacon put it, or argued that God's creative act was limited to setting up the laws that control history. Either way, God was seen as quite compatible with evolutionary theory. Moreover, Darwin's view of life need not produce a meaningless void. Instead, it helps to clarify our relationships with other humans, other species, and our environment. Those relationships, in turn, give meaning to our lives, just as linguistic relationships give meaning to our words. Thus, Darwin knew – and we would do well to recall – that evolutionary biology can be useful even if it yields no direct medical or technological applications. Even Huxley (1863), who was a very pragmatic Darwinian and coined the word 'agnostic', knew that the uniquely human quest to comprehend our place in nature is not driven by mere curiosity or technological imperatives, but by a profound need to understand ourselves, our purpose, our existence. Within that larger and enduring enterprise, evolutionary neuroscience will continue to play a crucial role.

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2 Phylogenetic Character Reconstruction

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Glossary

adaptation A feature or phenotype or trait that evolved to serve a particular function or purpose.

anagenesis The origin of evolutionary novelties within a species lineage by changes in gene allele frequencies by the processes of natural selection and/or neutral genetic drift.

character polarity The temporal direction of change between alternative (primitive and derived) states of a character.

character state reconstruction The process of estimating the ancestral or primitive condition of a character at a given node (branching point) in a phylogenetic tree.

clade A complete branch of the tree of life. A monophyletic group.

cladogenesis The origin of daughter species by the splitting of ancestral species; may or may not occur under the influence of natural selection.

cladogram A branching tree shaped diagram used to summarize comparative (interspecific) data on phenotypes or gene sequences. In contrast to a phylogeny, a cladogram has no time dimension.

comparative method The study of differences between species.

continuous trait A quantitatively defined feature with no easily distinguished boundaries between phenotypes (e.g., size, cell counts, and gene expression levels).

convergence Similarity of structure or function due to independent evolution from different ancestral conditions.

discrete trait A qualitatively defined feature with only a few distinct phenotypes (e.g., polymorphism; presence vs. absence).

homology Similarity of structure or function due to phylogeny (common ancestry).

homoplasy Similarity of structure or function due to convergence, parallelism or reversal.

monophyletic A systematic category that includes an ancestor and all of its descendants; a complete branch of the tree of life; a 'natural' taxon; a clade.

node An internal branching point in a phylogenetic tree.

optimization Methods for estimating ancestral trait values on a tree. Commonly used optimization criteria are: maximum parsimony (MP) which minimizes the amount of trait change, and maximum likelihood (ML) which maximizes the likelihood of a trait at a node given likelihood values for trait evolution.

parallelism Similarity of structure or function due to independent evolution from a common ancestral condition.

paraphyletic A systematic category that includes an ancestor and some but not all of its descendents (e.g., 'invertebrates', 'agnathans', 'fish', and 'reptiles' (*sans* birds)).

parsimony	A principle of scientific inquiry that one should not increase, beyond what is necessary, the number of entities required to explain anything.
phenotypic evolution	Change in the developmental pro gram descendents inherit from their ancestors.
phylogenetic character	A homologous feature or phenotype or trait of an organism or group of organisms.
phylogenetic systematics	A method for reconstructing evolu tionary trees in which taxa are grouped exclusively on the presence of shared derived features.
phylogenetic tree	Genealogical map of interrelation ships among species, with a measure of relative or absolute time on one axis. Also called a tree of life or a phylogeny.
phylogeny	The evolutionary history of a species or group of species that results from anagenesis and cladogenesis.
polyphyletic	A systematic category that includes taxa from multiple phylogenetic origins (e.g., 'homeothermia' con sisting of birds and mammals).
reversal	Change from a derived character state back to a more primitive state; an atavism. Includes evolu tionary losses (e.g., snakes which have 'lost' their paired limbs).
synapomorphy	A shared, derived character used as a hypothesis of homology.
taxon	A species or monophyletic group of species (plural taxa).
trait evolution	The sequence of changes of a feature or phenotype on a phylogeny.

2.1 Introduction to Character State Reconstruction and Evolution

Comparisons among the features of living organisms have played a prominent role in the biological sciences at least since the time of Aristotle. The comparative approach takes advantage of the enormous diversity of organismal form and function to study basic biological processes of physiology, embryology, neurology, and behavior. This approach has given rise to the widespread use of certain species as model systems, based on what has become known as the August Krogh Principle: "For many problems there is an animal on which it can be most conveniently studied" (Krebs, 1975).

From an evolutionary perspective, interspecific (between species) comparisons allow for the systematic study of organismal design. Rensch (1959) conceived of phylogeny as being composed of two distinct sets of processes: anagenesis, the origin of phenotypic novelties within an evolving species lineage (from the Greek *ana* = up + *genesis* = origin), and cladogenesis, the origin of new species from lineage splitting (speciation) (from the Greek *clado* = branch). Anagenetic changes arise within a population by the forces of natural selection and genetic drift. Cladogenesis may or may not arise from these population-level processes, and in fact many (or perhaps most?) species on Earth are thought to have their origins from geographical (allopatric) speciation under the influence of landscape and geological processes (Mayr, 1963; Coyne and Orr, 1989).

Because species descend from common ancestors in a hierarchical fashion (i.e., from a branching, tree-like process of speciation) closely related species tend to resemble each other more than they do more distantly related species. Patterns in the diversification of phenotypes have therefore been described as mosaic evolution, in which different species inherit distinct combinations of traits depending on the position of that species in the tree of life (McKinney and McNamara, 1990). Under this view, character evolution is regarded as a process of historical transformation from a primitive to a derived state, and study of this process necessarily presumes knowledge of primitive or ancestral conditions. In other words, because character evolution is perceived as trait change on a tree, it is necessary to estimate 'ancestral trait values'.

Direct observations of ancient phenotypes may be taken from fossils, which provide unique information on entirely extinct groups of organisms, and are usually associated with stratigraphic information pertaining to relative and absolute geological ages (Benton, 1993). Nonetheless, the fossil record has many well-known shortcomings, including the famously incomplete levels of preservation, and usually very limited information about the nature of soft tissues such as nerves and brains (but see Edinger, 1941; Stensiö, 1963). Paleontological information on ancient physiological and behavioral traits is even more scanty (but see Jerison, 1976; MacLeod and Rose, 1993; Rogers, 2005).

Recent years have seen great advances in the formulation of comparative methods to estimate or infer ancestral phenotypes from extant (living) species (Garland *et al.*, 1992, 1999; Martins, 2000). These methods use patterns in the mosaic of traits present among species in the context of an explicit hypothesis of interrelationships. These methods also address new topics, such as whether rates of

phenotypic evolution have differed among lineages (clades), the circumstances in which a phenotype first evolved, the selective and developmental mechanisms underlying the origin of new phenotypes, and the evolutionary lability of phenotypes (Albert *et al*., 1998; Blomberg *et al*., 2003; Blackledge and Gillespie, 2004).

In this article, I summarize the major recent developments in phylogenetically based methods of studying character evolution, with the goals of explaining both the strengths and weaknesses of alternative methods. Most of the empirical examples cited are among animals with the most complex central nervous systems (e.g., vertebrates) in which neurological and behavioral evolution has been (arguably) most extensively studied. A major goal of this article is to highlight some of the most exciting new developments in the study of character evolution now being explored in this fascinating area of comparative neurobiology.

2.2 Basic Concepts

2.2.1 Homology: Similarity Due to Common Ancestry

All methods of ancestral character state reconstruction make explicit assumptions about the homology of the traits under study. In comparative biology the term 'homology' refers to similarity in form or function arising from common ancestry. In other words, homologous features among organisms can be traced to a single evolutionary origin. In the language of Garstang (1922), a homologous trait is a unique historical change in the developmental program of an evolving lineage. Homologous similarities may be observed in any aspect of the heritable phenotype, from properties of genetic sequences (e.g., base composition and gene order), through aspects of development, including cellular, tissue, and organismal phenotypes, to aspects of behavior that emerge from the organization of the nervous system. Homology in behavioral traits has been examined in a number of taxa, and in a variety of contexts (de Queiroz and Wimberger, 1993; Wimberger and de Queiroz, 1996; Blomberg *et al*., 2003). Taxa are individual branches of the tree of life, and may include species or groups of species that share a common ancestor (the latter are also referred to as clades or monophyletic groups).

It is important to note that developmental, structural, positional, compositional, and functional features of phenotypes are all useful in proposing hypotheses of homology. Yet by the evolutionary definition employed above, only features that can be traced to a common ancestor in an explicitly phylogenetic context are regarded as homologues. Because phylogenies are the product of comparative analyses using many traits, it is in fact congruence in the phylogenetic distribution of characters that serves as the ultimate criterion for homology. By this criterion homologous characters are said to have passed the test of congruence. In other words, congruence in the phylogenetic distribution of numerous character states is regarded to be the ultimate evidence for homology (Patterson, 1982; see Primate Brain Evolution).

2.2.2 Homoplasy: Convergence, Parallelism, and Reversal

All other forms of phenotypic similarity that arise during the course of evolution are referred to collectively as homoplasy (similarity due to causes other than homology). Homoplastic characters may arise from several sources: convergence due to similar functional pressures and natural selection, parallel (independent) evolution to a common structure or function from organisms with similar genetic and developmental backgrounds, or convergent reversal to a common ancestral (plesiomorphic) condition. Some well-known examples of convergent evolution in the nervous system include: image-forming eyes of cephalopod mollusks (e.g., squids and octopods) and vertebrates (Packard, 1972), and the evolution of G-protein-coupled receptors as odorant receptors in many animal phyla (Eisthen, 2002). Examples of parallel evolution in the nervous system of vertebrates have been summarized in several recent reviews (Nishikawa, 2002; Zakon, 2002). These include: electric communication in mormyriform (African) and gymnotiform (South American) electric fishes (Albert and Crampton, 2005), prey capture among frogs (Nishikawa, 1999), sound localization among owls (Grothe *et al*., 2005), and thermoreception in snakes (Hartline, 1988; Molenaar, 1992).

Reversals are among the most common forms of homoplasy, and are often the most difficult to detect even in the context of a resolved phylogenetic hypothesis of relationships (Cunningham, 1999). The reason for this is the phenotypes of some reversals may be quite literally identical, as in the case of convergent loss of structures (e.g., the derived loss of paired limbs in snakes and limbless lizards).

2.2.3 Character State Polarity

A central task of ancestral character state reconstruction is determining the direction or polarity of evolutionary change between alternative states of a character. The ancestral state is referred to as

plesiomorphic or primitive, and the descendent state is referred to as apomorphic or derived. Establishing the polarity of a character state transformation is critical to understanding the functional significance of that event. Phenotypes determined to be primitive simply mean they precede the derived state in time and are not necessarily functionally inferior. It is often, although by no means always, the case that characters evolve from more simple to more complex states, or from the absence of a particular state to the presence of that state.

There are several methods in use to determine character state polarity. The most widely used method is the so-called outgroup criterion, which employs conditions observed in members of clades other than the clade in which the derived state is present. The basic idea of the outgroup criterion is that for a given character with two or more states within a group, the state occurring in related groups is assumed to represent the plesiomorphic state. In other words, the outgroup criterion states that if one character is found in both ingroup and outgroup, this character is then postulated to be the ancestral state (plesiomorphic). Of course, it is always possible that a given outgroup exhibits an independently derived state of a given character, which is why the condition in several outgroup taxa is regarded as a more reliable test of the plesiomorphic condition.

2.2.4 Character or Trait Data

Methods for estimating ancestral character states and analyzing phenotypic evolution may treat trait data either as continuous (quantitative) or discrete (qualitative) (Zelditch *et al.*, 1995; Rohlf, 1998; Wiens, 2001). Continuously distributed trait values have no easily distinguished boundaries between phenotypes. Examples of continuous traits include the sizes of brains and brain regions (e.g., nuclei), the number of cells in a brain region, pigment intensity, amplitude or timing of communication signals, and the amount of gene expression in a tissue. Continuous phenotypic variation typically reflects the additive effects of alleles at multiple loci and is frequently also influenced by environmental factors. Patterns of intraspecific (within species) continuous variation are often analyzed using parametric statistics, including such devices as the population mean and standard deviation. Methods for the analysis of interspecific (between species) continuous traits are useful for assessing the quantitative relationships among variables to address questions regarding, for example, the trade-offs and constraints among correlated traits.

Discontinuous traits have only a few distinct phenotypes. In many cases alternative alleles generate phenotypes that differ from each other in discrete steps, such that each phenotype can be clearly distinguished from the others. Many classes of phenotypic data are inherently discrete, such as meristic counts (e.g., number of body segments, rhombomeres, and cortical visual maps), and genetic polymorphisms (e.g., left- vs. right-handedness). Nucleotide bases at a locus are discrete states of a character. The presence (or absence) of derived traits on a phylogenetic tree also constitutes a class of discrete phenotypes. Such derived traits that underlie or explain subsequent evolutionary events are referred to as key innovations. Some widely cited examples of putative key innovations in the comparative neurosciences include arthropod cephalic tagmosis (Strausfeld, 1998), cephalopod eyes (Hanlon and Messenger, 1996), craniate neural crest (Northcutt and Gans, 1983), and ray-finned fish genome duplication (Taylor *et al.*, 2003; Postlethwait *et al.*, 2004). Each of these novelties is thought to have been critical in the diversification of the taxon in which it originated.

2.2.5 Adaptation

One of the most widely applied uses of ancestral character state reconstruction is in the study of adaptation. The word adaptation is derived from the Latin *ad* (to, toward) and *aptus* (a fit), and is used to imply a feature or phenotype that evolved to serve a particular function or purpose. For example, the function or purpose of an animal central nervous system is to coordinate sensory information and motor output patterns; that is to say, a centralized brain is an adaptation for sensory-motor coordination. Adaptation is therefore used both as a noun to describe the features that arose because of natural selection, and as a verb, the process of natural selection through which the features originated. In an evolutionary context, an adaptation is not only a static description of the match between form and function, but is also an explanation for the origin of that relationship (Russell, 1916).

It is important to distinguish among several distinct uses of the word 'adaptation' in the biological sciences. A physiological adaptation is an organismal response to a particular stress: if you heat up from the sun you may respond by moving into the shade (a behavioral adaptation), or you may respond by sweating (a physiological adaptation). In an evolutionary context, adaptation is also a change in response to a certain problem, but the change is genetic. Evolutionary adaptations that

result from the process of natural selection usually take place over periods of time considerably longer than physiological timescales. Traits are referred to as adaptations only when they evolved as the solutions for a specific problem; that is, for a particular function or purpose. A physiological response can itself be an adaptation in the evolutionary sense.

In reconstructing ancestral phenotypes it is important to bear in mind the primitive condition may be more or less variable than the conditions observed in living species. In some cases physiological or developmental plasticity is itself an evolutionary (genetic) specialization that permits organisms to adapt physiologically or behaviorally. For example, many species are characterized as eurytopic, or tolerant of a wide variety of habitats. Other species are stenotopic, or adapted to a narrow range of habitats. Similarly, individual characters may be more or less variable within a species, and this variability may itself be subject to evolutionary change. Flexible phenotypes may be more adaptive in a variable environment and stereotyped phenotypes more adaptive in a stable environment (van Buskirk, 2002).

2.2.6 Phylogenetic Trees

Implicit in all phylogenetic methods for studying character evolution is a tree-shaped branching diagram, alternatively called a dendrogram, cladogram, phenogram, or tree, depending on the methods used to construct the diagram, and the information content it is intended to convey. It is important to note that each of the many alterative methods for building trees that are currently available was designed to communicate different kinds of information. The methods grouped formally as 'phylogenetic systematics' (cladistics) exclusively use derived similarities (synapomorphies) to hypothesize genealogical relationships. This is to be contrasted with phenetic methods which use measures of overall similarity to group taxa, including both primitive and derived aspects of similarity. Cladistic methods generate branched diagrams referred to as cladograms, which should be viewed as summary diagrams depicting the branching pattern most consistent with a given data set (morphological or molecular). It is important to distinguish raw cladograms from phylogenetic trees; there is no time dimension to a cladogram *per se*, and the branch lengths are simply proportional to the minimum number of steps required to map all the character states onto that tree. A robust phylogenetic tree is usually the result of several or many phylogenetic analyses. The geological time frames associated with branching events are usually estimated from external paleontological, molecular, and biogeographic sources of information.

Figure 1 provides a conceptual overview for how phylogenetic trees may be used to study phenotypic evolution. All comparative approaches begin by assuming (or building) a hypothesis of genealogical interrelationships among the taxa of interest. There are many methods, even whole philosophies, of tree building, and the reader is referred to Page and Holmes (1998) for an introduction to this literature. Phylogenetic methods are then used to optimize character states at internal nodes of the tree; these nodes or branching points are hypothesized speciation events. Comparisons of trait values at ancestral and descendant nodes of the tree allow the history of phenotypic changes to be traced. The distribution of these phenotypic changes (also known as steps or transformations) can then be assessed, qualitatively or quantitatively, depending on the types of data examined and the analytical methods employed.

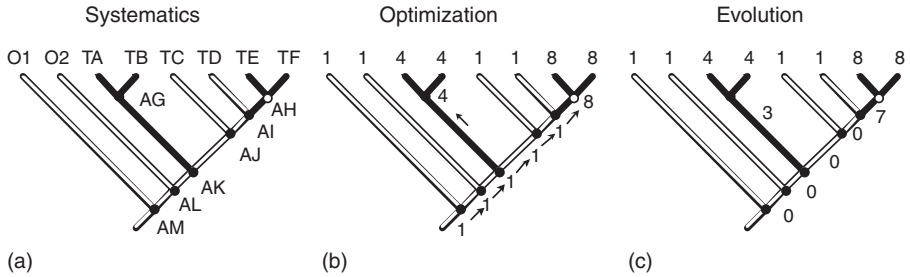

Figure 1 Summary of the comparative approach for inferring phenotypic evolution. a, Phylogenetic systematics (i.e., tree building): reconstruction of genealogical interrelationships among taxa (extant and/or fossil) using morphological and/or molecular sequence data. Taxa are species or clades (monophyletic groups of species): phylogeny includes six ingroup terminal taxa (TA TF) and two outgroup taxa (O1 and O2). b, Character state optimization at internal nodes (branching points or hypothesized speciation events). Observed trait values at tips of the tree. Seven internal tree nodes represented by ancestral taxa (AG AM) with trait values estimated by linear parsimony. c, Evolution: tracing the history of phenotypic changes along branches of the tree. Numbers indicate absolute amount of trait change on the branch.

A tree-shaped branching diagram conveys two kinds of information (whether they are intended or not): the tree topology, or the sequential order in which the taxa branch from one another, and the lengths of the individual branches (Figure 2). These two aspects of a tree correspond to the cladogenesis and the anagenesis of Rensch (1959). The tree topology (branching order) is reconstructed from the distribution of shared–derived traits among taxa. The traits examined may be morphological novelties or nucleotide substitutions. Branch lengths may be reconstructed from one or more sources of information, including alternative models (or modes) of character evolution, or from empirical data. Under models of constant (or near constant) evolution (e.g., molecular clocks), all terminal taxa are treated as equidistant from the root (or base) of the tree. Terminal taxa are those at the tips of the tree, as opposed to ancestral taxa at internal nodes (branching points) within the tree. Under models of punctuated equilibrium, all (or most) character evolution occurs at branching points (nodes), and all branches are therefore of equal (or almost equal) length. Branch lengths derived from empirical data sets may be treated as proportional to the amount of character state change on that particular tree topology, or from stochastic models of evolution assuming that DNA nucleotide substitutions occur at an equal rate (Sanderson, 2002). The constant evolution and punctuated equilibrium models represent extremes of branch-length heterogeneity, between which branch lengths derived from empirical data sets usually fall. Branch lengths for clades with known fossilized members can also be estimated from the geological age of these fossils (Benton *et al.*, 2000; Near and Sanderson, 2004). Calibrations based on molecular sequence divergence or fossil data can take one of two forms: assignment of a fixed age to a node, or enforcement of a minimum or maximum age constraint on a node. The latter option is generally a better reflection of the information content of fossil evidence.

It is important to recognize an analytical difference in the two kinds of information represented in a phylogeny: whereas the tree topology is transitive, the branch lengths are not. In the language of formal logic, 'transitive' means that a relationship necessarily holds across (i.e., it transcends) the particularity of data sets. In the case of phylogenetic trees, the branching order derived from analysis of one data set is expected to predict the branching order of independent data sets (e.g., those derived from different genes, genes and morphology, osteology and neurology). Branch lengths, however, are intransitive, meaning the branch length values derived from one data set are not expected to predict those of other data sets. The reason for this is that we believe there has been a single phylogenetic history of life; a unique sequence of speciation events that gave rise to the species richness of the modern world. This single history underlies the evolution of all aspects of organismal phenotypes. There are, however, no such expectations of homogeneity in the rates of phenotypic (or gene sequence) evolution; in fact,

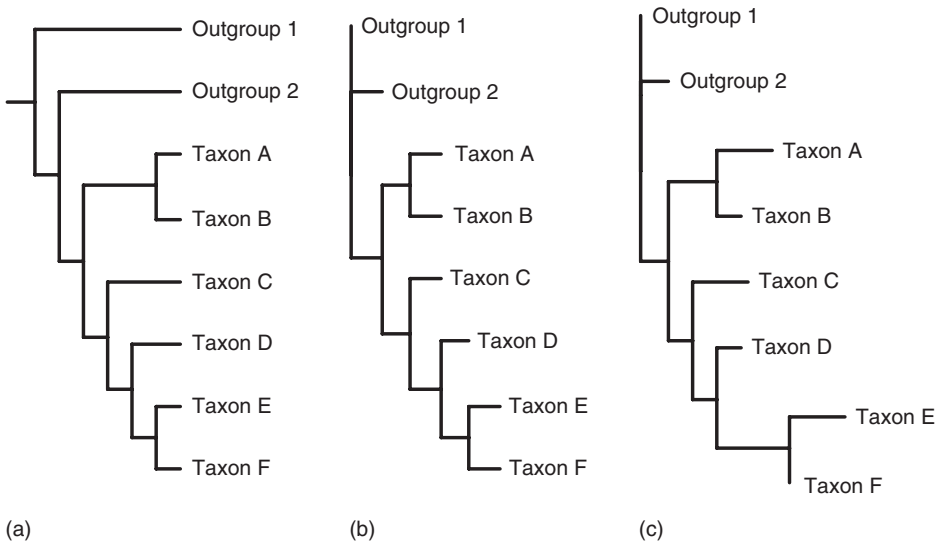

Figure 2 Alternative branch length models. a, Molecular clock: all terminal taxa equidistant from root to from an ultrametric tree. b, Equal branch lengths: all character evolution (anagenesis) occurs at branching events, as in punctuated equilibrium. c, Empirical: branch lengths proportional to amount of character evolution and/or geological ages determined from fossils. Note: tree topology is transitive; branch lengths are not.

the differential effects of directional and stabilizing selection on different phenotypes may be expected to result in longer or shorter branches for some traits than others.

2.3 Methods

2.3.1 Parsimony Optimization of Discrete Traits

The principle of parsimony (i.e., Occam's razor) is widely used in the natural sciences as a method for selecting from among numerous alternative hypotheses. The principle of parsimony underlies all scientific modeling and theory building. The basic idea is that one should not increase, beyond what is necessary, the number of entities required to explain anything. In this context, parsimony means that simpler hypotheses are preferable to more complicated ones. It is not generally meant to imply that Nature itself is simple, but rather that we as observers should prefer the most simple explanations.

Maximum parsimony (MP) is a character-based method used in phylogenetic systematics to reconstruct phylogenetic trees by minimizing the total number of evolutionary transformations (steps) required to explain a given set of data. In other words, MP minimizes the total tree length. The steps may be nucleotide base or amino acid substitutions for sequence data, or gain and loss events for restriction site and morphological data. MP may also be used to infer ancestral states of a character within a phylogenetic tree (this is discussed in the following).

2.3.2 Binary and Multistate Characters

Discrete characters may be characterized as either binary (coded into two mutually exclusive alternative states) or as multistate (a transformation series of three or more discrete states). The alternative states of a binary character are generally (although not necessarily) explicit hypotheses of the primitive and derived (advanced) states of a single evolutionary transformation event, such as the origin (or loss) of a novel feature. A multistate character is a more complex intellectual device with many more interpretations of meaning. Multistate characters may be presented as many stages of a long-term phylogenetic trend (e.g., larger relative brain size, larger body size) or as independent alternative trends from a common ancestral plan (e.g., large brains evolving from enlargement of the cerebellum in chondrichthyans vs. the telencephalon in mammals). An ordered transformation series models a preconceived phylogenetic sequence of changes, such that in the series 1–2–3, state 3 is only permitted to be derived from state 2. In an unordered transformation series, state 3 may be derived from either of states 1 or 2. Following a similar logic, reversals (e.g., from 2 to 1) may be allowed, penalized, or prohibited, depending on the preconceptions of the investigator. Of course, building *a priori* conceptions of order or reversibility into an analysis of character state change precludes the use of that analysis as an independent test of those assumptions. To summarize this section, treating all characters as unpolarized and unordered means that all transitions among states are regarded as equally probable.

2.3.3 Squared-Change and Linear Parsimony

There are two general types of MP widely used in tracing the evolution of continuous traits: squared-change parsimony and linear parsimony. Squared-change algorithms (Rogers, 1984) seek to minimize the amount of squared change along each branch across the entire tree simultaneously, using a formula in which the cost of a change from state x to y is $(x - y)^2$. Squared-change parsimony assigns a single ancestral value to each internal node to minimize the sum of squares change over the tree (Maddison, 1991). When using squared-change parsimony, the absolute amount of evolution over the whole tree is not necessarily minimized, and some degree of change is forced along most branches. Linear parsimony reconstructs ancestral node values by minimizing total changes (Figure 3). Linear-parsimony algorithms (Kluge and Farris, 1969) seek to minimize the total amount of evolution and consider only the three nearest nodes when calculating the ancestral character states. In linear parsimony the cost of a change from x to y is $|x - y|$. The result of this local optimization is that changes are inferred on very few or single branches. Linear parsimony therefore permits the accurate reconstruction of discontinuous events, or of large changes in trait values on a tree. Although evolutionary change is often thought of as gradual, large changes on a tree may result from a variety of real biological processes, not the least of which is the extinction of taxa with intermediate trait values (Butler and Losos, 1997).

2.3.4 Maximum Likelihood and Bayesian Optimization

Maximum likelihood (ML) methods for tracing character evolution select ancestral trait values with highest likelihood on a given phylogenetic hypothesis given a model of trait evolution (defined

Taxon	*Character*
Outgroup 1	1
Outgroup 2	1
Taxon A	4
Taxon B	4
Taxon C	1
Taxon D	1
Taxon E	8
Taxon F	8

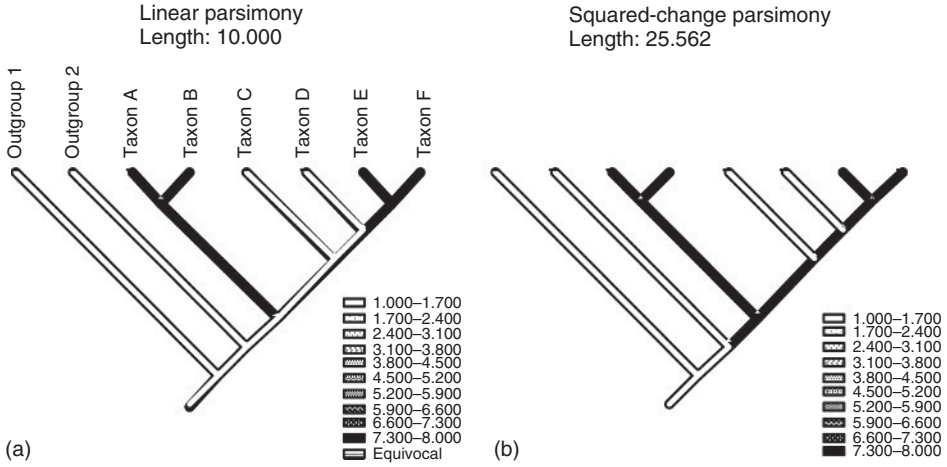

Figure 3 Alternative methods for estimating ancestral character states. a, Linear parsimony. b, Squared-change parsimony. Character state data by taxon reported in the table.

by user). Bayesian analysis (BA) selects the ancestral trait value with the highest posterior probability, given the probabilities of priors (external evidence) and assumptions of trait evolution (defined by user). Because they are model-based approaches, ML and BA optimization methods are more commonly used in the analysis of gene sequence data, using explicit models of changes between nucleotide bases (Liò and Goldman, 1998; Sullivan *et al.*, 1999). ML has been used in the analysis of continuous character evolution where the models may vary from very simple (e.g., Brownian motion) to quite complex; there is a large literature regarding methods to test the validity of using particular models (Diaz-Uriarte and Garland, 1996; Oakley, 2003).

2.3.5 Which Optimization Approach to Use?

Empirical studies using simulated data sets and those derived from evolution in a test tube have concluded that model-driven approaches like ML and BA give more accurate results than MP when the modeled parameters (i.e., likelihood or probability of nucleotide substitutions) are known, but can be positively misleading when the parameters are unknown (Hillis *et al.*, 1992; Oakley and Cunningham, 2000). MP often provides less resolution (more interior tree nodes reconstructed with ambiguous states), than ML or BA methods, which usually give very precise estimates with high confidence levels even under circumstances in which available data are insufficient to the task. In this regard, MP methods are regarded as more conservative, with lower risk of recovering false positives (Webster and Purvis, 2002).

Most studies on the evolution of neural characters use MP approaches because, unlike molecular sequence data, it is not straightforward how to pose or parametrize models on the evolution of complex phenotypes. Continuously varying aspects of neural features, like the size or shape of structures, have been modeled as simple Brownian motion or random walk processes, under the assumptions that the trait has not experienced selection and that there are no constraints on variance through time (Butler and King, 2004). Whether or not the assumptions of Brownian motion or any other specific model are satisfied by real neural or behavioral data is almost completely unknown.

A general conclusion reached by a number of review studies is that, under most circumstances faced by comparative morphologists, linear parsimony is the most conservative method for reconstructing ancestral trait values (Losos, 1999). Unlike squared-change parsimony, linear parsimony does not average out change over the interior nodes of a tree, but rather permits discontinuous changes along a branch. This has the

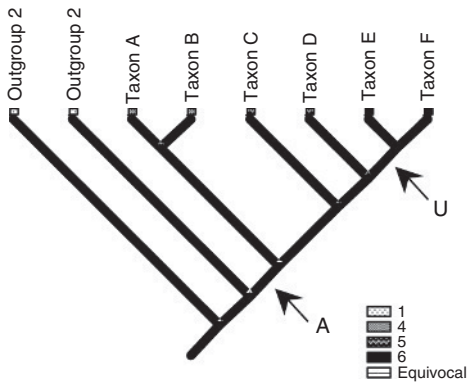

Figure 4 Ambiguous (A) vs. unambiguous (U) optimizations.

advantageous effect of not forcing gradual trait evolution on the tree, and also of not forcing unnecessary trait reversals (Figure 3). A methodological advantage of linear over squared-change parsimony is that it permits the reconstruction of ambiguous ancestral character state reconstructions (Figure 4). This is a desirable property in cases where the available data are in fact insufficient to resolve the trait value at a specified internal nodes (Cunningham, 1999). A methodological disadvantage of linear parsimony is that, computationally, it requires a completely resolved tree topology in which all branching events are divided into only two daughter clades. Unfortunately, fully resolved trees are unusual in most studies with many (>30) species. By contrast, squared-change parsimony can be calculated on a tree with unresolved multichotomies (also called polytomies), and therefore often becomes the method of choice by default. One alternative to using squared-change parsimony when faced with an incompletely resolved tree is to use linear parsimony on numerous (100, 1000) arbitrarily resolved trees, and then report statistics (e.g., minimum and maximum) of the trait values obtained. Software for this procedure is available in the freely available Mesquite software package (see 'Relevant Website').

2.3.6 Correlative Comparative Methods

Ordinary least-squares regression allows one to investigate relationships between two variables in order to ask if change in one of these variables is associated with change in the other. One may ask, for example, how is variation in brain size related to body size, ecological role (predator vs. prey), climate, life history mode, or locomotion (Albert *et al*., 2000; Safi and Dechmann, 2005). The least-squares fitting procedure is commonly used in data analysis in comparative studies, and conventional regression analysis has been one of the main tools available to comparative neurobiology and ecological physiology to study form–function relationships and adaptation (Garland and Carter, 1994). However, it is now widely recognized that interspecific observations generally do not comprise independent and identically distributed data points, thus violating fundamental assumptions of conventional parametric statistics (Felsenstein, 1985, 1988; Pagel and Harvey, 1989; Harvey and Pagel, 1991).

Phylogenetically based statistical methods allow traditional topics in comparative neuroanatomy and physiology to be addressed with greater rigor, including the form of allometric relationships among traits and whether phenotypes vary predictably in relation to behavior, ecology, or environmental characteristics (Brooks and McLennan, 1991; Frumhoff and Reeve, 1994; Losos, 1996). In a conventional regression analysis the data points represent terminal taxa. In a phylogenetic regression the data points represent sister-taxon comparisons (Grafen, 1989). These two methods are compared in Figure 5, in which identical data are analyzed using conventional and phylogenetic regression methods. The phylogeny of Figure 5 includes six terminal taxa (TA–TF) and two outgroup taxa (O1 and O2), which are represented by two continuously distributed characters (C1 and C2). The tree topology has been determined from data other than characters 1 and 2, and the branch lengths are treated as equal (under a model of punctuated equilibrium). There are seven internal tree nodes represented by ancestral taxa (AG–AM) with trait values estimated by least-square parsimony. By removing psuedorepilcates, the phylogenetic regression compares fewer taxa, has fewer degrees of freedom, and has a lower correlation coefficient (R^2 value) than does the conventional regression. The phylogenetic regression, therefore, provides a better quantitative measure of correlated evolution between the two traits, and is a more conservative measure of the strength of adaptive pressures.

Relationships between brain size and the volume of frontal and visual cortices in mammals have recently been studied using the methods of phylogenetic regression analysis (Bush and Allman, 2004a, 2004b). These studies found that size has a profound effect on the structure of the brain, and that many brain structures scale allometrically; that is, their relative size changes systematically as a function of brain size. They also conclude that the three-dimensional shape of visual maps in anthropoid primates is significantly longer and narrower than

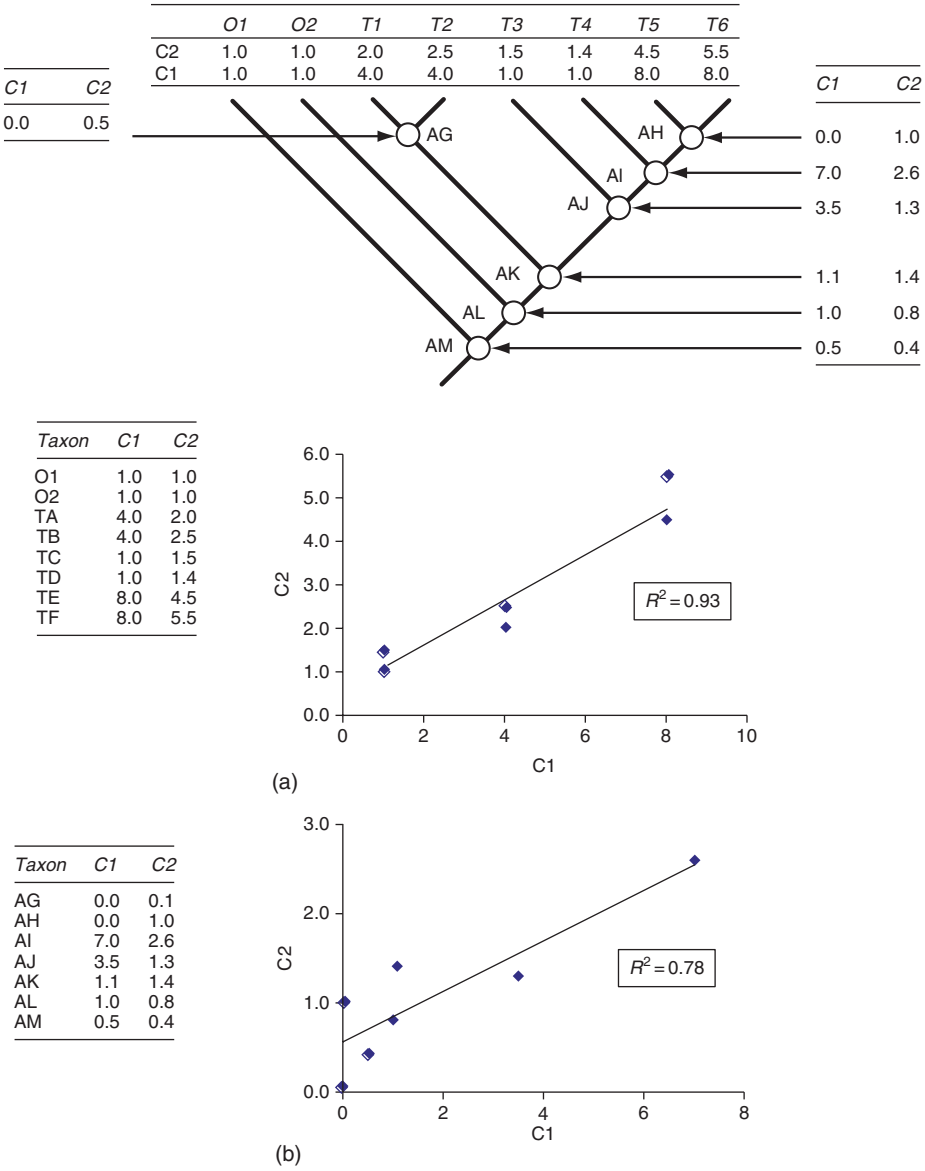

Figure 5 Comparison of conventional and phylogenetic regression analyses. Phylogeny of six terminal taxa (TA TF) and two outgroup taxa (O1 and O2), represented by two continuously distributed characters (C1 and C2). Tree topology determined from data other than characters 1 and 2, and branch lengths treated as equal. Seven internal tree nodes represented by ancestral taxa (AG AM) with ancestral trait values estimated by least-squares parsimony. a, Conventional regression of trait values from terminal taxa. b, Phylogenetic regression of trait values at internal tree nodes using the method of independent contrasts. Note that by removing psuedoreplicates, the phylogenetic regression compares fewer taxa, has fewer degrees of freedom, and has a lower correlation coefficient (R^2 value) than does the conventional regression. The phylogenetic regression, therefore, provides a more conservative quantitative measure of correlated evolution between the two traits.

in strepsirrhine primates. Using conventional regression analyses, von Bonin (1947) showed that frontal cortex hyperscales with brain size, and humans have "precisely the frontal lobe which [we deserve] by virtue of the overall size of [our] brain." These are, of course, precisely the qualitative conclusions arrived at by Bush and Allman using analysis of phylogenetic regressions. In fact, many studies reviewing the uses of phylogenetic methods for reconstructing ancestral states conclude that all methods will recover a very strong historical signal (Losos, 1999).

2.4 Limitations of Methods

The accuracy of ancestral reconstructions has been investigated by comparisons with known phylogenies (e.g., viruses, computer simulations; Oakley and Cunningham, 2000). It is well known that all phylogenetically based methods perform

poorly when taxon sampling is low and when rates of evolution in the character of interest are unequal among branches of the tree (Garland *et al*., 1993; Sullivan *et al*., 1999; Hillis *et al*., 2003). Further, all methods for studying character evolution on a tree make certain assumptions about the capacity of trees to faithfully record the actual history of character change. These include the assumptions that: phenotypic diversification results largely from speciation and that the effects of extinction have not erased the signal, that taxon sampling faithfully represent the history of diversification, and that genealogical history is largely or entirely bifurcating (vs. multifurcating or converging). Of course, all methods assume we know the 'true' (or 'nearly true') tree topology. In addition, each of the optimization methods makes assumptions about critical parameters, including branch lengths, models of character evolution, absolute rates of evolution, homogeneity (vs. heterogeneity) of evolutionary rates, reversibility (or the lack thereof), and the orderedness (or unorderedness) of multistate characters.

The accuracy of ancestral trait reconstruction also depends strongly on parameter estimation (e.g., tree topology, branch lengths, and models of trait evolution). ML and BA perform well when model assumptions match real parameters. ML and BA are positively misleading when model assumptions are violated. MP is more conservative, recovering fewer false positives than ML and BA when biological parameters are not known. Squared-change parsimony, ML, and BA minimize large changes, spreading evolution over the internal tree branches. Linear parsimony permits reconstructions at ancestral nodes with no change, and permits ambiguous reconstructions. 'Independent contrasts' assumes that selection operates in the origin but not maintenance of derived traits.

Both conventional and phylogenetic correlations of interspecific character data make assumptions about critical parameters. These assumptions are often of unknown validity, and in some cases are known to be incorrect. Conventional statistics assume that each terminal taxon (tips of the tree) may be treated as independent sample of the relationship under investigation. This means that the character value (phenotype) observed in that taxon evolved independently (without inheritance) from the values in other taxa in the analysis. In an evolutionary context, this is equivalent to assuming that trait values result primarily from stabilizing selection in each species that acts to maintain trait values, rather than from directional selection at the origin of the trait in an ancestral species (Hansen, 1997). In other words, conventional statistics assume traits to be highly labile and without significant phylogenetic inertia. Phylogenetic correlations make converse assumptions, that trait values are due largely or entirely to directional selection at the origin of a feature and that the influence of stabilizing selection is negligible. Phylogenetic correlations also must make particular assumptions about branch lengths and models of trait evolution.

2.5 Conclusions

As in all aspects of historical inquiry, the study of character evolution is exceptionally sensitive to the amount of information that has actually survived up to the present. The reality of neural evolution was in most cases almost certainly very complex, and may be reliably regarded to have included vastly more numbers of independent transformations than has been recorded in the distribution of phenotypes preserved among living species. The signature of many historical events has been overwritten by reversals and convergences, or eliminated altogether by extinctions. Paleontologists estimate that more than 99% of all species that have ever lived are now extinct (Rosenzweig, 1995). This figure, of course, includes higher taxa (e.g., trilobites, placoderms, plesiosaurs) that are now entirely extinct, bringing up the aggregate percentage of extinction for all taxa. The proportion of living species that persists within certain targeted taxa may be much higher (e.g., Lake Victoria cichlid fishes). Nevertheless, in comparative studies of neural, physiological, or behavioral phenotypes, it is rare to have information on all extant species. Whether it is from extinction or incomplete surveys, taxon sampling remains one of the greatest sources of error in phylogenetic estimates of character evolution (Sullivan *et al*., 1999; Zwickl and Hillis, 2002).

Despite all these reservations, we must continue to estimate ancestral traits in order to study phenotypic evolution. None of the methods reviewed in this article should be regarded as a magic bullet, but rather there are advantages and disadvantages of each method as they are applied under different circumstances. All the methods reviewed here have proved to be useful tools in the phylogenetic toolbox. As in other aspects of science, it is important to make our assumptions explicit, and to use reasonable assumptions. Further, as in other aspects of evolutionary biology, critical insights into the evolution of neural characters will come from a better understanding of the biology of the phenotypes themselves, and the organisms in which they have evolved.

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3 Basic Nervous System "Types": One or Many?

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Glossary

Bilateria A monophyletic group of metazoan animals that is characterized by bilateral symmetry. This group comprises all of the Metazoa except for the Radiata (Ctenophores and Cnidaria) and the Parazoa (sponges).

blastopore The site of gastrulation initiation.

coelom Fluid filled body cavity found in animals that is lined by cells derived from mesoderm tissue in the embryo and provides for free, lubricated motion of the viscera.

Deuterostomia (From the Greek: *mouth second*) A major group of the Bilateria including echinoderms and chordates. In deuterostomes, the first opening (the blastopore) becomes the anus and the mouth derives from a secondary invagination.

Ecdysozoa Major group of protostome animals, including the arthropods (insects, arachnids, crustaceans, and relatives), roundworms, and several smaller phyla, which are characterized by a trilayered cuticle, composed of organic material, which is periodically molted as the animal grows by a process called ecdysis.

Gastroneuralia A subdivision of the Bilateria defined by the location of the nerve cord, Gastroneuralia are characterized by a ventral nerve cord and include most protostomes except the Tentaculata.

homeodomain A 60 amino acid part of proteins that corresponds to the homeobox sequence found in homeobox genes that are involved in the regulation of the development (morphogenesis) of animals, fungi, and plants.

homology Correspondence or relation in type of structure because of shared ancestry.

Lophotrochozoa Major group of protostome animals, including mollusks, annelids, nemerteans, brachiopods, and several other phyla characterized either by the production of trochophore larvae, which have two bands of cilia around their middle, or by the presence of a lophophore, a fan of ciliated tentacles surrounding the mouth.

Notoneuralia A subdivision of the Bilateria defined by the location of the nerve cord, Notoneuralia are characterized by a dorsal nerve cord and include most deuterostomes except the Echinodermata, Chaetognatha, and Enteropneusta.

phylogeny The origin and evolution of a set of organisms, which reveals ancestral relationships, such as monophyly (common origin) or polyphyly (independent origin), among known species.

Protostomia (From the Greek: *first the mouth*) A major group of the Bilateria including the Lophotrochozoa and the Ecdysozoa. In protostomes, the mouth forms at the site of the blasto pore and the anus forms as a second opening.

Urbilateria The animal that preceded all recent bilateral symmetric animals.

3.1 Introduction

The diversity of nervous systems is enormous. In terms of structural and functional organization as well as in terms of levels of complexity, nervous systems range from the simple peripheral nerve nets found in some of the basal invertebrate taxa to the centralized nervous systems and highly complex brains that characterize vertebrates and cephalopods. Starting in the eighteenth century, numerous attempts were undertaken to reconstruct the evolutionary origin of the diverse nervous system types found in the animal kingdom (see Origin and Evolution of the First Nervous System). However, initially none of these attempts resulted in consensus, in part because of the uncertain and ambiguous nature of the postulated phylogenetic relationships among the various animal groups considered. At the beginning of the twentieth century, it became evident that the bilaterally symmetrical animals, the Bilateria, could be phylogenetically subdivided into two major branches (Fioroni, 1980). This subdivision of the Bilateria into the protostome and the deuterostome animals remains valid (Brusca and Brusca, 1990) and has been confirmed by molecular analyses (e.g., Adoutte *et al*., 2000).

Do the general nervous system types that characterize the protostome and deuterostome animals also follow this binary subdivision? Classical neuroanatomical and embryological studies suggest that this is the case, at least in part. Accordingly, most bilaterian animals can be subdivided into two major groups with different central nervous system (CNS) morphologies. These are the Gastroneuralia, which are characterized by a ventral nerve cord and include major protostome groups such as arthropods, annelids, and mollusks, and the Notoneuralia, which are characterized by a dorsal nerve cord and include all (deuterostome) chordates (e.g., Nielsen, 1995). The two groups often manifest different modes of CNS development. In gastroneuralians such as arthropods, the ganglionic masses detach from the ventral neuroectoderm to form a rope-ladder nervous system of connectives and commissures, whereas in notoneuralian chordates the neuroectoderm folds inwardly as a whole to form a neural tube (Figure 1). As a result of the Gastroneuralia/Notoneuralia subdivision, the notion of an independent evolutionary origin of the CNS of protostomes versus deuterostomes gained general acceptance and accordingly a polyphyletic origin of bilaterian nervous systems was proposed.

The alternative notion, namely, that bilaterian nervous systems might have a common evolutionary origin, was rejected precisely because of the evident dissimilarities in the mode of development, topology, and adult morphology of the nervous systems in major protostome versus deuterostome groups. However, starting in the 1980s, a number of key

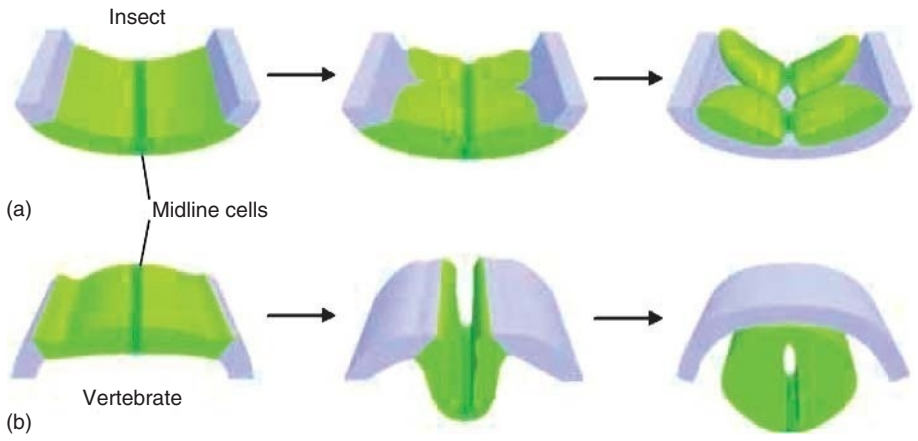

Figure 1 Morphogenesis of the ventral nerve cord in a prototype insect (a) and of the dorsal neural tube in a prototype vertebrate (b). Arrows indicate ontogenetic sequences; yellow-green, neurogenic ectoderm; blue, epidermal ectoderm. Reproduced from Arendt, D. and Nübler-Jung, K. 1999. Comparison of early nerve cord development in insects and vertebrates. *Development* 126, 2309 2325, with permission from The Company of Biologists Ltd.

findings resulting from developmental biological analyses of animal body axis formation began to call into question the validity of the Gastroneuralia/Notoneuralia subdivision and, in doing so, provided initial support for the idea of a monophyletic origin of the bilaterian nervous system. In a nutshell, these findings demonstrated that the molecular genetic mechanisms of anteroposterior axis formation are shared among all bilaterians and that the molecular genetic mechanisms of dorsoventral axis formation in vertebrates are similar to those that operate in insects, only that their dorsoventral topology is inverted, upside-down. If dorsal in vertebrates corresponds to ventral in insects, might not the dorsal nerve cord of Notoneuralia in fact correspond to the ventral nerve cord of Gastroneuralia?

This axial inversion hypothesis was remarkable not only because it was based on unequivocal molecular genetic evidence, but also because it provided support for an old and much-derided view that emerged in the early nineteenth century. Its first proponent was the French zoologist Geoffroy Saint-Hilaire, in opposition to his countryman, the comparative anatomist Cuvier. Both engaged in a debate about a fundamental issue in the biological sciences, namely, whether animal structure ought to be explained primarily by reference to function or rather by morphological laws. At the heart of this debate was the question of whether a common structural plan, or Bauplan, underlies all animal development, thus indicating homology of structures across different animal phyla. Contemporary developmental biological studies based on analyses of expression and function of homologous regulatory control genes in various animal model systems have revived this fundamental question and contributed novel insight into the issue of homology of nervous systems. In this article, we will begin with this famous debate, consider the impact of molecular developmental genetics on a bilaterian nervous system Bauplan, and then discuss the current data for and against a common evolutionary origin of the nervous system. Though our main emphasis will be on conserved mechanisms of anteroposterior and dorsoventral patterning of the nervous system in insect and vertebrate model systems, we will also consider gene expression studies in invertebrates such as hemichordates and cnidarians.

3.2 The Cuvier–Geoffroy Debate

3.2.1 A Common Bauplan for Animal Development?

In 1830, a series of eight public debates were held at the Académie Royale des Sciences in Paris. The two opponents, George Cuvier (1769–1832) and Étienne Geoffroy Saint-Hilaire (1772–1844), were prominent and internationally renowned scientists. Both had made major contributions in many areas of natural history, including comparative anatomy and paleontology. Cuvier divided the animal kingdom into four completely separate branches or *embranchements*: vertebrates, articulates (largely arthropods and annelids), mollusks (which at the time meant all other soft, bilaterally symmetrical invertebrates), and radiates (echinoderms, cnidarians, and various other groups). According to Cuvier, there was no affinity whatsoever between the four *embranchements*. Any similarities between organisms were due to common functions, not to common ancestry. Function determines form; form does not determine function. Thus, even within these divisions, he allowed structural similarity to result solely from the same functional demands.

Geoffroy, by contrast, insisted that function was always dependent on structure and by no means sufficed to determine structure. What counted were the interconnections between parts; structures in different organisms were the same if their parts were connected to one another in the same pattern. Eventually Geoffroy developed the doctrine of unity of composition, applicable at least within each class of animals. Each animal is formed from a structural blueprint based on a common plan, and although animal structure is modified extensively because of functional requirements, the modification is constrained by the unity of composition (which later came to be known as the basic Bauplan). This doctrine of Geoffroy's came to be known as philosophical anatomy and was founded on analogy between structures (homology in modern terminology). Geoffroy's main criterion for determining true analogies was the connectivity between structures and this could often be better determined from the embryo rather than from the adult. The value of the theory of analogues was that it offered a scientific explanation for differences in structure.

Initially, these ideas related primarily within each class of animals or *embranchements*, but Geoffroy imagined that the principle could be extended to the animal kingdom as a whole. After having established a common scheme for vertebrates, he extended this principle across the boundaries of Cuvier's four *embranchements* to articulates. In 1822, Geoffroy published a paper entitled *Considérations générales sur la vertèbre*, in which he proposed that the ventral side of arthropods was analogous to the dorsal side of the vertebrates. This dorsoventral axis inversion hypothesis was based on a dissected crayfish that he had placed upside down and, as he noted, in this orientation the organization

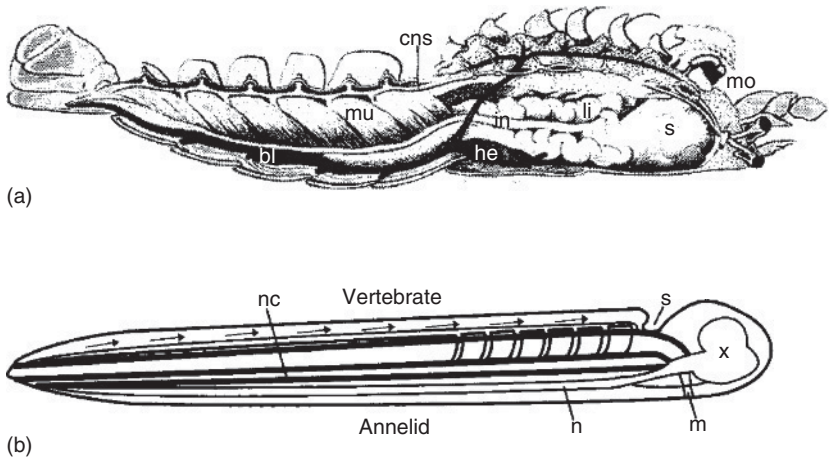

Figure 2 The dorsoventral inversion hypothesis. a, Geoffroy Saint-Hilaire's dissected lobster. In this dissection, the animal is presented in the orientation opposite to the orientation that it would normally have with respect to the ground. The central nervous system (cns) is at the top and is traversed by the mouth (mo). Below this is the digestive tract with the stomach (s), liver (li), and intestine (in). Below the gut are the heart (he) and main blood vessels (bl). Muscles (mu) flank the CNS. In this orientation, the body plan of the arthropods resembles that of the vertebrate. b, Inverted relationship of the annelid and vertebrate body plans; only the mouth changes position with inversion, making a new opening in the chordate lineage. m, mouth; n, nerve cord; nc, notochord (only in chordates); s, stomodeum (secondary mouth); x, brain. Arrows show direction of blood flow. a, Reprinted by permission from Macmillan Publishers Ltd: *Nature* (De Robertis, E. M. and Sasai, Y. 1996. A common plan for dorsoventral patterning in Bilateria. *Nature* 380, 37 40), copyright (1996). b, Modified textbook diagram; see, for example, Romer and Parsons (1977).

of the main body system of the lobster resembled that of a mammal (see Figure 2). One objection readily raised against such an attempt to link arthropods and vertebrates was that the nervous system in arthropods was nevertheless found on the ventral side, whereas in vertebrates it was located on the dorsal side. Geoffroy's solution to this problem was that the definitions of dorsal and ventral were purely arbitrary, because they were based solely on the orientation of the animal to the sun. If it was assumed that the arthropod walked with its ventral side rather than its dorsal side toward the sun, then all of the organs of the arthropod would have the same topological arrangement as the organs of vertebrates.

As expected, Cuvier rejected such interpretations. For him, animals shared similar basic plans only because they carried out a similar combination of interrelated functions. Because the fundamental plan was completely different in each *embranchement,* there were no and could be no transitional forms leading from one *embranchement* to the next. Moreover, no one had ever observed the transformation of one species into another. The differences between the scientific approaches of Geoffroy and Cuvier came to a head when two young naturalists, Meyranx and Laurencet, submitted to the academy a comparison of the anatomy of vertebrates and cephalopods (squids, cuttlefish, and octopi), claiming that they were based on the same basic structural plan. Geoffroy, who was chosen by the academy to review the paper, enthusiastically adopted this claim as proof of his unity of composition shared by all animals. Cuvier could not reconcile this with the results of his careful anatomical research, and in the ensuing debates, he showed convincingly that many of Geoffroy's supposed examples of unity of structure were not accurate; the similarities between vertebrates and cephalopods were contrived and superficial. As an immediate consequence, the results of Meyranx and Laurencet never went to press (for details, see Appel, 1987).

3.2.2 From Unity of Composition to Unity of Nervous Systems?

Although Cuvier was considered to have won the 1830 debates, Geoffroy's philosophical anatomy remained remarkably influential during the subsequent decades. A resolution of the conflicting ideas was achieved, in part, by Darwin's evolutionary theory in which structural homology became an important criterion for establishing phylogenetic relationships. Moreover, with the advent of molecular developmental genetics, it has become clear that homology is a concept that applies not only to morphology, but also to genes and developmental processes. Indeed, and rather unexpectedly, more than 150 years after the famous debate, developmental genetics has provided experimental evidence for Geoffroy's unity of composition and specifically for his dorsoventral axis inversion

hypothesis that appeared to be so convincingly refuted by Cuvier.

The discovery that a common developmental genetic program underlies dorsoventral axis formation in both insects and vertebrates was based on the analysis of two sets of homologous genes that encode morphogens in the model systems *Drosophila* and *Xenopus* (Holley *et al*., 1995; Schmidt *et al*., 1995; De Robertis and Sassai, 1996; Holley and Ferguson, 1997). The transforming growth factor β (TGFβ) family member encoded by the *decapentaplegic* (*dpp*) gene is expressed dorsally and promotes dorsal fate in *Drosophila*, whereas its vertebrate orthologue *Bone morphogenetic protein* (*Bmp4*) is expressed ventrally and promotes ventral fate in *Xenopus*. These morphogens are antagonized by the secreted products of the orthologous genes *short gastrulation* (*sog*) in *Drosophila* and *Chordin* in *Xenopus*. Importantly, the site of action where *sog/Chordin* expression inhibits *dpp/Bmp4* signaling corresponds in both insects and vertebrates to the region of the dorsoventral body axis that gives rise to the embryonic neuroectoderm from which the nervous system derives (see below).

These results provide strong evidence that the molecular interactions that occur on the ventral side of insects are homologous (in Geoffroy's sense, analogous) to those that occur on the dorsal side of vertebrates – an observation that revitalizes Geoffroy's initial proposition of the unity of composition between arthropods and mammals and supports the hypothesis of a dorsoventral inversion of their body axes during the course of evolution (Arendt and Nübler-Jung, 1994). Moreover, these results also provide strong evidence that the molecular interactions that lead to the formation of the ventral CNS in insects are homologous to those that lead to the formation of the dorsal CNS in vertebrates, indicating a dorsoventral body axis inversion as the most parsimonious explanation for the dorsoventrally inverted topology of the CNS that characterizes Gastroneuralia versus Notoneuralia.

Comparable molecular genetic studies on other sets of homologous genes in various model systems ranging from annelids and arthropods to mammals are providing further evidence that Geoffroy's unity of composition might be the result of a developmental construction plan that is shared by all bilaterian animals. Thus, evolutionarily conserved developmental control genes act not only in dorsoventral axis specification but also in anteroposterior axis formation, segmentation, neurogenesis, axogenesis, and eye/photoreceptor cell development through comparable molecular mechanisms that appear to be conserved throughout most of the animal kingdom. The implications of these findings are far-reaching. They suggest that, although diverse in their mode of development and adult morphology, bilateral animals derived by descent from a common ancestor, the Urbilateria, which may already have evolved a rather complex body plan (De Robertis and Sasai, 1996). Accordingly, the urbilaterian nervous system may already have evolved structural features that prefigured elements of the nervous systems of the descendent bilaterian animals. If this were indeed the case, then the ventrally located arthropod nervous system may be homologous to the dorsally located chordate nervous system; the insect brain may be composed of structural units homologous to those of the vertebrate brain; the visual system of a fly may be homologous to the visual system of a mammal. The plausibility of this scenario is particularly evident with regard to the conserved mechanisms of anteroposterior and dorsoventral patterning of the nervous system that operate in insects and vertebrates.

3.3 Conserved Mechanisms for Anteroposterior Patterning of the CNS

3.3.1 *Hox* Genes Are Involved in the Regional Specification of Neuronal Identity

Along the anteroposterior axis, the insect and vertebrate neuroectoderm is subdivided into compartment-like regions, each of which expresses a specific combination of conserved developmental control genes. In both animal groups, regions of the posterior brain and the nerve cord are specified by the expression and action of homeodomain transcription factors encoded by the *Hox* genes (see Figure 3). *Hox* genes were first identified in *Drosophila* and *Hox* gene orthologues have subsequently been found in all other bilaterian animals, including mammals. During embryonic development, these developmental control genes are involved in anteroposterior patterning of features such as the morphology of segments in *Drosophila* or the morphology of axial mesoderm derivatives in mammals. *Hox* genes generally respect the co-linearity rule: they are expressed along the body axis in the same order as they are found clustered on the chromosome. Their role in anteroposterior regionalization may have evolved early in metazoan history (Carroll, 1995).

In both invertebrates and vertebrates, *Hox* gene expression is especially prominent in the developing CNS, and the nervous system may be the most ancestral site of *Hox* gene action. In animal taxa

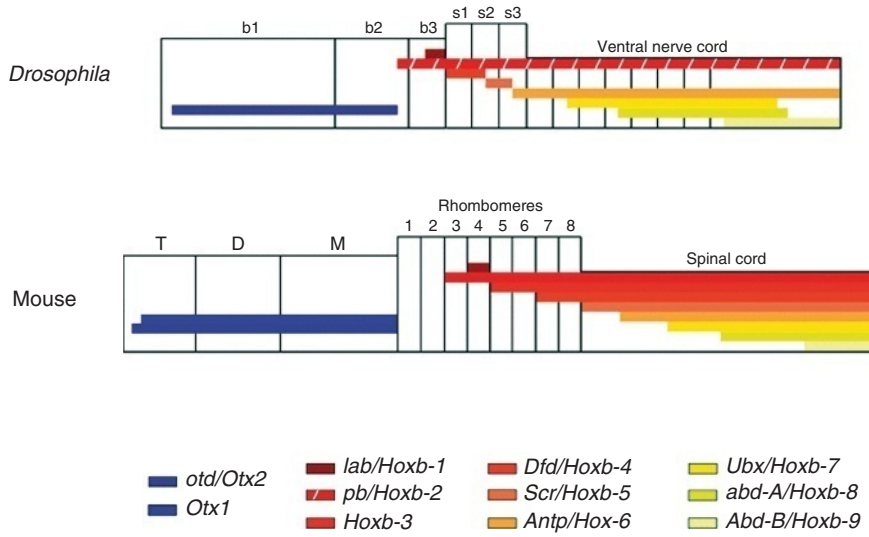

Figure 3 Conserved anteroposterior order of gene expression in embryonic brain development. Schematic diagram of *Hox* and *otd/Otx* gene expression patterns in the developing CNS of *Drosophila* and mouse. Expression domains are color-coded. (Top) Gene expression in embryonic stage 14 *Drosophila* CNS. Borders of the protocerebral (b1), deutocerebral (b2), tritocerebral (b3), mandibular (s1), maxillary (s2), labial (s3), and ventral nerve cord neuromeres are indicated by vertical lines. In contrast to the other Hox genes, *pb* is expressed only in small segmentally repeated groups of neuronal cells; this difference is indicated by a diagonally striped bar to denote the *pb* expression domain. (Bottom) Gene expression in embryonic day 9.5 12.5 mouse CNS. Borders of the telencephalon (T), diencephalon (D), mesencephalon (M), and rhombomeres are indicated by vertical lines. Reproduced from Hirth, F. and Reichert, H. 1999. Conserved genetic programs in insect and mammalian brain development. *Bioessays* 21, 677 684, with permission from John Wiley & Sons, Inc.

investigated thus far, such as planarians (Orii *et al*., 1999), nematodes (Kenyon *et al*., 1997), annelids (Kourakis *et al*., 1997; Irvine and Martindale, 2000), mollusks (Lee *et al*., 2003), arthropods (Hirth and Reichert, 1999; Hughes and Kaufman, 2002), urochordates (Ikuta *et al*., 2004), cephalochordates (Wada *et al*., 1999), hemichordates (Lowe *et al*., 2003), and vertebrates including zebra fish, chicken, mouse, and human (Lumsden and Krumlauf, 1996; Vieille-Grosjean *et al*., 1997; Carpenter, 2002; Moens and Prince, 2002), the *Hox* gene expression patterns in the developing CNS consist of an ordered set of domains that have a remarkably similar anteroposterior arrangement along the neuraxis.

The function of *Hox* genes in CNS development has been studied through loss- and gain-of-function experiments primarily in *Drosophila*, zebra fish, chicken, and mouse. In *Drosophila*, loss-of-function studies have shown that *Hox* genes are required for the specification of regionalized neuronal identity in the posterior brain (Hirth *et al*., 1998). Comparable results have been obtained through loss-of-function studies in vertebrates, where *Hox* genes are involved in specifying the rhombomeres of the developing hindbrain. For example, in the murine *Hoxb1* mutant, rhombomere 4 (r4) is partially transformed to r2 identity (Studer *et al*., 1996), whereas in *Hoxa1* $^{/}$; *Hoxb1* $^{/}$ double mutants, a region corresponding to r4 is formed, but r4-specific neuronal markers fail to be activated, indicating the lack of neuronal identity of the remaining territory between r3 and r5 (Studer *et al*., 1998; Gavalas *et al*., 1998). This suggests that *Hoxa1* and *Hoxb1* act synergistically in the specification of r4 neuronal identity – a mode of action remarkably similar to that of their fly orthologue, *labial*, in specifying segmental neuronal identity during *Drosophila* brain development (Figure 4).

This evolutionarily conserved *Hox* gene action is underscored by experiments that show that even *cis*-regulatory regions driving the specific spatiotemporal expression of *Hox* genes appear to operate in a conserved manner in insects and vertebrates. Thus, the enhancer region of the human *Hoxb4* gene, an orthologue of *Drosophila Deformed*, can function within *Drosophila* to activate gene expression in a *Deformed*-specific pattern, whereas the enhancer region of *Drosophila Deformed* activates *Hoxb4*-specific expression in the mouse hindbrain (Malicki *et al*., 1992). Similar results have been obtained for *Hox1* orthologues (Pöpperl *et al*., 1995), suggesting that the expression, function, and regulation of *Hox* genes in the specification of segmental neuronal identity during CNS development may be an ancestral feature of this gene family.

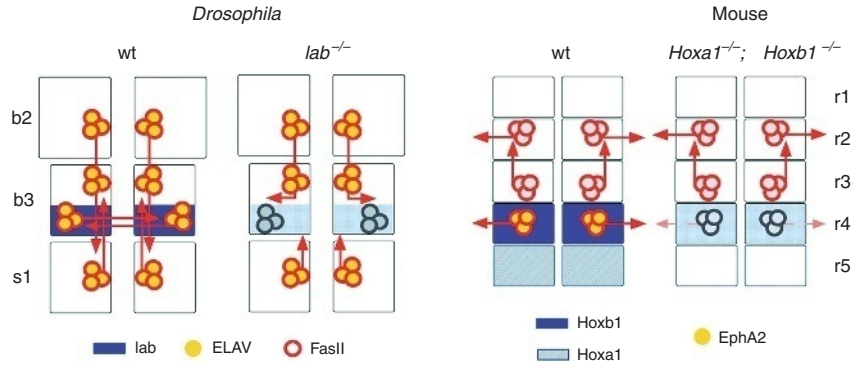

Figure 4 Comparable brain phenotypes in *lab/Hox1* loss-of-function mutants in *Drosophila* and mouse. (Left) Simplified scheme of the deutocerebral (b2), tritocerebral (b3), and mandibular (s1) neuromeres of the *Drosophila* brain. In the wild type (wt) cells in the posterior tritocerebrum express lab (blue) and also express the neuron-specific marker ELAV and the cell adhesion molecule FasII. In the *lab* null mutant (*lab* $^{-/-}$), cells in the mutant domain are present but do not extend axons and fail to express the neuron-specific marker ELAV and the cell adhesion molecule FasII, indicating a total loss of neuronal identity. Axons from other parts of the brain avoid the mutant domain. (Right) Simplified scheme of rhombomeres r1–r5 of the mouse hindbrain. In the wild type (wt) cells in r4 co-express *Hoxa1* and *Hoxb1* and also express the r4-specific marker EphA2. In the *Hoxa1* $^{-/-}$; *Hoxb1* $^{-/-}$ double homozygous mutant, cells in r4 are present but the r4-specific marker EphA2 fails to be activated in r4, indicating the presence of a territory between r3 and r5 with an unknown identity. The double mutant also exhibits multiple defects in the motor neuron axonal projections; facial motor neurons are scarce and exit randomly from the neural tube. Reproduced from Hirth, F. and Reichert, H. 1999. Conserved genetic programs in insect and mammalian brain development. *Bioessays* 21, 677–684, with permission from John Wiley & Sons, Inc.

3.3.2 Cephalic Gap Genes in Regionalization of the Anterior Brain: The *otd/Otx* Genes

In none of the animal species investigated to date are *Hox* genes expressed in the most anterior regions of the developing CNS. This suggests that the developing CNS is subdivided into a posterior Hox region and a more anterior non-Hox region. In both invertebrates and vertebrates, the non-Hox region of the anterior brain is characterized by the expression and action of the cephalic gap genes *tailless* (*tll*)/*Tlx*, *orthodenticle* (*otd*)/*Otx*, and *empty spiracles* (*ems*)/*Emx* (Arendt and Nübler-Jung, 1996). The most prominent example of cephalic gap genes acting in brain development is that of the *otd/Otx* genes. As is the case of the *Hox* genes, the CNS-specific expression of the *otd/Otx* genes is conserved throughout most of the animal kingdom.

otd/Otx genes are expressed in the anterior part of the developing nervous system in planarians (Umesono *et al.*, 1999), nematodes (Lanjuin *et al.*, 2003), annelids (Bruce and Shankland, 1998; Arendt *et al.*, 2001), mollusks (Nederbragt *et al.*, 2002), arthropods (Hirth and Reichert, 1999; Schröder, 2003), urochordates (Wada *et al.*, 1998), cephalochordates (Tomsa and Langeland, 1999), hemichordates (Lowe *et al.*, 2003), and vertebrates (Acampora *et al.*, 2001b; Schilling and Knight, 2001).

Functional studies, carried out primarily in *Drosophila* and mouse, have shown that *otd/Otx* gene activity is essential for the formation of the anterior neuroectoderm. In *Drosophila*, *otd* is expressed in the developing brain throughout most of the protocerebrum and adjacent deutocerebrum. In *otd* mutants, the protocerebrum is deleted due to defective neuroectoderm specification and the subsequent failure of neuroblast formation (Hirth *et al.*, 1995; Younossi-Hartenstein *et al.*, 1997). Loss-of-function analyses for *Otx* genes carried out in the mouse show that these genes are also critically required at different stages in the development of the anterior brain. *Otx2* null mice are early embryonic lethal and lack the rostral neuroectoderm that is normally fated to become the forebrain, midbrain, and rostral hindbrain due to an impairment in early specification of the anterior neuroectoderm by the visceral endoderm. *Otx1* null mice show spontaneous epileptic seizures and abnormalities affecting the telencephalic dorsal cortex and the mesencephalon, as well as parts of the cerebellum and certain components of the acoustic and visual sense organs (Acampora *et al.*, 2001b).

These essential roles of the *otd/Otx* genes in anterior brain development of insects and vertebrates suggest an evolutionary conservation of *otd/Otx* genes in embryonic brain development that extends beyond gene structure to patterned expression and function (Figure 5). A direct experimental demonstration of this functional conservation has been carried out in genetic cross-phylum rescue experiments. Thus, human *Otx* transgenes have been expressed in *Drosophila otd* mutants (Leuzinger *et al.*, 1998) and, conversely, the murine *Otx1* and

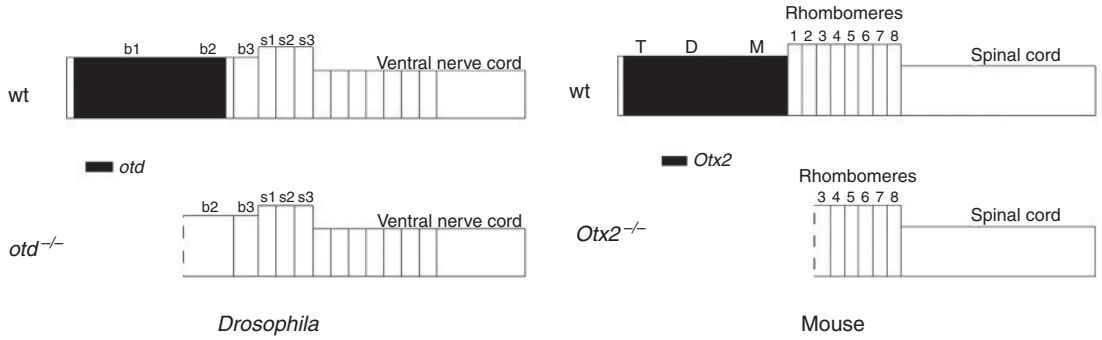

Figure 5 Conserved expression and function of the *otd/Otx2* genes in embryonic brain development. Schematic diagram of *otd* and *Otx2* gene expression patterns and *otd* and *Otx2* mutant phenotypes in the developing CNS of *Drosophila* and mouse. (Top) *otd* gene expression in the wild type (wt) and brain phenotype of *otd* null mutant in embryonic stage 14 *Drosophila* CNS. Borders of the protocerebral (b1), deutocerebral (b2), tritocerebral (b3), mandibular (s1), maxillary (s2), labial (s3), and some of the ventral nerve cord neuromeres are indicated by vertical lines. (Bottom) *Otx2* gene expression in the wild type (wt) and brain phenotype of *Otx2* homozygous null mutant in embryonic day 12.5 mouse CNS. Borders of the telencephalon (T), diencephalon (D), mesencephalon (M), and rhombomeres are indicated by vertical lines. Reproduced from Hirth, F. and Reichert, H. 1999. Conserved genetic programs in insect and mammalian brain development. *Bioessays* 21, 677 684, with permission from John Wiley & Sons, Inc.

Otx2 genes have been replaced with the *Drosophila otd* gene in the mouse (Acampora *et al*., 1998a, 2001b). Intriguingly, despite the obvious anatomical differences between mammalian and *Drosophila* brains, the human *Otx1* and *Otx2* genes complemented the brain defects in *otd* mutant *Drosophila* and, similarly, the // *Drosophila otd* gene was able to rescue most of the CNS defects of *Otx1* and *Otx2* mutant mice (Acampora *et al*., 1998a, 1998b, 2001a; Leuzinger *et al*., 1998).

3.3.3 A Tripartite Organization of the Insect and Chordate Brain?

The conserved expression and function of *otd/Otx* and *Hox* genes suggest that invertebrate and vertebrate brains are all characterized by a rostral region specified by genes of the *otd/Otx* family and a caudal region specified by genes of the *Hox* family. However, in ascidians and vertebrates, a *Pax2/5/8* expression domain is located between the anterior *Otx* and the posterior *Hox* expression regions of the embryonic brain (Holland and Holland, 1999; Wada and Satoh, 2001). In vertebrate brain development, this *Pax2/5/8* expression domain is an early marker for the isthmic organizer positioned at the midbrain–hindbrain boundary (MHB), which controls the development of the midbrain and the anterior hindbrain (Liu and Joyner, 2001; Rhinn and Brand, 2001; Wurst and Bally-Cuif, 2001). The central role of this MHB region in brain development together with the conserved expression patterns of *Pax2/5/8* genes in this region have led to the proposal that a fundamental characteristic of the ancestral chordate brain was its tripartite organization characterized by *Otx*, *Pax2/5/8*, and *Hox* gene expressing regions (Wada *et al*., 1998).

An analysis of brain development in *Drosophila* has uncovered similarities in the expression and function of the orthologous genes that pattern the vertebrate MHB region (Hirth *et al*., 2003). Thus, a *Pax2/5/8* expressing domain was found to be located between the anterior *otd/Otx* expressing region and the posterior *Hox* expressing region in the embryonic brain. In *Drosophila*, as in vertebrates, this *Pax2/5/8* expressing domain is positioned at the interface between the *otd/Otx2* expression domain and a posteriorly abutting *unplugged/Gbx2* expression domain. Moreover, inactivation of *otd/Otx* or of *unplugged/Gbx2* results in comparable effects on mispositioning or loss of brain-specific expression domains of orthologous genes in both embryonic brain types. These developmental genetic similarities indicate that the tripartite ground plan, which characterizes the developing vertebrate brain, is also at the basis of the developing insect brain (Figure 6). This, in turn, has led to the suggestion that a corresponding, evolutionarily conserved, tripartite organization also characterized the brain of the last common ancestor of insects and chordates (Hirth *et al*., 2003).

3.4 Conserved Mechanisms for Dorsoventral Patterning of the CNS

3.4.1 Antagonistic Activity of Dpp/BMP-4 and sog/Chordin

As briefly mentioned above, among the significant molecular control elements involved in the embryonic establishment of the dorsoventral body axis are

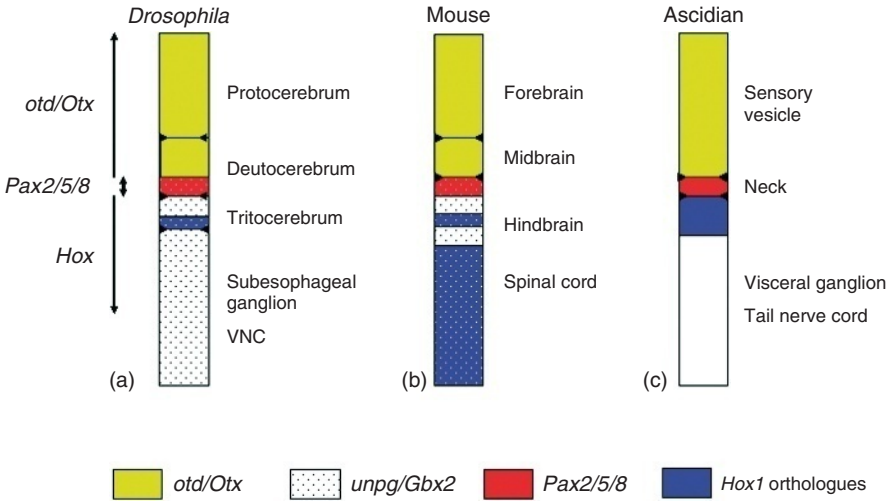

Figure 6 Tripartite organization of the (a) *Drosophila*, (b) mouse, and (c) ascidian brain, based on expression patterns of orthologous genes. The expression of *otd/Otx2*, *unpg/Gbx2*, *Pax2/5/8*, and *Hox1* gene orthologues in the developing CNS of (a) stage 13/14 *Drosophila* embryo, (b) stage E10 mouse embryo, and (c) neurula ascidian embryo. In all cases, a *Pax2/5/8*-expressing domain is located between an anterior *otd/Otx2* expressing region and a posterior *Hox* expressing region in the embryonic brain. Moreover, in *Drosophila*, as in mouse, a *Pax2/5/8*-expressing domain is positioned at the interface between the *otd/Otx2* expression domain and a posteriorly abutting *unplugged/Gbx2* expression domain. This *otd/Otx2 unpg/Gbx2* interface displays similar developmental genetic features in both *Drosophila* and mouse. Reproduced from Hirth, F., Kammermeier, L., Frei, E., Walldorf, U., Noll, M., and Reichert, H. 2003. An urbilaterian origin of the tripartite brain: Developmental genetic insights from *Drosophila. Development* 130, 2365 2373, with permission from The Company of Biologists Ltd.

signaling molecules of the TGFβ family such as Dpp, studied most extensively in *Drosophila*, and BMP-4, one of the vertebrate homologues of Dpp (De Robertis and Sasai, 1996). These proteins establish dorsoventral polarity in the insect embryo and in the vertebrate embryo. In both cases, they are restricted in their spatial activity by antagonistically acting extracellular signaling proteins. These antagonists are Sog in *Drosophila* and its homologue Chordin in vertebrates. The two groups of interacting signaling molecules, Dpp/BMP-4 and Sog/Chordin, act from opposing dorsoventral poles in both insects and vertebrate embryos (Holley *et al.*, 1995). Remarkably, in *Drosophila*, Dpp exerts its activity on dorsal cells and Sog on ventral cells, whereas in vertebrates BMP-4 acts on ventral cells and Chordin activity is found in dorsal cells. In both cases, it is the region of the embryo that attains neurogenic potential and forms neuroectoderm in which Sog/Chordin is expressed and inhibits the action of invading Dpp/BMP-4 signals.

Thus, despite the morphological differences between embryos of the two species, the *Sog/Chordin* gene is expressed on the side from which the CNS arises, whereas the *dpp/Bmp-4* gene is expressed on the opposite side of the embryo where it promotes ectoderm formation. This functional conservation of the Sog/Chordin and the Dpp/BMP-4 morphogens suggests an evolutionarily conserved, homologous mechanism of dorsoventral patterning. This suggestion is further substantiated by experimental studies showing that injection of *Chordin* RNA (from *Xenopus*) promotes ventralization of cell fates in *Drosophila* embryos, including the formation of ectopic patches of CNS. Correspondingly, injection of *sog* RNA (from *Drosophila*) causes dorsal development in *Xenopus*, including the formation of notochord and CNS (Holley *et al.*, 1995; Schmidt *et al.*, 1995). Thus, the function of *sog/Chordin* is reversed in insects and vertebrates; in both cases, injection of the gene product promotes the development of the side of the embryo that contains the CNS: dorsal in vertebrates, ventral in insects. This pervasive equivalence of gene structure and function points to an essential role of *Sog/Chordin* and *Dpp/BMP-4* in CNS induction/specification in insects and vertebrates, irrespective of the location along the dorsoventral axis at which the CNS forms (Figure 7).

3.4.2 vnd/Nkx, ind/Gsh, and msh/Msx: Specification of Longitudinal Columns

Beyond the mechanisms of early neuroectoderm formation, a further set of genetic elements involved in early dorsoventral patterning of the CNS appears to be evolutionarily conserved (Cornell and Ohlen, 2000). These genetic regulatory elements are three

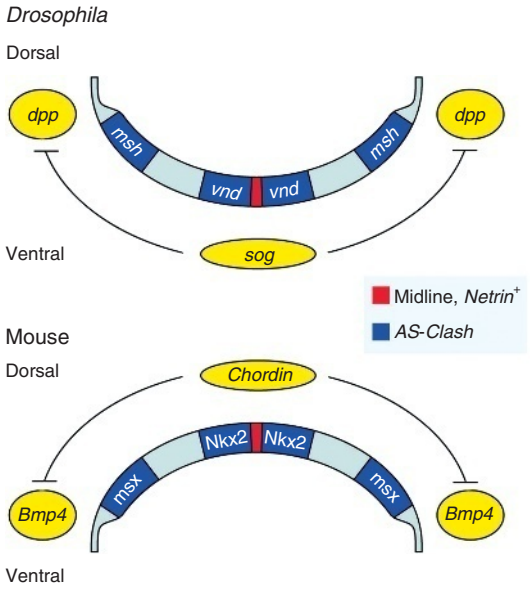

Figure 7 Transverse sections through the *Drosophila* and mouse CNS primordia showing similar dorsoventral regulation of pattern by the *sog* (*short gastrulation)/Chordin*, *dpp* (*decapentaplegic)/BMP4*, *Msx/msh*, *Nkx2/vnd, AS-C* (*achaete scute complex)/ash* (*AS-C homologues*), and *Netrin* gene families. Reproduced from Sharman, A. C. and Brand, M. 1998. Evolution and homology of the nervous system: Cross-phylum rescues of *otd/otx* genes. *Trends Genet.* 14(6), 211 214, with permission from Elsevier.

sets of homeobox genes that control the formation of columnar dorsoventral domains in the ventral neuroectoderm of *Drosophila*; their homologues may act in a similar fashion in dorsoventral patterning in the neural plate of vertebrates (Figure 7). In *Drosophila*, the homeobox genes are *ventral nerve cord defective* (*vnd*), *intermediate nerve cord defective* (*ind*), and *muscle-specific homeobox* (*msh*) and they are expressed in longitudinal stripes along the ventral (*vnd*), intermediate (*ind*), and dorsal (*msh*) columns in the neuroectoderm (Isshiki *et al.*, 1997; McDonald *et al.*, 1998; Chu *et al.*, 1998; Weiss *et al.*, 1998). In each column, expression of the appropriate homeobox gene is required for neuroblast formation and for specification of columnar identity. Comparable expression patterns have been reported for the beetle *Tribolium* (Wheeler *et al.*, 2005).

In vertebrates, homologues of the *Drosophila* columnar genes that belong to the *Nkx* (*vnd*), *Gsh* (*ind*), and *Msx* (*msh*) gene families have been identified. These genes are expressed in columnar domains in the neural plate and neural tube of the embryonic CNS. (Invagination of the vertebrate neural plate to form the neural tube results in translocation of the lateromedial position into the dorsoventral position.) In vertebrates, several *Nkx* family members are expressed in ventral regions of the neural tube and at least one of these is expressed earlier in the corresponding medial region of the neural plate (Qiu *et al.*, 1998; Pera and Kessel, 1998; Pabst *et al.*, 1998; Shimamura *et al.*, 1995). Similarly, expression of vertebrate *Msx* family members is seen in the lateral neural plate, which later forms the dorsal neural tube (Wang *et al.*, 1996). Finally, vertebrate *Gsh* family genes are expressed at dorsoventrally intermediate levels in the neural tube (Valerius *et al.*, 1995; Hsieh-Li *et al.*, 1995). Functional studies suggest that some of these genes are involved in controlling regional identity along the dorsoventral axis of the neural tube (Briscoe *et al.*, 1999; Sussel *et al.*, 1999). These findings indicate that in the developing CNS of insects and vertebrates, the expression domains of columnar genes in the neuroectoderm/neural plate are comparable (Figure 7). This, in turn, has led to the proposal that the medial, intermediate, and lateral neurogenic columns of the *Drosophila* embryonic neuroectoderm correspond to the medial, intermediate, and lateral columns of the vertebrate neural plate, albeit in dorsoventral inverted orientation (D'Alessio and Frasch, 1996; Weiss *et al.*, 1998).

3.4.3 The CNS Midline: Pattern Formation and Axonal Guidance

In the nervous systems of bilaterians, specialized cells located at the midline of the neuroectoderm play an essential role in organizing the development of the CNS (Tessier-Lavigne and Goodman, 1996; Dickson, 2002). In insects and vertebrates, cells of the CNS midline are known to represent inductive centers for the regional patterning of the neuroectoderm. Moreover, the CNS midline represents an important intermediate target where growing axons either cross and project contralaterally or remain on the same side of the body. The midline cells express at their surface membrane-bound guidance molecules and secrete diffusible factors that act as attractive or repulsive guidance cues and guide growing axons from a distance; under the influence of these molecules, some axons avoid the midline, whereas others grow toward it and cross it once.

The developmental control genes that specify these midline cell populations appear to differ between insects and vertebrates. In *Drosophila*, formation of midline cells requires the specific expression of the *single-minded* gene (Nambu *et al.*, 1990), whereas in vertebrates, the formation of midline cells requires the specific expression of *HNF3beta* (Ang and Rossant, 1994; Weinstein *et al.*, 1994). Also, the morphogens that mediate

the inductive interactions of the midline cells differ in vertebrates versus insects. In vertebrates, *Sonic hedgehog* signaling from the floor plate exerts its patterning function on the adjacent dorsal neuroectoderm (Ho and Scott, 2002), whereas in *Drosophila*, *EGF* signaling exerts patterning on the adjacent ventral neuroectoderm (Skeath, 1999).

In contrast, many aspects of midline cell-mediated axon guidance are controlled by functionally and evolutionarily conserved ligand–receptor systems that include the *Netrin*, *DCC*, *Slit*, and *Robo* gene families (Araujo and Tear, 2002; Kaprielian *et al*., 2001). Homologous *Netrin* genes encode soluble attractor molecules that are detected in the floor plate and ventral neural tube of vertebrates as well as in the midline glial cells of *Drosophila* and that serve to guide commissural axons toward the midline. In both cases, the *Netrins* are expressed at a time when first commissural growth cones, which express the homologous *frazzled/DCC* genes that encode transmembrane receptors, are extending toward the midline. *Netrin* mutant embryos exhibit defects in commissural axon projections in mice and flies, indicating similar functional roles of these attractants. Moreover, in *Drosophila* as well as in vertebrates, axonal projections away from the midline depend on the presence at the midline of a repellent molecule, which binds and interacts with axonal receptors. In *Drosophila*, the midline repellent that expels commissural axons and prevents them from recrossing is the ligand Slit, which mediates its repulsive effects via receptors of the Roundabout (Robo) family that are dynamically expressed on commissural axons. In vertebrates, three *Slit* homologues (*Slit1*, *Slit2*, and *Slit3*) and three *Robo* homologues (*Robo1*, *Robo2*, and *Rig-1*) have been identified, with expression patterns reminiscent of their *Drosophila* counterparts. The vertebrate *Slit* genes are expressed in the floor plate at the ventral midline of the spinal cord, and their corresponding *Robo1* and *2* receptors are expressed by commissural axons. Studies indicate that vertebrate commissural axons become insensitive to floor plate attraction and sensitive to *Slit*-mediated repulsion after crossing the midline; this modulation of repulsion at the midline is reminiscent of the situation in the *Drosophila* CNS.

3.5 Evolutionary Origin of the CNS

3.5.1 Molecular Phylogeny: Several Possibilities

The similarities in anteroposterior and dorsoventral patterning genes as well as their conserved relative topological expression patterns and functional roles implicate a common genetic program underlying insect and mammalian nervous system development (Hirth and Reichert, 1999; Arendt and Nübler-Jung, 1999; Reichert and Simeone, 2001). This suggests that orthologous genes were already involved in neural specification in the insect and vertebrate stem species, if not already in a common bilaterian ancestor. Does this mean that the insect and chordate CNS are homologous structures and therefore of monophyletic origin? Two alternative hypotheses, which are not mutually exclusive, can be envisaged. The first of these postulates that the ancestral bilaterian nervous system was already centralized and had its development governed by conserved genetic mechanisms that are still apparent in extant insects and mammals (monophyletic origin of the brain). The second hypothesis is that the ancestral bilaterian nervous system was controlled by conserved genetic mechanisms that still operate in arthropods and vertebrates, but that centralization of the nervous system occurred independently in protostome and deuterostome lineages (polyphyletic origin of the brain).

Based on classical phylogeny, which places acoelomates, such as platyhelminthes, and pseudocoelomates, such as nematodes, nearer to the base of the Bilateria than the coelomate protostomes and deuterostomes, the first hypothesis seems more likely (Figure 8a). Since flatworms and nematodes have a CNS with a brain and a ventral nerve cord, a comparable centralized nervous system would be likely to reflect the ancestral state for both Protostomia and Deuterostomia, and indeed for all Bilateria.

In this view, the evolutionary advance of centralizing the nervous system occurred only once. In contrast, molecular phylogenetic analyses no longer provide evidence that preferentially supports one of the two hypotheses. According to studies based on 18S rRNA sequence comparisons, there are no longer any living bilaterians that can be considered to be evolutionary intermediates between the radially (or biradially) symmetric animals and the bilaterally symmetric protostomes and deuterostomes (Figure 8b). Invertebrate lineages such as platyhelminthes and nematodes, which were considered to be near the base of the bilaterian tree in classical phylogeny, are now placed next to protostome groups with highly complex body and brain morphology such as mollusks and arthropods in the two new protostome subgroupings, the lophotrochozoans and ecdysozoans (Adoutte *et al*., 2000). Thus, although neurons and nervous systems, which are present in radiate cnidarians and ctenophores, apparently existed before the origin of bilaterian animals, the evolutionary origin of nervous system

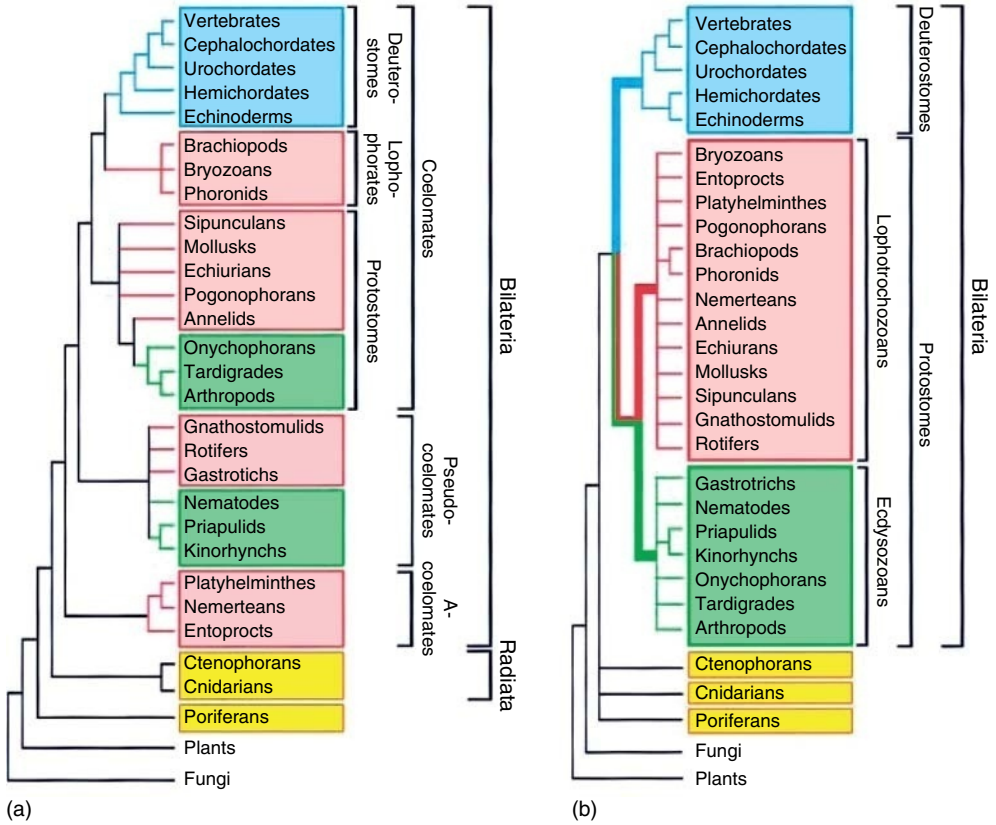

Figure 8 Metazoan phylogenies. a, The traditional phylogeny based on morphology and embryology. b, The new molecule-based phylogeny. Reproduced from Adoutte, A., Balavoine, G., Lartillot, N., Lespinet, O., Prud'homme, B., and de Rosa, R. 2000. The new animal phylogeny: Reliability and implications. *Proc. Natl. Acad. Sci. USA* 97, 4453 4456. Copyright 2000 National Academy of Sciences, USA, with permission.

centralization and brain formation cannot be deduced from molecular phylogenetic data alone (see Origin and Evolution of the First Nervous System). This means that in terms of nervous system organization of the last common ancestor of modern bilateral animals, current molecular phylogeny is compatible with a number of possibilities (see, for example, Arendt and Nübler-Jung, 1997; Adoutte *et al.*, 2000; Gerhart, 2000; Shankland and Seaver, 2000; Meinhardt, 2002; Erwin and Davidson, 2002; Holland, 2003; and references therein).

3.5.2 Do Specialized Gene Expression Patterns Predict Specialized Brain Structures?

Since molecular phylogeny does not support preferentially either of the two hypotheses for the evolutionary origin of the CNS, we are left with the molecular data provided by comparative developmental genetic studies. Given the conserved molecular patterning mechanisms, or at least the conserved gene expression patterns, that characterize brain development in all bilaterians examined, what inferences can be made about the evolution of the CNS? The hypothesis of a monophyletic origin of the CNS is underscored by the notion that specialized developmental patterning mechanisms and patterned anatomical complexity evolved together (Tautz, 2003). Since comparative developmental genetics indicates that a complex set of conserved and specialized anteroposterior and dorsoventral patterning genes were operative in the nervous system of the urbilaterian ancestor of protostomes and deuterostomes, it is reasonable to assume that these genes generated an urbilaterian nervous system that also manifested complex anatomical specializations along the anteroposterior and dorsoventral axes (Hirth and Reichert, 1999; Arendt and Nübler-Jung, 1999; Reichert and Simeone, 2001). Thus, the conservation of expression and function of the dorsoventral columnar genes, including their dorsoventral inversion, provides strong evidence for the existence of an urbilaterian nervous system that was already dorsoventrally regionalized. Moreover, the observed dorsoventral inverted expression of these genes in the CNS of insects versus vertebrates is

precisely what would be predicted by the body axis inversion hypothesis, which in turn is substantiated by independent molecular evidence from gene expression data on heart development and gastrulation (e.g., Cripps and Olson, 2002; Arendt and Nübler-Jung, 1997).

Alternative scenarios for the evolution of centralized nervous systems in protostomes and deuterostomes have been proposed in which the CNSs occurred independently, after the split of the two groups, and without a dorsoventral inversion (reviewed in Gerhart, 2000; Holland, 2003; Lacalli, 2003). An implicit assumption of these proposals is that the bilaterian ancestor did not exert a dorsoventrally centralized nervous system but instead already had a structured map of patterning gene expression, which was then independently used for generating the CNS in different phyla. In the *Auricularia* hypothesis originally put forward by Garstang (1894; see also Nielsen, 1999), the evolutionary origins of the chordate nervous system are thought to be found in the ciliary bands of a deuterostome dipleurula-type larval ancestor resembling an echinoderm *Auricularia* larva. During the evolution of the chordate CNS, bilateral rows of cilia and the associated nerves were said to have converged through complex morphogenetic movements to the dorsal midline and fused to form the neural tube. Evidence for this view was found in comparative anatomical studies between echinoderms (particularly *Auricularia* larvae), hemichordates, and urochordates, and data show that a number of genes involved in chordate CNS development, including *SoxB3, Nkx2.1*, and *Otx*, are expressed in ciliary bands of larval hemichordates and/or echinoderms (Taguchi *et al.*, 2002; Takacs *et al.*, 2002; Tagawa *et al.*, 2001). Thus far, however, the ciliary band derivatives have not been shown to give rise to cells of the adult nervous system after metamorphosis. Furthermore, the *Auricularia* hypothesis does not take into account the molecular genetic similarities between the CNS of protostomes and that of chordates.

A comparative study on an enteropneust hemichordate has shown that the anteroposterior expression pattern of a large number of genes, which are involved in axial patterning of the vertebrate and arthropod CNS, is conserved in the apparently diffuse nervous system of the enteropneust acorn worm. The body-encircling basiepithelial nerve net of the directly developing hemichordate *Saccoglossus kowalevskii* expresses a complex set of regulatory genes in circumferential networks (Lowe *et al.*, 2003). Among these are the orthologues of the *otd/Otx*, *tll/Tlx*, *ems/Emx*, *unpg/Gbx*, *dll/Dlx*, *Pax*, *En*, *Lim*, *Hox*, and other highly conserved gene families, which reveal an anteroposterior order of domains that is remarkably similar to the insect and mammalian gene expression patterns (Figure 9). Unfortunately, almost nothing is known about the expression of hemichordate *dpp/BMP-4* and *sog/Chd* homologues and whether they might possess a neural/antineural antagonism that could limit and/or condense the nerve net into a CNS to one side of the body. Only in the indirectly developing hemichordate *Ptychodera flava* has a BMP2/4 homologue been described; however, no expression was observed during embryogenesis, suggesting that it is not involved in axis formation (Harada *et al.*, 2002). Moreover, little is currently known about *vnd/Nkx, ind/Gsh*, and *msh/Msx* orthologous gene expression and whether these genes might possess any early dorsoventral patterning functions in longitudinal column formation of the hemichordate nervous system. Thus far, only the expression of a hemichordate *Nkx2.1* homologue, which is specifically expressed in a ventral sector of the anterior ectoderm, is known (Lowe *et al.*, 2003).

Based on the gene expression studies in *Saccoglossus*, Lowe and co-workers have proposed that the nervous system of the deuterostome ancestor of hemichordates and chordates was also organized in a diffuse, body-encircling, basiepithelial nerve net (Lowe *et al.*, 2003). According to molecular phylogeny, this indicates that the bilaterian ancestor preceding protostomes and deuterostomes also possessed a diffuse, body-encircling, basiepithelial nerve net. Independent centralization events in protostomes and deuterostomes without dorsoventral inversion could then have resulted in anteroposteriorly oriented CNSs with similar gene expression domains (Holland, 2003).

Alternatively, the diffuse nervous system of *Saccoglossus* may represent the secondary loss of a centralized nervous system. Like cnidarians and ctenophores, hemichordates exhibit only neuroepidermal fibers without organized ganglia, brain, or other obvious specialized neural structures. Indeed, most of the data of Lowe *et al.* (2003) are equally compatible with a secondary reduction scenario, in which the ancestor of the deuterostomes would have had a centralized nervous system, which was lost in the hemichordates due to their peculiar lifestyle as sediment-burrowing worms. Moreover, the apparently simple, nerve net-like nervous system of hemichordates may display further substructures, including CNS elements, as suggested

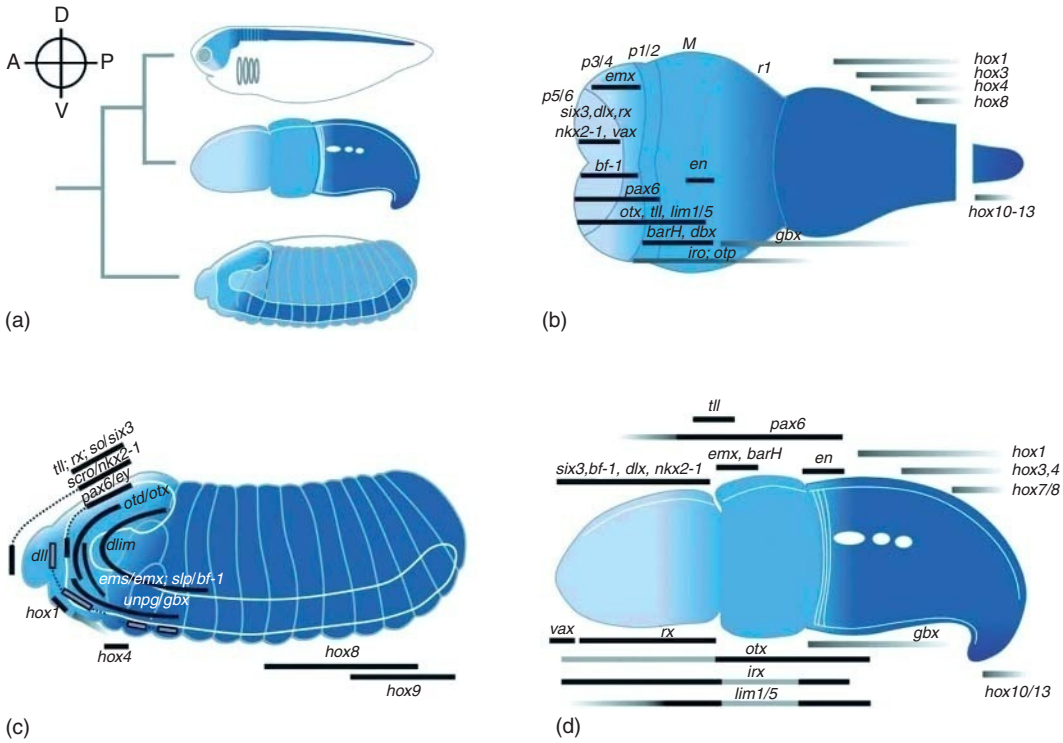

Figure 9 Comparison of the neural gene domain maps of hemichordates, chordates, and *Drosophila*. In addition to individual gene domains, the color gradient in each panel indicates general similarities of gene expression domains. a, Representation of the general organizational features of the CNSs of chordates and arthropods and the diffuse nervous system of hemichordates arranged on a phylogram. The compass indicates the axial orientation of each model. b, Representation of a dorsal view of a vertebrate neural plate (see Rubenstein *et al.*, 1998). p1/2, prosomeres 1 and 2; p3/4, prosomeres 3 and 4; p5/6, prosomeres 5 and 6; M, midbrain; r1/2, rhombomeres 1 and 2. The discontinuous domain represents the postanal territory of the nerve cord. All 22 expression domains are shown. c, *Drosophila* late stage 12 embryo model with 14 expression domains shown (lateral view, post-germ-band retraction, before head involution). All models are positioned with anterior to the left. d, The acorn worm (lateral view), with its diffuse nervous system, is shown with a blue color gradient of expression in the ectoderm; the anterior domains, the midlevel domains, the posterior domains, and the postanal territory are color matched to the anteroposterior dimension of the chordate model. Reproduced from Lowe, C. J., Wu, M., Salic, A., *et al.* 2003. Anteroposterior patterning in hemichordates and the origins of the chordate nervous system. *Cell* 113, 853 865, with permission from Elsevier.

by earlier neuroanatomical analyses: nerve fiber tracts are formed in the epithelium, including major ventral and dorsal tracts (Bullock, 1945; Knight-Jones, 1952).

3.5.3 A Simple Nerve Net at the Base of Nervous System Evolution?

There is some evidence that a basiepithelial, noncentralized nerve net, perhaps comparable to those found in extant hemichordates, may indeed represent the basal evolutionary state from which bilaterian nervous systems evolved. Basiepithelial nervous systems exist in some gastroneuralians, and the subepithelial nervous systems, as in insects, often go through a basiepithelial state during their development (Nielsen, 1995; Arendt and Nübler-Jung, 1999). However, the question remains of how such a simple nerve net condensed into a centralized nervous system and when this occurred in evolution. Paleontological evidence can provide a reasonable estimate of when CNSs were already formed in protostome and deuterostome animals. A conservative estimate is a date of 530–540 Mya in the early Cambrium, when a complex variety of bilaterian forms representing most of the modern major animal groups was present (Grotzinger *et al.*, 1995; Conway-Morris, 2000). These forms included arthropods such as trilobites and early agnathan-like stem vertebrates and the fossil record for both of these animal forms indicates that they already had brains and CNS with features typical for arthropods and vertebrates (Fortey, 2000; Holland and Chen, 2001). Thus, centralization of nervous systems must have occurred earlier, probably after the split between the cnidarians and the bilaterians, which is thought to have occurred between 600 and 630 Mya (Peterson *et al.*, 2004). If this is the case, then the cnidarian nervous system might be

more informative of early CNS evolution in stem Bilateria than that of hemichordates.

The basic organization of the nervous system in cnidarians (and ctenophores) is that of a diffuse nerve net that can also manifest centralized elements such as nerve rings and ganglionic centers. Moreover, many of the conserved developmental control genes that operate in the insect and vertebrate nervous system are also present in Cnidaria and thus at least some of these differentiation gene batteries date to the last common ancestor of cnidarians and bilaterians (Finnerty *et al*., 2004; Ball *et al*., 2004; Finnerty, 2003; Galliot, 2000). Among these are anterior and posterior *Hox* genes, an asymmetrically expressed *dpp* gene, and an *Otx* gene. However, the expression patterns of these genes differ among cnidarian species and are inconclusive as far as anteroposterior or dorsoventral axis determination is concerned (Yanze *et al*., 2001; Finnerty *et al*., 2004). For example, the typical bilaterian head gene *Otx* is expressed along the entire primary body axis in cnidarians. In *Hydra*, the *CnOtx* gene is expressed at a low level in the ectodermal epithelial cells of the body, during early budding in the region of the parental body column from which cells will migrate into the developing bud, and *CnOtx* is strongly upregulated during reaggregation, in contrast to head or foot regeneration where it is downregulated (Smith *et al*., 1999). In *Podocoryne*, the *Otx* gene displays two types of expression: in the gonozooid polyp at every developmental stage of the budding medusa and in the mature medusa, restricted to the striated muscle cells (Müller *et al*., 1999). These data suggest that *Otx* is not involved in axis determination or head specification in *Hydra* and *Podocoryne*. Thus, ambiguous species-specific gene expression data in cnidarians make comparisons between cnidarian and bilateral nervous systems difficult and thus far are inconclusive concerning CNS evolution.

3.6 Conclusions

Contemporary experimental studies analyzing the expression and function of homologous genes in various animal model systems are reviving a fundamental question raised more than 150 years ago in the famous academic debate between Cuvier and Geoffroy Saint-Hilaire: does a common Bauplan underlie animal development, indicating homology of structures such as the ventrally located insect and the dorsally located chordate nervous system? Comparisons of the expression, function, and regulation of genes and genetic networks involved in anteroposterior and dorsoventral patterning of the insect and vertebrate nervous systems suggest that orthologous genes were already involved in neural specification in the insect and vertebrate stem species. Thus, the pervasive equivalence of the *Dpp/BMP-4* and *sog/Chd* antagonism in executing the distinction between neural and non-neural, the *vnd/Nkx, ind/Gsh*, and *msh/Msx* gene network involved in early dorsoventral columnar patterning, the role of the *otd/Otx* genes in anterior CNS regionalization, and the action of *Hox* genes in the specification of segmental neuronal identity are all conserved in both insect and mammalian CNS development. This strongly suggests that these molecular genetic mechanisms were already apparent in an urbilaterian ancestor and that the insect and vertebrate nervous systems evolved from a common ancestral urbilaterian brain.

However, it is also conceivable that complex gene expression characteristics pre-dated the generation of morphological complexity in the course of nervous system evolution. The analysis of developmental control gene expression in a hemichordate demonstrates that complex gene expression patterns, comparable to those observed in the CNS of insects and vertebrates, are compatible with the existence of a diffuse basiepithelial nerve net. Nevertheless, the hemichordate body plan is clearly derived and its basiepithelial nerve net may be the result of a secondary reduction or loss of an ancestral CNS. Some of the developmental control genes that operate in CNS development in arthropods and chordates are also expressed during cnidarian development. Although a diffuse, net-like nervous system is apparent in Cnidaria, the ambiguous data on orthologous gene expression in these animals impede any conclusive comparisons between cnidarian and bilateral nervous systems. The available data therefore suggest that only one ancestral, albeit rather complex, nervous system type was at the origin of bilaterian CNS evolution.

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4 Origin and Evolution of the First Nervous System

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Glossary

anterior class Hox *genes* — Group of *Hox* genes that are involved in the specification of the anterior most part of the anteroposterior body axis of bilaterians. The bilaterian Hox cluster genes are believed to be descended from an ancestral ProtoHox cluster which included four genes, the ancestor of the present day Hox classes (anterior, group 3, central, and posterior).

basal Metazoa — Here used to refer to Porifera, Cnidaria, Ctenophora, and Placozoa. Other authors include the Platyhelminthes (flatworms).

Bilateria — A monophyletic group of metazoan animals that is characterized by bilateral symmetry. Traditionally, this group includes deuterostomes (e.g., chordates, echinoderms, and hemichordates), and protostomes (e.g., arthropods, nematodes, annelids, and mollusks).

Coelenterata — Cnidaria and Ctenophora were traditionally joined together as Coelenterata based on the presence of a single gastrovascular system serving both nutrient supply and gas exchange.

deuterostome — A bilaterian animal whose mouth forms embryonically as a secondary opening, separate from the blastopore. Deuterostomes include chordates, hemichordates, and echinoderms.

effector cell/organ — Single cells or group of specialized cells transducing external stimulation or neuronal signals into a specific response like contraction, secretion, bioluminescence, or electricity.

Eumetazoa — A monophyletic group of animals including all metazoans except the phylum Porifera.

excitable epithelia — Epithelia which can conduct electrical signals over wide areas without decrement.

expressed sequence tag (EST) — A nucleic acid sequence that is derived from cDNA as part of sequencing projects.

four domain Na^+ channel — A single protein ion channel composed of four linked domains, each of which consists of six transmembrane segments. The whole protein folds up into a channel forming a pore that is selective for Na^+ ions. The four domain Na^+ channels are believed to have evolved from structurally similar Ca^{2+} channels.

gap junctions — Membrane protein complexes (connexons) that join the plasma membranes of two neighboring cells creating a communication between the cytoplasm of the two cells. This allows the exchange of molecules and the direct propagation of electrical signals.

higher Metazoa — We use these terms as a synonym of Bilateria.

homologue — A gene related to a second gene by descent from a common ancestral DNA sequence. The term, homologue, may apply to the relationship between genes separated by the event of speciation (see orthologue) or to the relationship between genes separated by the event of genetic duplication (see paralogue).

hypostome — The terminal region of a polyp, on which the mouth is situated.

low resistance pathway — A tract of multiple cells which are cytoplasmically connected through specialized pores in the cell membranes allowing the fast conduction of electrical signals.

medusa — Mobile form (jellyfish) of life history in the cnidarian classes Hydrozoa, Scyphozoa, and Cubozoa (Medusozoa).

Medusozoa — Comprises three of the four cnidarian classes (Hydrozoa, Scyphozoa, and Cubozoa), which produce a sexually reproducing medusa (jellyfish) as part of the life cycle.

mesenteries — Longitudinal sheets of tissues that extend radially from the body wall of polyps into the body cavity.

mesogloea (also known as mesoglea) — The body layer between ectoderm and endoderm in cnidarians, ctenophores and acoelomates, which is traditionally distinguished from mesoderm on the basis of the former being acellular and the latter cellular.

myoepithelium — A single layered tissue of contractile cells.

orthologue — Orthologues are genes in different species that evolved from a common ancestral gene by speciation. Orthologues often retain the same function in the course of evolution.

pacemaker — Single cell or group of cells (neuronal or muscular) that spontaneously drive rhythmic activity in neighboring cells.

paralogue — Paralogues are genes related by duplication within a genome. Paralogues may evolve new functions.

planula — The free swimming, ciliated larva of a cnidarian.

polyp — The sessile form of life history in cnidarians; for example, the freshwater *Hydra*.

posterior class Hox *gene* — Group of *Hox* genes that is involved in the specification of the posterior part of the antero posterior body axis of bilaterians. The bilaterian Hox cluster genes are believed to be descended from an ancestral ProtoHox cluster which included four genes, the ancestor of the present day Hox classes (anterior, group 3, central, and posterior).

protomyocyte — An evolutionary antecedent of muscle cells.

protoneuron — Term coined by Parker (1919) for the type of nervous cell from which modern ganglionic neurons evolved.

protostome — A bilaterian animal whose mouth and anus develop embryonically from the same invagination (the blastopore) during embryogenesis.

Radiata — Animals that are traditionally considered to have radial symmetry. This group includes the Ctenophora and the Cnidaria.

Siphonophora — Cnidarian order of marine colonial hydrozoans.

statocyst — The statocyst is a balance organ and consists of a pouch lined with sensory hairs, within which sits a heavy granule called the statolith. The sensory hair cells are connected by nerve fibers to the animal's nervous system. The sensed motion of the statolith in response to gravity allows the animal to orientate itself.

4.1 Introduction

4.1.1 Tracing Back the First Nervous System

By definition, the first nervous system evolved after the evolutionary shift from unicellular to multicellular life forms. Complex, coordinated behavior controlled by a primitive nervous system in early metazoan animals must have conferred strong selective advantages and thus contributed significantly to the evolutionary success of nervous systems within metazoan animals. Ultimately, more advanced nervous systems, including our own, evolved into the most complex structures found in living matter. In order to learn more about the origins of complex nervous systems in highly evolved animal species, research on the more simple nervous systems that characterize basal metazoan phyla was initiated more than two centuries ago. Then, as today, understanding the origin and early evolution of these simple nervous systems may lead to more profound insight into fundamental principles of development, organization, and function of modern nervous systems.

It is highly likely that the emergence of the first nervous system predated the evolutionary divergence of Bilateria and Radiata 600–630 Mya (Peterson *et al.*, 2004) given the fact that neurons and nervous systems are present in both animal groups. However, the independent evolution of the Bilateria and Radiata during this long period of time implies that most extant animals cannot be regarded as primitive in terms of the organization of their nervous systems. Moreover, for the Radiata, which are generally considered to be basal eumetazoan groups, the fossil record is poor and does not allow reconstruction of fossil nervous systems (Chen *et al.*, 2002). Thus, in the quest to understand the origin of the first nervous systems, it seems best to pursue a comparative approach, in which the structure, function, and development of nervous systems in several basal metazoan phyla are considered and compared in terms of key molecular, cellular, and morphological aspects.

In this review, we will begin by defining what neurons and nervous systems are and then present a current version of the phylogenetic relationships that characterize the systematic groups that are relevant for subsequent considerations. Following this, we will give a brief historical overview of the ideas concerning the origin and evolution of the first nervous system. The main part of the review will then present a detailed comparative analysis of nervous systems in the basal metazoan phyla which may have participated in the origin of the nervous system. Here the main emphasis will be on Cnidaria, but Porifera, Ctenophora, and Placozoa will also be presented, and electrical conduction outside of the animal kingdom will be considered. Finally, we will discuss the implications of recent molecular genetic findings on neurogenesis and axial patterning in cnidarians and bilaterians for our current understanding of the origin of the first nervous system.

4.1.2 Definition of the Nervous System

All living cells respond to stimuli and engage in signal processing. Thus, even in the absence of a nervous system, reactions to external stimuli do occur. In most metazoans however, a discrete subset of specialized somatic cells form an interconnected network, called the nervous system, in which multiple sensory stimuli can be processed and conducted to specific effector organs, achieving coordination of complex behaviors. A useful general definition of nervous systems has been given by Bullock and Horridge (1965): "A nervous system is an organized constellation of cells (neurons) specialized for the repeated conduction of an excited state from receptor sites or from other neurons to effectors or to other neurons." An additional aspect was put forward by Passano (1963), who pointed out that the ability to generate activity endogenously is as much a part of the definition of a nervous system as is the ability to respond to stimulation. It follows, from these considerations, that connectivity, specialization for propagating an excited state, and spontaneous generation of activity are important anatomical and physiological criteria for a true nervous system.

The functional units of nervous systems are nerve cells or neurons, which are specialized for the reception of stimuli, conduction of excitation, and signal transmission to other cells. Neurons appear in the most simple animals as specialized conducting, secreting, and spontaneously active cells within epithelia which themselves may show sensory, conducting, and pacemaker features. Given their role in conduction, a key point about neurons is that they are elongated, which enables them to transmit beyond their immediate neighbors without exciting all the interspersed cells (Horridge, 1968).

Some extant animals have a diffuse nerve net representing either an ancestral organization or a secondary loss of centralized structures as often observed in parasitic or sedentary life forms. A nerve net has been defined by Bullock and Horridge (1965) as "a system of functionally connected nerve cells and fibers anatomically

dispersed through some considerable portion of an animal and so arranged as to permit diffuse conduction of nervous excitation, that is, in relatively direct paths between many points. The paths, as opposed to indirect routing through a distant ganglion or central structure, are multiple and confer a tolerance of incomplete cuts."

4.1.3 Basal Metazoan Phylogeny

A comparative approach to nervous system structure, function, and origin requires an understanding of the phylogeny that underlies the animal groups considered. It is now commonly agreed that all metazoan phyla including Porifera have a monophyletic origin (reviewed in Müller, 2001; Müller *et al.*, 2004). In this section the phylogenetic relationships of major extant taxonomic groups at the stem of bilaterian animals will be presented (Figure 1).

Choanoflagellata, which show a striking structural resemblance to the choanocytes found in sponges, have been hypothesized to be the closest relative to multicellular animals, and Porifera have been proposed to derive from a colonial form of choanoflagellates (James-Clark, 1867). Recent molecular phylogenetic data provide further support for this hypothesis, indicating that choanoflagellates are indeed more closely related to animals than are fungi and, thus, form a monophyletic sister group of metazoans (Medina *et al.*, 2001; Brooke and Holland, 2003).

Porifera represent the earliest known metazoan phylum and consist of three major taxa: Hexactinellida, Demospongiae, and Calcarea. The molecular sequence analysis of key proteins from these three poriferan classes, suggest that Hexactinellida are the phylogenetically oldest taxon, while Calcarea represent the class most closely related to higher metazoan phyla (Medina *et al.*, 2001; Müller *et al.*, 2004).

The relative positions of the potential sister groups to the bilaterians, namely Cnidaria, Ctenophora, and Placozoa are controversial. Classically the Cnidaria and Ctenophora have been grouped together as the sister group to bilaterians. Together, they are also referred to as the Radiata based on their radially symmetrical appearance (this term may be inappropriate given that biradial and even bilateral symmetry are also common among these animals). On morphological and embryological grounds, such as the presence of mesoderm as a third germ layer, multiciliated cells or a simplified through gut, Ctenophora have been suggested to be the closest relative to Bilateria (Nielsen, 1997; Martindale and Henry, 1999). However, recent molecular phylogenetic analyses support the notion that Cnidaria are more closely related to Bilateria than are Ctenophora, and Cnidaria are therefore often considered as the true sister group of Bilateria (Collins, 1998; Kim *et al.*, 1999; Medina *et al.*, 2001; Martindale *et al.*, 2002). Within Cnidaria recent molecular data based on ribosomal

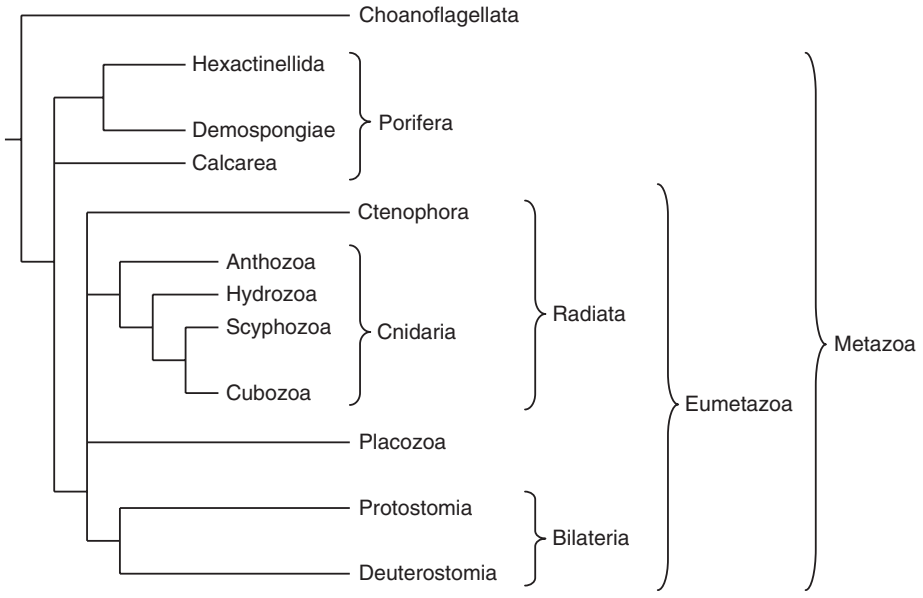

Figure 1 Phylogeny of metazoan animals at the stem of Bilateria. Choanoflagellata have been included as the closest unicellular relatives to the metazoans. The phylogeny is based on widely accepted molecular data and the currently uncertain relationships between the different sponge classes as well as among the potential bilaterian sister groups (Ctenophora, Cnidaria, and Placozoa) have been left open. Terms used in the text for higher classification of animal phyla are indicated on the right-hand side.

DNA sequence analysis and mitochondrial genome organization are in agreement with the view that the Anthozoa, which have only a polyp stage, are basal to the other three classes, Hydrozoa, Scyphozoa, and Cubozoa, which are characterized by an additional medusa stage in their life cycle (Medusozoa; Petersen, 1979).

The Placozoa, represented by a single known species, *Trichoplax adhaerens*, were long believed to be cnidarians with a simple organization as the result of secondary reduction (Bridge *et al.*, 1995). Analysis of molecular data, however, has shown that Placozoa are not derived cnidarians (Ender and Schierwater, 2003). Furthermore, Bilateria and Placozoa may have a more recent common ancestor than either does to Cnidaria (Collins, 2002).

The rapidly increasing amount of molecular data from basal metazoans such as sponges, ctenophorans, cnidarians, placozoans are expected to further clarify the phylogenic relationships among these groups in the coming years. A robust phylogeny based on different sets of molecular data and, importantly, including a large number of representing species for each taxonomic group will be essential to understand early metazoan evolution and, thus, gain more insight into the origin of the first nervous system.

4.2 Historical Concepts and Theories about the Evolutionary Origin of Nervous Systems

4.2.1 The Elementary Nervous System

The cornerstone for studies of the evolution of nervous systems at the cellular level was the application of the cell theory (Schleiden, 1838; Schwann, 1839) to the anatomical units of the nervous system in the neuron doctrine which was put forward by Cajal, Kölliker, Waldeyer, and others at the end of the nineteenth century (reviewed in Shepherd, 1991). Subsequently, with improved anatomical staining methods, it became possible to specifically label nervous structures in basal metazoan organisms. With experimental access to the neurons and nervous systems of basal metazoans, it became conceivable to address the question of which cell lineages originally gave rise to nerve cells and how the first nervous system was organized at the cellular level. Hypothetical considerations were initially based on the conceptual model of an elementary nervous system, defined as "a group of nerve cells with the minimal number of specializations required to perform the basic functions of nervous tissue" (Lentz, 1968). However, Lentz pointed out that this simplified conceptual approach does not necessarily determine the actual characteristics of an evolutionarily early, simple system.

Nerve cells are likely to have arisen in multicellular organisms from epithelial cells that turned out to become able to transduce external information (pressure, light, and chemicals) into chemical and electric signals, and then transmit these signals to neighboring cells (Mackie, 1970; Anderson, 1989). Assuming an epithelial layer of equivalent cells, all having the potential of receiving stimuli and producing some form of effector response, different evolutionary theories on the origin of specialized sensory cells, nerve cells, and muscle cells have been proposed. In the following, a brief historical overview of the most influential theories about the evolution of the first nervous system will be given.

4.2.2 Proposals for the Evolution of the First Nervous System

One of the earliest theories on the origin of the nervous system was that of Kleinenberg (1872), which he based on the discovery of 'neuromuscular cells' in the freshwater hydrozoan *Hydra*. He viewed this cell type as a combination of receptor, conductor, and effector cell. The apical ends of the described cells were exposed on the surface of the epithelium and were believed to act as nervous receptors. Their basal ends were drawn out into muscular extensions and supposedly served as effectors which received signals from the cell bodies. Kleinenberg postulated that comparable 'neuromuscular cells' gave rise to nerve and muscle cells in the course of evolution. In 1878 the Hertwig brothers described sensory cells, ganglionic cells, and muscular cells in Cnidaria, and postulated that each element was differentiated from a separate epithelial cell but still in a physiologically interdependent way (Hertwig and Hertwig, 1878). In contrast to this notion, Claus (1878) and Chun (1880) suggested that nerve and muscle cells arose independently and became associated only secondarily.

The theory of the Hertwigs in which nerve and muscle were thought to have evolved simultaneously was generally accepted until Parker's publication of *The Elementary Nervous System* in 1919. In this influential publication, Parker proposed a succession of three major evolutionary stages in the organization of the neuromuscular system (Figure 2; Parker, 1919). In sponges, which Parker considered as extant representatives of the first evolutionary stage, muscle is present at the absence of nerve cells. This stage is characterized by the appearance of 'independent effectors' such

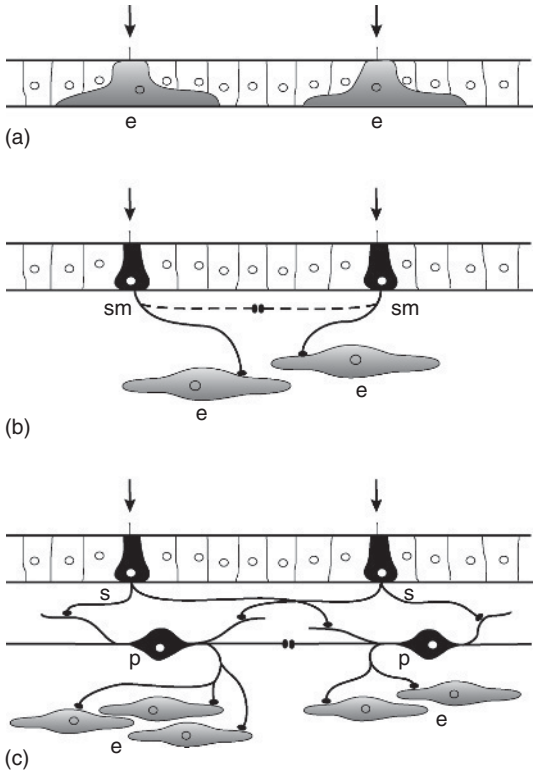

Figure 2 Succession of three evolutionary stages of neuromuscular organization according to Parker (1919). a, Independent effectors. Single contractile effector cells surrounded by epithelial cells are directly stimulated, which leads to a response in the cell. b, Receptor effector system. Sensory motor neurons directly conduct external stimuli to the underlying muscle cells. In a more complex form, sensory motor neurons can be interconnected among each other (dashed lines). c, Nerve net. A second type of neuronal cell termed "protoneuron" by Parker intercalates between the sensory cells and the muscle cells and forms a highly interconnected neuronal network. Parker proposed that nerve cells of higher animals had their origin in protoneurons. e, effector/muscle cell; p, protoneuron; s, sensory cell; sm, sensory-motor neuron. Arrows indicate the site of stimulation.

as the contractile cells of the oscula sphincters in sponges, which respond directly to environmental stimuli. Although sponges lack nerves, Parker pointed out that they do have a slow type of conduction due to elementary protoplasmic transmission, and he suggested that this 'neuroid transmission' might be considered the forerunner of nervous activity. The second stage of evolution was postulated to be a receptor–effector system such as that believed to exist "in the tentacles of many cnidarians" (Parker, 1919). Receptors were thought to arise from epithelial cells that were in close proximity to the already differentiated muscle cells and, in its simplest form, directly connected to the subjacent muscle cells. However, the separate existence of this type of receptor–effector system has never been directly observed and even Parker admitted that this organizational level might frequently be complicated by the fact that receptor cells not only innervate muscle cells but are also interconnected among each other. In the final stage of early nervous system evolution, a third type of cell, termed "protoneuron" by Parker, was intercalated between the sensory and effector cells forming a true nerve net. This stage was thought to be represented by the nerve nets of extant Cnidaria, and Parker suggested that nerve cells of higher animals were derived from this third type of protoneuronal cell. In a nutshell, Parker proposed that the first nervous system evolved as a consequence of the selective advantage obtained by coordinating independent effectors.

In the second half of the twentieth century, a number of alternative theories for the evolutionary origin of the nervous system were put forward. Based on morphological and physiological studies on sea anemone nerve nets, Pantin (1956) proposed that nervous systems functioned from the beginning to coordinate the behavior of the whole animal. He argued that the nervous system did not evolve on the basis of single cells, but rather originated as whole networks innervating multicellular motor units. Only later would specific conducting tracts have become associated with specific reflexes in the nerve net and given rise to the reflex arc, which according to this view, is not primitive. Pantin's major objection to Parker's theory was the lack of evidence for the independent existence of a receptor–effector system. Based on studies of *Hydra* and scyphomedusae, Passano (1963) postulated that the nervous system evolved from specialized pacemaker cells whose function was to generate contractions within groups of protomyocytes from which they derived. In this view, nerve cells would have derived from pacemaker cells, retaining rhythm generation as their primary function, and only later becoming specialized for conduction over long distances and as sensory receptors. Grundfest (1959, 1965) postulated that the ancestral neuron was derived from a secretory cell that developed a conducting segment between its receptive and secretory poles. Accordingly, true neurons were originally formed when the secretory activity became confined to the terminations of the cells' processes. Thus, this theory is based on the notion that secretion is a primitive feature of the nervous system (Figure 3). A few years earlier, Haldane (1954) proposed that signaling by means of neurotransmitters and hormones had its origin in chemical signaling in protists exemplified by the chemical signals involved in the control of conjugation among different mating types in ciliates. Lentz (1968) noted that protists

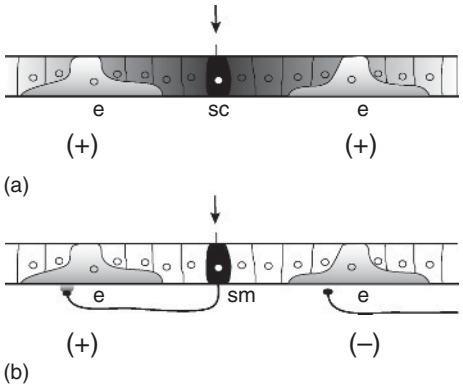

Figure 3 Cell signaling by diffuse secretion preceded synaptic innervation according to Grundfest (1959, 1965). a, Ancestral state. Single cells secrete biologically active substances upon stimulation, which diffuse throughout the epithelium and activate all surrounding effector cells. b, Emergence of neurons. Upon stimulation, sensory neurons specifically activate their target cells by local synaptic release of neurotransmitters. e, effector cell; sc, secretory cell; sm, sensory-motor neuron. Arrows indicate the site of stimulation, (+) stands for an active state and ( ) for an inactive state of the effector cell.

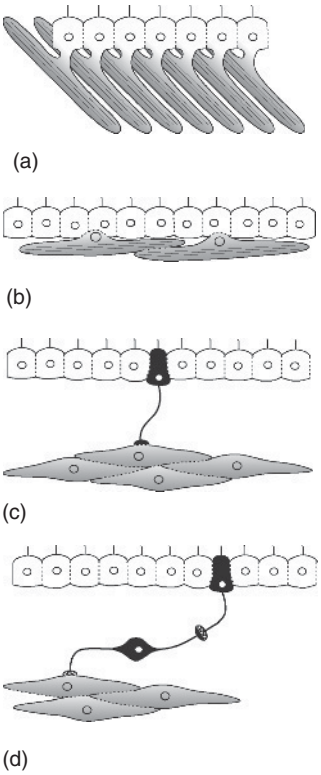

Figure 4 The evolution of nerve and muscle cells from electrically coupled myoepithelial cells according to Mackie (1970). a, Primordial myoepithelium. b, Protomyocytes start to leave the epithelium and move into the interior. c, Protoneurons evolve, conveying excitation to the myocytes from the exterior. All cells are still shown as electrically coupled. d, Neurosensory cells and neurons evolve. They are connected to one another and to the myocytes by chemically transmitting, polarized synapses. Electrical coupling persists in many epithelia and muscles. However, conduction of impulses becomes increasingly a property of the nervous system. Dashed lines at junctions between cells indicate low resistance pathways through which electrical currents can flow. Modified from Mackie, G. O. 1970. Neuroid conduction and the evolution of conducting tissues. *Q. Rev. Biol.* 45, 319 332, Copyright 1970, The University of Chicago Press.

as well as many non-nervous cells have excitable and conductile properties and, furthermore, that 'neurohumors' occur in protists, indicating that these substances could have evolved before the appearance of neurons. He therefore suggested "that the nerve cell arose by the coupling of electrical activity with secretion of biologically active substances so that a chain of events in response to stimuli resulted in alteration of effector activity." In contrast to Grundfest's proposal that the ancestral neuron was a secretory cell which developed specialized receptive surfaces and a conductile intermediate component, Lentz proposed that both neuronal functions evolved simultaneously.

Horridge (1968) and Mackie (1970) described excitable epithelia in hydromedusae and siphonophores, which conduct action potentials and serve as pathways mediating certain types of behavior. Based on this discovery, they proposed that nerves evolved from tissue whose cells were already interconnected by pathways for metabolic exchange and electrical current flow, thus making cell-to-cell propagation of action potentials possible. According to Horridge, the primary function of neurons was neurosecretory or growth regulatory and only later did their elongated axons become effective in impulse propagation. Nerve cells, with their elongated form and functional isolation from surrounding tissues, would have arisen in response to a need for a more selective type of excitation within conductile epithelia in which effector subgroups could be controlled independently (Horridge, 1968). Mackie proposed that the starting point for a metazoan nervous conducting system resembled a myoepithelial tissue sheet in coelenterates. The cells in the tissue capable of reception, transmission, and contraction were connected by cytoplasmic pathways, which also served metabolic exchange among the cells (Figure 4). Specialized muscle cells arose by segregation from the primordial epithelium, whereas cells that lost their contractile component but retained their conducting ability gave rise to nerve cells (Mackie, 1970). Westfall propagated the idea that receptive, electrogenic, and neurosecretory functions co-evolved in primitive protoneurons. This proposal was based on his demonstration with

electron microscopic resolution that nerve cells in *Hydra* not only have receptor poles with a sensory cilium and basal neurites making synaptic contact with effectors but also contain neurosecretory material (Westfall, 1973; Westfall and Kinnamon, 1978). He further proposed that specialized neurons found in modern higher animals derived from multifunctional neuronal ancestors comparable to those found in *Hydra* (e.g., Grimmelikhuijzen, 1996).

In more recent studies, Seipel *et al.* (2004), working on the development of the hydrozoan *Podocoryne carnea*, have found molecular evidence supporting the hypothesis that muscle and nerve cells derive from a common myoepithelial precursor. In bilaterian animals, neuronal determination and differentiation is controlled by genes encoding basic-helix-loop-helix (bHLH) transcription factors and among these are the genes of the Atonal gene family (reviewed in Lee, 1997; Dambly-Chaudiere and Vervoort, 1998). In *Podocoryne*, the cnidarian Atonal-like 1 (Atl1) gene is expressed in a subset of nerve cell precursors of the medusa and additionally in developing striated muscle cells. Simlarly, the neuronal marker gene coding for the cnidarian RFamide neuropeptide is expressed not only in mature nerve cells but also transiently in the developing muscle of *Podocoryne* (Seipel *et al.*, 2004). Based on these developmental genetic similarities, the authors propose that nerve and muscle cells are likely to have been linked closely in evolution and share a common ancestor. In contrast, Miljkovic-Licina *et al.* (2004) studying regulatory genes involved in differentiation of neuronal cell lineages in *Hydra* have proposed a scenario in which mechanoreceptor cells would have preceded neuronal cell types in evolution. Their work shows that the nematocyte and neuronal cell differentiation pathways share regulatory genes that exhibit a high level of conservation during metazoan evolution (Miljkovic-Licina *et al.*, 2004). Nematocytes can sense chemical and mechanical stimuli, transduce these signals, and react to them through nematocyst discharge. The authors propose that this type of fast and cell-autonomous response was a hallmark of very primitive nerve cells and that nematocytes were a derived cnidarian byproduct of these ancestral 'neuro-epithelial' cells. In subsequent evolutionary steps, the 'neuro-epithelial' cells could have differentiated into neuronal cells with elongated processes that began to establish connections with myoepithelial cells and involve them in the response to the stimulus. During later stages, neuronal cells would have become progressively more interconnected with each other in a nervous system allowing coordinated behavior.

In summary, a variety of alternative theories implying different origins of the nervous system have been suggested in the last 150 years. Most of these theories are based on extrapolations of observations made on extant protists, sponges, and cnidarians. The origin of neurons is generally attributed to epithelial cells; however, the characteristics of these ancestral cells are variously considered to have been contractile, neurosecretory, conductile, chemoreceptive, or mechanoreceptive, and each theory emphasizes one or several of these features as being driving force for the evolution of the nervous system. While many of these proposals appear plausible and inspiring for further discussion, it seems impossible to rate one of the theories as more relevant than the others. However, all of the proposed scenarios for the evolution of the nervous system do focus attention on the cell biology of excitable cells in the basal animal groups, and this focus will be explored in more depth in the following pages.

4.3 Origin of the First Nervous System: A Comparative Phylogenetic Approach

4.3.1 Introduction

Although the nervous system must have arisen in a multicellular organism, unicellular organisms such as protists show a variety of behavioral programs in response to their environment. In protists, behavioral responses to external stimuli are achieved at a subcellular level by organelles specialized for signal reception, signal conduction, and effector response (Deitmer, 1989; Febvre-Chevalier *et al.*, 1989; Hennessey, 1989). Thus, molecular machineries capable of reception of chemical, mechanical, or light stimuli, secretion of biologically active substances, propagation of electrical potentials along membranes, and conversion of stimuli into effector responses were probably already present in the ancestor of metazoans. Assuming colonial protists with equivalent cells as an intermediate form between unicellular protists and early metazoans, an increasing specialization of subgroups of cells must have occurred during evolution. Porifera represent the most basal extant metazoan phylum and are thought to have derived from a colonial form of choanoflagellates. Although a variety of different cell types can be found in sponges, no nerve cells could be identified so far (Jones, 1962; Pavans de Ceccatty, 1974; Mackie, 1979). Nevertheless, contractile cells encircling the oscular openings in sponges are able to react upon mechanical stimulation. In cnidarians and ctenophores, the closest metazoan relatives of sponges,

nerve cells are present and can form sophisticated nervous systems capable of solving complex behavioral tasks. This evolutionary step from poriferan to cnidarian or ctenophoran organization may harbor the emergence of nerve cells and nervous systems.

4.3.2 Non-Nervous Conduction Outside of the Animal Kingdom

Many key characteristics of nerve cells can be found in non-nervous cells of metazoans, plants, fungi as well as in unicellular organisms like protists and even prokaryotic bacteria. These characteristics include reception and transmission of signals to other cells, intercellular communication by secretion of biologically active substances, and the propagation of electrical potentials. Nevertheless, the combined appearance of these features in morphologically and functionally specialized nerve cells is unique to the nervous systems of metazoan animals.

Ion channels, which can be gated by ligands, voltage, or mechanical forces and are permeable to specific ions, such as K^+, Ca^{2+}, Na^+, and Cl , play a major role in the generation of neuronal excitability in higher animals. Moreover, ionic fluxes across cellular membranes mediate a great variety of biological processes that are essential for viability of most life forms. A large number of genes presumably coding for ion channels have been identified in prokaryotes, but although structural or electrophysiological information has been obtained for some of these proteins, their biological roles are mostly unknown. Presumably, prokaryote channels are involved in metabolic function, osmoregulation, and motility (Ranganathan, 1994; Kung and Blount, 2004). In the bacterium *Escherichia coli*, genome sequencing suggests the presence of six putative mechanosensitive channels, one putative voltage-gated K^+ channel, and two Cl channel-like structures. Three of the mechanically gated channels are involved in osmoregulation and release solutes upon osmotic down-shock, whereas Cl channels apparently function in short-term acid tolerance. Although, the function of the K^+ channel is still unknown, its protein shares extensive topological and structural similarity with eukaryotic K^+ channels suggesting a common ancestral origin from which K^+ and later probably Ca^{2+} and Na^+ channels evolved (Milkman, 1994; Ranganathan, 1994; Kung and Blount, 2004). Voltage-dependent and stretch-activated ion channels have been found in the plasma membrane of yeast (Gustin *et al.*, 1986, 1988; Zhou *et al.*, 1995). In addition, the yeast genes involved in the pheromone response show high similarity to signal transduction genes of higher animals. For example, the mating factor receptor STE2 of *Saccharomyces cerevisiae* belongs to the rhodopsin/beta-adrenergic receptor gene family (Marsh and Herskowitz, 1988), and the alpha-type mating factor shows amino acid sequence similarities with the vertebrate reproductive gonadotropin-releasing hormone (Loumaye *et al.*, 1982).

In addition to the transmission of information through substrate flux, plants have electrical and hormonal signaling systems. Action potentials in plants were described for the first time in 1873 by Burdon-Sanderson. He recorded electrical signals from a specimen of the Venus's flytrap, *Dionaea muscipula*, which he received from Charles Darwin (Burdon-Sanderson, 1873; Sibaoka, 1966). The leaves of *Dionaea* are divided into two lobes, each of which carries three tactile sense hairs functioning as trigger for an all-or-nothing electrical signal that is followed by the fast closing of the lobes entrapping the prey. In plants like the Venus's flytrap, action potentials are part of a signaling system that responds to mechanical stimulation by changing cell turgor, which leads to relatively rapid movements. Propagation of action potentials from the site of stimulation to the effector cells has been studied in the seismonastic movements of the leaves of *Mimosa pudica* (Sibaoka, 1966; Simons, 1992). Non-nervous electrical conduction in plants involves low-resistance pathways (plasmodesmata) between the phloem cells, comparable with gap junctions that electrically couple cells in excitable epithelia and muscles in animals. Action potentials in plants have been studied in detail in the giant internodial cells of the freshwater algae *Chara* and *Nitella*. In these large cells, a motility system based on actin and myosin drives cytoplasmic streaming, which serves to equally distribute organelles and nutrients around the central vacuole. Upon mechanical or electrical stimulation, an action potential is generated, which spreads in both directions along the shoot and immediately stops the cytoplasmic streaming probably to avoid leakage of the cell in case of injury. In contrast to the action potentials of higher animals where the influx of Na^+ and Ca^{2+} supports the depolarizing phase, in *Chara* and *Nitella* Ca^{2+} and Cl are the key components of depolarization, a situation which is typical for plant action potentials. A fast initial influx of Ca^{2+} ions is followed by the efflux of Cl through Ca^{2+} activated Cl channels across the vacuolar and plasma membranes. The falling phase of the action potential is due to an increase in K^+ permeability,

similarly to what occurs in nervous cells of higher animals (Sibaoka, 1966; Simons, 1992; Wayne, 1994; Kikuyama, 2001). Although molecules that act as neurotransmitters in higher animals such as glycine, GABA, glutamate, and acetylcholine have been isolated from plants, no chemical transmission of electrical signals between cells of plants has been observed. Rather, these substances are involved in a variety of functions related to metabolism, circadian rhythm, or light response of plants (Simons, 1992; Mackie, 1990; Hille, 1984).

A number of neuroactive substances including adrenalin, noradrenalin, 5-HT, DOPA, dopamine, and beta-endorphin as well as receptors for acetylcholine, catecholamines, and opiates have been reported in protists (Zipser *et al.*, 1988; Carr *et al.*, 1989; Görtz *et al.*, 1999). Furthermore, receptor tyrosine kinase genes, known to be involved in cell–cell signaling in metazoans, have been recently isolated from choanoflagellates, suggesting that this family of signal receptor molecules evolved before the origin of multicellular animals (King and Carroll, 2001; Brooke and Holland, 2003; King *et al.*, 2003). Some protists can respond to mechanical stimulation with depolarizing or hyperpolarizing membrane potentials. Their membranes are equipped with ion channels gated mechanically, or by ligand or voltage, and in some cases, action potentials are elicited when the cell membrane is depolarized up to a threshold level by receptor potentials. In most protists, Ca^{2+} ions are responsible for carrying ionic currents and coupling membrane excitation to motile response or contractile activity (Febvre-Chevalier *et al.*, 1989). In some ciliates, ion channels are not distributed uniformly over the cell membrane; this is reminiscent of neuronal cell membranes that have distinct channel populations in dendrites, soma, axon, and presynaptic terminals. For example, in *Paramecium* and *Stylonychia*, different ion channels can be found at the front and back poles of the cell generating different ion currents, which lead to opposed escape behaviors away from the source of mechanical stimulation (Kung, 1989; Deitmer, 1989; Kung and Blount, 2004). Behavioral responses in protists elicited by action potentials often involve changes of cell shape or alterations in the pattern of ciliary or flagellar beating (Febvre-Chevalier *et al.*, 1989; Hennessey, 1989). The complexity of effector responses driven by different types of electrical potentials within a unicellular organism is nicely illustrated by the dinoflagellate *Noctiluca*. Two different kinds of flagellar movements and a bioluminescent light response are controlled through different action potentials involving different ion currents across the cytoplasmic and vacuolar membrane. In this manner, multiple bioelectric activities in *Noctiluca* are able to control altered effector responses within a single cell (Oami, 2004). Thus, in the absence of a nervous system, protists exhibit complex behaviors which incorporate features of sensory receptors and effectors into a single, highly structured eukaryotic cell.

4.3.3 Porifera: Specialized Cells and Electrical Conduction

Sponges, the most basal extant metazoans, probably evolved from a colonial choanoflagellate. At this stage of phylogeny a number of specialized cell types including muscle-like contractile cells has made its appearance, however, nerve cells are lacking (Jones, 1962; Pavans de Ceccatty, 1974; Mackie, 1979). Some of the actin-containing contractile cells (myocytes) are concentrated as sphincters around the osculum and pore canals of sponges. To contract, the sphincters have to be directly stimulated and they thus represent "independent effectors" as proposed by Parker (1919). Slow contractile responses that spread over short distances have been described in several sponge species, but the responsible cells do not seem to be electrically excitable, and there is no evidence of associated changes in membrane potentials (Mackie, 1979). Thus, some form of mechanical interaction between neighboring cells seems likely. The sponge epithelial cells that build the external and internal boundary of the mesenchyme are not joined together with occluding junctions and, therefore, the internal milieu may not be very well isolated from the external. Nevertheless, the mesenchyme provides an environment in which electrical and chemical gradients could be generated and nutrients and hormones diffuse without excessive leakage through the body wall (Mackie, 1990). Acetylcholinesterase, catecholamines, and serotonin have been shown, by histochemical techniques, to be present in sponges (Lentz, 1968). Further, some neuroactive substances have been demonstrated to influence the water circulation in the sponge *Cliona celata* (Emson, 1966), but so far there is no clear evidence that they are involved in intercellular signaling processes. Interestingly, a recent finding has shown that cells isolated from the marine sponge *Geodia cydonium* (Demospongiae) react to the excitatory amino acid glutamate with an increase in intracellular calcium concentration (Perovic *et al.*, 1999). Extracellular agonists as well as antagonists known from metabotropic glutamate/GABA-like receptors in mammalian nerve cells were found to

elicit similar effects in these sponge cells. In addition, a cDNA coding for a seven-transmembrane receptor was isolated from *Geodia*, which has high sequence similarity to metabotropic glutamate/GABA-like receptors in mammals. Although these findings suggest that Porifera possess a sophisticated intercellular communication and signaling system, so far there is no evidence for the type of specialized intercellular signal transmission in sponges that might foreshadow the evolutionary origin of nervous systems.

The tissue of glass sponges (Hexactinellida) is syncytial, allowing the rapid propagation of electrical events, which is a fundamental difference between this class and the other two cellular sponge classes, Demospongiae and Calcarea (Müller, 2001). All-or-nothing electrical impulses were recorded from the glass sponge *Rhabdocalyptus dawsoni*. Tactile and electrical stimuli evoke impulses, which lead to the abrupt arrest of water flow through the body wall, presumably due to the coordinated cessation of beating of the flagella in the flagellated chambers. From the superficial pinacoderm, impulses are conducted through the trabecular reticulum, a multinucleate syncytial tissue draped around the spicules of the sponge skeleton, to the flagellated chambers. Impulses are propagated diffusely at $0.27 \pm 0.1\,\mathrm{cm\,s^{-1}}$, a value that falls within the lower range of action potential conduction velocities in non-nervous tissues. It is assumed that signal propagation through the syncytium depends on Ca^{2+} influx and that Ca^{2+} channels may also mediate the flagellar arrest (Leys *et al.*, 1999). The trabecular syncytium seems to be a derived feature specific to the most ancient sponge class Hexactinellida. Since calcareous sponges and demosponges lack comparable syncytial tissue, they would require low-resistance pathways equivalent to eumetazoan gap junctions to conduct electrical signals from cell to cell, but no similar structures have been found so far (Leys *et al.*, 1999; Müller, 2001).

Larvae of many sponge species exhibit rapid responses to external stimuli including light, gravity, and current (reviewed in Wapstra and van Soest, 1987). Demosponge larvae have a spheroid body shape and consist of an outer epithelial layer of monociliated cells and a solid center of amoeboid cells in an extracellular matrix of collagen. The spheroid-shaped body is polarized anteroposteriorly with respect to the swimming movement of the larvae, and a ring of pigmented cells that gives rise to long cilia is located at the posterior end. In the demosponge *Reneira*, directional swimming is mediated by the long cilia of the posterior pigmented cells and incorporates an asymmetric response of these cells to different light intensities (Figure 5). Increased light intensity causes a bending of the cilia such that they shield the pigment vesicles, whereas decreased light intensity reverses this process. This results in steering the larva away from bright light (Leys and Degnan, 2001). Interestingly, re-analysis of the action spectrum of the ciliary response to light reveals that the photoreceptive pigment in the sponge larva has the characteristics of rhodopsin, similar to the situation in other metazoans that have a rhodopsin-like protein as their primary photoreceptive pigment (Leys *et al.*, 2002; Leys and Meech, 2006). In *Reneira* the light response of the posterior cells has been suggested to depend on the depolarization of the membrane potential and the influx of Ca^{2+} into the cilium. Since sponge larvae lack neurons or gap junctions that would allow coordination of signals among cells with long cilia, each posterior cell appears to respond independently to changes in light intensity. On the other hand, no intercellular coordination seems to be required, given the inherent photokinetic responses of each ciliated cell depending on its

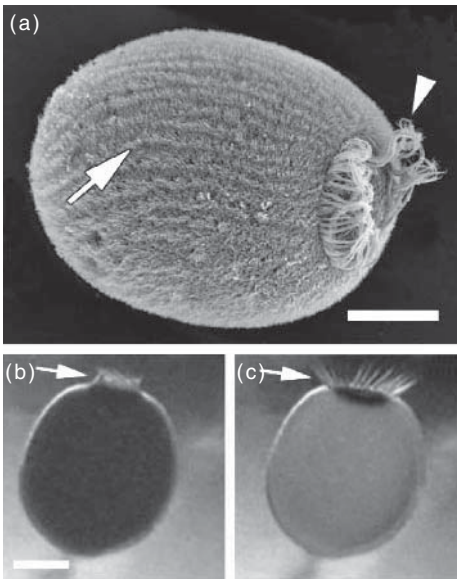

Figure 5 Photosensitive cells and ciliary light response of the sponge *Reneira* larva. a, Scanning electron micrograph showing the structure of the demosponge larva. Monociliated epithelial cells form most of the outer layer (arrow). The posterior pole is circumscribed by a ring of long cilia (arrowhead). Video recording of bending (b) and straightening (c) of the long posterior cilia (arrows) in response to shutting and opening of a shutter in front of the light source. Scale bars: 100 μm. Reproduced from *J. Comp. Physiol. A*, vol. 188, 2002, pp. 199 202, Spectral sensitivity in a sponge larva, Leys, S. P., Cronin, T. W., Degnan, B. M., and Marshall, J. N., figures 1a and 2b (I + II). With kind permission of Springer Science and Business Media.

position relative to the light source (Leys and Degnan, 2001). Therefore, in some cases 'independent effectors' in sponges may mediate coordinated behavior. Although sponges emerged at an early level in multicellular animal evolution when nervous systems had not yet evolved, they do represent the oldest extant metazoans with specialized cells responding to different stimuli and performing behavioral tasks.

4.3.4 Ctenophora and Cnidaria: The Oldest Extant Nervous Systems

Ctenophora and Cnidaria are the lowest animal phyla that have a nervous system. The two phyla were traditionally joined together in one group, termed Coelenterata, based on the presence of a single gastrovascular system serving both nutrient supply and gas exchange among the body parts. Molecular phylogenetic data, however, suggest an independent origin of the two phyla in the prebilaterian line, and their relative position in early metazoan phylogeny is controversial (Martindale and Henry, 1999; Medina *et al.*, 2001; Podar *et al.*, 2001; Ball *et al.*, 2004). Whereas most molecular data support the more basal position of ctenophores with cnidarians forming the sister group to bilaterians, other evidence, including the presence of true subepithelial muscles and multiciliated cells, supports the view that ctenophores are more closely related to bilaterians than cnidarians (Nielsen, 1997). Thus, it is presently not clear whether Ctenophora or Cnidaria are the closest extant metazoan relatives of Porifera. Nevertheless, it is likely that the first nervous system evolved at the evolutionary step from Porifera to either of the two coelenterate phyla.

Ctenophores are medusoid gelatinous animals, which generally have two tentacles for capturing prey and eight ciliary comb rows on their outer surface for locomotion. The nervous systems of ctenophores are organized into diffuse nerve nets, which show some local tract-like accumulations below the ciliary comb rows and around the mouth and pharynx. At the ultrastructural level, polarized as well as symmetrical chemical synapses have been shown to be present in these nerve nets. Sensory nerve cells are interspersed among the epithelial cells, except at the aboral pole where sensory and nerve cells constitute, together with a statocyst, the apical organ. Locomotory movements of ctenophores involve metachronal beating of eight comb plate rows radiating from the aboral region. The apical organ serves as pacemaker of the comb plate rows and coordinates geotactic responses (Satterlie and Spencer, 1987). Transmission of ciliary activity among comb plate cells is non-nervous by mechanical coupling (Tamm, 1982). In addition, comb cells are electrically coupled through gap junctions, probably allowing the synchronous response of neighboring cells to modulatory synaptic input (Hernandez-Nicaise *et al.*, 1989). In *Pleurobrachia* different inhibitory and excitatory pathways coordinate the electromotor behavior of comb plate cells with tentacle movements during prey capture and ingestion (Moss and Tamm, 1993). In their basic elements the ctenophoran nervous systems already share many features with nervous systems of higher animals, thus allowing well-coordinated behavioral programs in a basal metazoan animal.

4.3.5 Cnidarian Nervous Systems: Multiple Levels of Organization

It is often assumed that nervous systems probably evolved first in Cnidaria or a closely related ancestor, and their nervous systems are, thus, often considered to be among the simplest forms and reflect an early stage of evolution. This view prevailed until few decades ago and is still present in many textbooks (Brusca and Brusca, 1990; Ruppert and Barnes, 1994). However, cnidarians have been evolving independently for some 600–630 million years, and have therefore had plenty of time to develop sophisticated solutions for comparable behavioral tasks and under similar conditions as have many higher animals. During this long evolutionary time period, a wide spectrum in nervous system complexity emerged within the cnidarian phylum, ranging from the diffuse nerve nets of sessile polypoid species to the multiple ring-shaped nerve tracts, giant axons, and highly specialized sensory organs in actively swimming medusoid species. Thus, in some cases, the complexity of nervous systems in modern cnidarians may reflect more the behavior tasks of the species considered than any ancestral organization. Many physiological and structural solutions found exclusively in the nervous systems of cnidarians deal with the problem of generating coordinated behavior in a radially symmetrical animal (Mackie, 1990). Ring-shaped nerve nets or diffuse epithelial conduction may, therefore, represent adequate systems for specific behavioral functions rather than remnants of a primitive nervous system. Nevertheless, many basic features of bilaterian nervous systems can be found in cnidarian nervous systems and consequently are likely to have been present in their common ancestors in which the first nervous system probably evolved. These features, which have been the subject

of considerable research, are considered in more detail below.

Different levels of nervous system organization are encountered in the phylum Cnidaria and often even in the same animal. The spectrum of levels ranges from independent effector cells, as already found in sponges, to the first trends of centralization of integrative and coordinative functions in the nerve rings of some medusae (Bullock and Horridge, 1965; Mackie, 2004). In many aspects, the cnidarian nematocytes can be considered as 'independent effectors' (Miljkovic-Licina *et al.*, 2004). Nematocytes are mechanoreceptor cells found in the ectodermal tissue of cnidarian tentacles that discharge the toxic content of a highly specialized capsule named the cnidocyst upon contact with the prey. Although most nematocytes are innervated, they are still able to discharge in the absence of nerve cells (Aerne *et al.*, 1991) and thus respond to direct stimulation. Another example of an 'independent effector' in cnidarians are the photoreceptor cells of the cubozoan *Tripedalia* planula. These unicellular photoreceptors contain the photoreceptor and shielding pigment granules within the same cell, which in addition carries a motor cilium that enables the larva to perform phototactic behavior. Ultrastructural analysis further reveals that there is no nervous system to which these photosensitive cells transmit visual information. These cells are thus self-contained sensory-motor entities that respond directly without a coordinating nervous system (Nordström *et al.*, 2003). The unicellular photoreceptors of the *Tripedalia* larva represents an interesting parallel to the photosensitive ciliated cells of sponge larvae in that each cell has a well-developed motor-cilium, which directly responds to light stimulation (Leys and Degnan, 2001; Leys *et al.*, 2002; Nordström *et al.*, 2003). However, since no similar autonomous photosensory motor cells have been described in more basal cnidarian larvae, the homology of these two structures can be most likely excluded.

Excitable epithelia are another non-nervous element involved in signal conduction that can be found in Cnidaria side by side with highly specialized nervous conduction pathways. Excitable epithelia are present in the endodermal radial canals of hydrozoan medusae where they conduct signals involved in motor control of behavioral responses such as 'crumpling' (protective involution), feeding, or swimming. In the pelagic jellyfish *Aglantha*, this epithelial pathway is preserved despite the presence of a highly complex nervous system consisting of several neuronal conduction systems that include diffuse nerve nets, nerve rings, and giant axons (Mackie, 2004). Thus, relatively slow, non-nervous signal conduction of the type known from sponges and even plants can offer alternative pathways in parallel to highly specific, fast nervous conduction. Epithelial conduction consisting of electrically coupled equivalent cells, from which more specific pathways evolved with the emergence of elongated nerve cells, has been proposed as a characteristic of the hypothetical metazoan ancestor (Horridge, 1968; Mackie, 1970). Whether epithelial conduction is indeed an ancient feature or rather arose several times during evolution is unclear. Nevertheless, this mode of conduction can be found throughout the animal kingdom, from ctenophores to the early tadpole larvae of amphibians (Roberts, 1969; Mackie, 1970).

A diffuse, two-dimensional nerve net formed by bi- or multipolar neurons is considered to be a simple form of nervous system organization. A classical example of this simple type of neural ground plan is found in *Hydra*. This cnidarian has a network of multifunctional nerve cells, which combine sensory and motor tasks and have processes that conduct impulses bidirectionally. Traditionally, the nervous system of *Hydra* has been illustrated with a simple meshwork of equally spaced neurons, as it is still the case in many textbooks (e.g., Brusca and Brusca, 1990). However, detailed neuroanatomical analysis of the *Hydra oligactis* nerve net shows that its neurons are not equally distributed throughout the polyp body wall but rather form a ring-shaped area between tentacles and mouth opening and local concentrations in the peduncle suggesting a level of regional specialization (Figure 6a; Grimmelikhuijzen and Graff, 1985). Furthermore, distinct neuronal subsets can be distinguished morphologically or neurocytochemically based on neuropeptide expression (Grimmelikhuijzen *et al.*, 1996). In *Hydra*, new nerve cells are constantly generated by interstitial cells in a specific zone of the polyp body column and migrate toward the body extremities where old nerve cells are lost. As they migrate, nerve cells can undergo morphological and neurochemical transformations and give rise to the different neuronal subsets (Bode *et al.*, 1988; Grimmelikhuijzen *et al.*, 1996). In addition to their roles in behavior, nerve cells in *Hydra* are directly involved in the regulation of growth and in the production of chemical morphogenetic gradients (Schaller *et al.*, 1996). Thus, the nervous system of *Hydra* is not a simple, diffuse meshwork of interconnected nerve cells and it is unlikely to represent an ancestral situation within the Cnidaria. In the sea pansy, *Renilla koellikeri*, belonging to the phylogenetically basal cnidarian class of Anthozoa, the

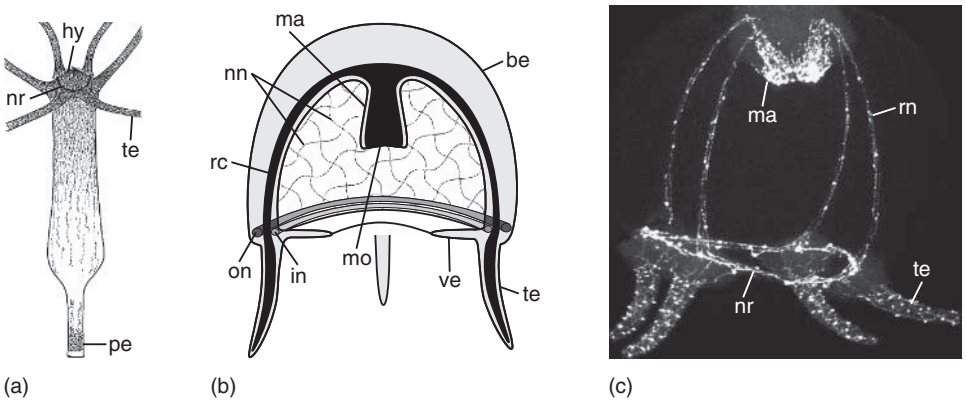

Figure 6 Nervous system organization of hydrozoan polyps and medusae. a, Drawing showing the RFamide-positive nervous system in *Hydra oligactis*. This species has a dense plexus of immunoreactive neurites in the hypostome and a nerve ring between hypostome and tentacle bases. A collar of neurons can be found in the peduncle. b, Nerve net and nerve rings in a hydromedusa. Nerve nets underlying the ectodermal and endodermal tissues span the inner surface of the bell. An inner and an outer nerve ring encircle the bell near the margin. These nerve rings connect with fibers innervating the tentacles, muscles, and sensory organs. c, Fluorescent RFamide staining of the hydromedusa *Podocoryne carnea*. Nerve cells expressing RFamide can be detected in the nerve ring around the margin of the bell and the radial nerves which line the four radial canals. In addition many RFamide positive cells are found around the mouth opening at the tip of the manubrium and scattered over the surface of the tentacles. be, bell; hy, hypostome; in, inner nerve ring; ma, manubrium; mo, mouth; nn, nerve net; nr, nerve ring; on, outer nerve ring; pe, peduncle; rc, radial canal; rn, radial nerve; te, tentacle; ve, velum. a, Reproduced from *Cell Tissue Res*., vol. 241, 1985, pp. 171 182, Antisera to the sequence Arg-Phe-amide visualize neuronal centralization in hydroid polyps, Grimmelikhuijzen, C. J. P., figure 9b. With kind permission of Springer Science and Business media. c, Courtesy of V. Schmid.

nervous system is also found to consist of multiple interconnected nerve nets with local concentrations at specific organs involved in feeding or reproduction (Pernet *et al.*, 2004; Umbriaco *et al.*, 1990). Indeed, it appears that the simplest form of nervous system organization found in extant cnidarians is that of multiple interconnected nerve nets formed by different neuronal subtypes and showing local concentrations.

An important feature of nerve nets is diffuse conduction, characterized by the spreading of an impulse in all directions from the site of stimulation. Symmetric synapses are frequently seen in cnidarian nerve nets, especially in Scyphomedusae, where they can transmit excitation bidirectionally (Anderson and Spencer, 1989). Although, bidirectionality can often account for diffuse conduction, symmetrical synapses are apparently not an absolute requirement for this and diffuse conduction can also be obtained by the distributed arrangement of many unidirectional pathways (Bullock and Horridge, 1965). Asymmetrical as well as symmetrical chemical synapses have been identified in all cnidarian classes, whereas electrical synapses have been demonstrated only in hydrozoans by electrical and dye coupling and by the presence of conventional gap junctions (Anderson and Mackie, 1977; Spencer and Satterlie, 1980; Westfall *et al.*, 1980). In the multiple nerve net system of hydrozoans, neurons belonging to the same nerve net are generally electrically coupled by gap junctions or even represent true syncytia, whereas chemical synapses are restricted to the interfaces between different nerve nets or utilized for excitation of epithelia, including myoepithelia (Satterlie and Spencer, 1987; Mackie, 2004). The restriction of gap junctions within the phylum Cnidaria to Hydrozoa raises the question of whether electrical signaling between neighboring cells via gap junctions could have preceded the evolution of true nervous conduction. If gap junctions evolved before neurons did, the ancestors of Anthozoa and Scyphomedusae must have independently lost their gap junctions secondarily during evolution, which is rather unlikely. Alternatively, gap junctions arose *de novo* in the ancestor of Hydrozoa after nervous cells had already evolved (Mackie, 1990).

Cnidarian nerve rings and nerve tracts have been proposed to correspond to 'compressed nerve nets' (Spencer and Schwab, 1982), although nerves consisting of parallel axon bundles, which are not interconnected by synapses have also been described (Mackie, 2004). A nerve ring, which has been taken as a simple example of neuronal centralization in Cnidaria, is located near the oral pole of the polyp *Hydra oligactis* (Figure 6a; Grimmelikhuijzen and Graff, 1985). Even more obvious is the presence of nerve rings in medusae at the margin of the bell (Figures 6b and 6c). These nerve rings are integrative centers, where different peripheral pathways

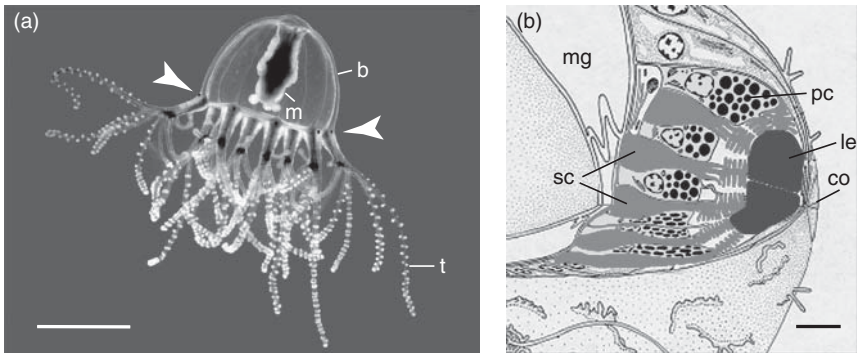

Figure 7 Photosensitive organs in the hydromedusa *Cladonema radiatum*. a, Photograph of an adult medusa with lens eyes located at the base of the tentacles at the margin of the bell (arrowheads). b, Structure of the lens eye in a schematic cross section. The lens eye consists of sensory cells, pigment cells, and a tripartite lens that is covered by a cornea. b, bell; co, cornea; le, lens; m, manubrium; mg, mesogloea; pc, pigment cell; sc, sensory cell; t, tentacle. Scale bar: 700 μm (a); 10 μm (b). Reproduced from Stierwald, M., Yanze, N., Bamert, R. P., Kammermeier, L., and Schmid, V. 2004. The sine oculis/six class family of homeobox genes in jellyfish with and without eyes: Development and eye regeneration. *Dev. Biol.* 274(1), 70 81, with permission from Elsevier.

from sensory organs converge and where activity patterns that result in coordinated behavior are generated. A further striking example is found in the two marginal nerve rings of *Aglantha*, a pelagic hydrozoan medusa. In these interconnected nerve rings, information from 14 conduction systems, including multiple nerve nets, giant axons, and two epithelial pathways, are processed and result in the generation of complex behavioral patterns (Mackie, 2004). A ring-shaped central nervous system (CNS) has been proposed to be appropriate for a radially symmetrical organism, where the term 'central' is not meant morphologically but rather in terms of the functions carried on within it (Spencer and Arkett, 1984; Mackie, 1990). Cnidarian nerve rings may therefore represent the first integrating concentrations of nervous tissue in the animal kingdom (Bullock and Horridge, 1965).

Ganglionic centers, which contain a variety of sensory structures including statocysts, ocelli, or even, lens eyes, can be found spaced around the bell margin at the base of the tentacles of many medusae (Figure 7a). The occurrence of photosensitive structures in Cnidaria includes a wide range of complexity and specializations. The sessile polyps of all cnidarian classes respond to light (Tardent and Frei, 1969) but until now no photoreceptive structures or specialized cells for light detection have been identified in polyps. The free-swimming medusa stage, however, can have differentiated photoreceptor organs, which range from simple ocelli to highly evolved lens eyes (Figure 7b; Land and Fernald, 1992; Stierwald *et al.*, 2004; Piatigorsky and Kozmik, 2004; Gehring, 2005). The diversity of photosensitive structures is illustrated by the cubozoan *Tripedalia cystophora*, where the planula develops unicellular photoreceptors scattered over the posterior epidermis of the larva, whereas the adult jellyfish forms elaborate multicellular lens eyes (Nordström *et al.*, 2003).

The presence of giant axons is another feature of nervous systems that is common to cnidarians and higher invertebrates. Giant axons are distinguishable from normal axons by their large diameter and relatively high speed of signal conduction. Indeed, the first intracellular neuronal recordings in Cnidaria have been carried out from the giant axons in the stem of the siphonophoran *Nanomia*, a colonial hydrozoan (Mackie, 1973). Giant axons may have evolved independently in different cnidarian groups, most probably by axonal fusion within nerve nets or endomitotic polyploidy (Mackie, 1989). In the hydromedusa *Aglantha*, several giant axons have been shown to be involved in rapid escape behavior. Interestingly, motor giant axons of *Aglantha*, which synapse onto swimming muscles, can conduct two types of action potentials. Rapidly conducted Na^+-dependent action potentials result in fast swimming associated with escape behavior, whereas slow swimming movements depend on low-amplitude Ca^{2+} action potentials. Thus, two kinds of impulse propagation within the same giant axon subserve different behavioral responses in *Aglantha* (Mackie and Meech, 1985), showing that structural simplicity does not allow inference of functional simplicity in Cnidaria.

4.3.6 Cnidarian Nervous Systems: Ion Channels and Neuroactive Substances

Cnidarian nervous systems have electrophysiological properties which are similar to those of higher animals. Neurons exhibit conventional action

potentials with Na^+ inward currents and K^+ outward currents, miniature end-plate potentials, Ca^{2+}-dependent quantal transmitter release, and with spatial and temporal synaptic summation and facilitation (Spencer, 1989). Typical four-domain Na^+ channels are found in Cnidaria, although these channels are not tetrodotoxin sensitive as in higher metazoans (Mackie, 1990). Whereas most protists use Ca^{2+} as the inward charge carrier, purely Na^+-dependent action potentials are common to metazoans, including cnidarians. This prompted Hille (1984) to speculate that Na^+ channels evolved from Ca^{2+} channels in parallel with the evolution of the first nervous system. With the emergence of voltage gated Na^+-selective channels, neurons that generate action potentials at high frequency would have become possible; if Ca^{2+} were the only positive charge carrier, high-frequency discharges would probably cause intracellular Ca^{2+} to accumulate to toxic levels (Anderson and Greenberg, 2001). Hille further suggested that oubain-sesitive Na^+–K^+ ATPase molecules, involved in maintaining the electroosmotic gradient of these two ions, evolved coincidentally with Na^+ channels (Hille, 1984).

Two different classes of neuroactive substances, classical neurotransmitters and neuropeptides, have been detected in cnidarian tissues. The major difference between these two classes is their mode of synthesis. While classical transmitters are synthesized in nerve terminals, neuropeptides are synthesized in neuronal cell bodies, processed within vesicles and then transported along the axons to the nerve terminals. A large percentage of cnidarian neurons show immunoreactivity with antisera against neuropeptides that have either an Arg-Phe-NH2 or Arg-Trp-NH2 carboxyterminus (LWamide, RFamide). Furthermore, from a single anthozoan species, *Anthopleura elegantissima*, 17 different neuropeptides have been isolated so far, some of which are specifically expressed in at least six identified neuronal subpopulations (Grimmelikhuijzen *et al.*, 1996). Cnidarian neuropeptides occur only in neurons and have been shown to have behavioral effects in several species. Interestingly, some of these neuropeptides also play an important role in growth regulation, morphogenesis, and the induction of metamorphosis (Schaller *et al.*, 1996). This dual role is exemplified in the planula of the hydrozoan *Hydractinia echinata*, where LWamide and RFamide neuropeptides form an antagonistic system that influences both planula migratory behavior and initiation of larval metamorphosis in response to environmental cues (Katsukura *et al.*, 2003, 2004; Plickert *et al.*, 2003). Although, the cnidarian nervous system is primarily peptidergic, there is growing evidence for the involvement of classical neurotransmitters in signal transmission. This is supported by the presence of biogenic amines and acetylcholine in the tissues of several cnidarian species and the role of these substances in modulating behavior. Furthermore, serotonin-immunoreactive neurons have been described in the colonial anthozoan *Renilla*, and GABA and glutamate receptors mediate a modulatory function of pacemaker activity and feeding response in *Hydra* (Umbriaco *et al.*, 1990; Concas *et al.*, 1998; Kass-Simon *et al.*, 2003; Pierobon *et al.*, 2004). However, it remains controversial to what extent neuronal signal transmission in Cnidaria is accomplished by the use of classical transmitters since their action at the synaptic level has not yet been demonstrated (Mackie, 1990; Grimmelikhuijzen *et al.*, 1996; Anctil, 1989). Nevertheless, the presence of both aminergic and peptidergic neurotransmitters in cnidarians indicates a parallel evolution of the two transmitter systems (Prosser, 1989).

4.3.7 Placozoa versus Cnidaria

The phylogenetic position of Placozoa, which is currently represented by a single-known species, *T. adhaerens*, is controversial. Recent evidence, however, favors the localization of Placozoa between Cnidaria and Bilateria, rather than within medusozoan cnidarians. Placozoa have a low level of tissue organization consisting of only four different somatic cell types arranged in a functional lower and upper side enfolding a number of intermediate cells (Grell and Ruthman, 1991). Although *Trichoplax* apparently lacks nerve cells, some cells react with antibodies raised against the neuropeptide RFamide (Schuchert, 1993). The possible presence of neuropeptides in *Trichoplax* may indicate a secondary loss of a nervous system, in accordance with the notion that placozoans are reduced derivatives of an early metazoan. Alternatively, RFamides could have a primitive prenervous role in growth regulation or differentiation. Be that as it may, extant placozoans do not have neurons and do not have nervous systems. Thus, we are left with the Cnidaria.

The analysis of signal conducting systems in cnidarians representing the most basal extant phyla with nervous systems, leads to the conclusion that many basic features characterizing nervous systems of higher animals were already present in the last common ancestor of cnidarians and bilaterians. Cnidarian neurons structurally resemble those of higher animals. Furthermore, the biophysical basis of electrogenesis in neurons is conventional, and chemical and electrical

synapses are similar to those found in all higher metazoans, although the common use of bidirectional synapses in cnidarians is somewhat unusual. Therefore, the 'simplicity' of the cnidarian nervous system does not lie at the level of individual neurons, but rather in the organization of such cells into conducting systems, such as nerve nets. The evolutionary origin of the neuron remains elusive.

4.4 Origin of the First Nervous System: A Comparative Developmental Genetic Approach

4.4.1 Conserved Genes in Neuronal Development

In 1990, Mackie relaunched Parker's discussion of the elementary nervous system and proposed that the evolutionary origin of the nervous system should be reconsidered in the light of recent results form molecular biology and developmental genetics (Mackie, 1990). Indeed, over 80 years after Parker first put forward his theoretical views, it seems appropriate to consider not only the origin of the cell lineages that initially gave rise to neurons, but also the origin of the genes involved in neurogenesis and neuronal differentiation. Ideally this type of molecular evolutionary developmental approach should allow identification of a basal set of genes that are likely to have been involved in generating the first nervous system. Thus, a novel and promising approach to nervous system evolution is the comparative analysis of the genes that control neuronal proliferation and differentiation in key metazoan phyla. Which key phyla should be subjected to such a molecular genetic analysis? Although, impulse conduction and sensitivity to neuromodulatory substances have been shown in different Porifera, extant sponges lack nerve cells and a nervous system and are therefore not ideal for studies on the molecular genetics of neuronal development. In contrast, true neurons as well as different levels of nervous system organization can be found in the Cnidaria, and, in consequence, a comparative developmental genetic analysis of cnidarian versus bilaterian nervous systems is likely to be useful. In the following, the evolution and origin of the first nervous system will be considered in light of the molecular genetic control elements for neurogenesis, axial patterning, and eye development that are conserved between Cnidaria and Bilateria. A caveat for all of these considerations is, however, the fact that functional analyses of key control genes are still lacking in the Cnidaria.

4.4.2 Genetic Control of Neurogenesis in Cnidaria and Bilateria

Key genetic regulators of neurogenesis have been studied in a number of vertebrate (mouse, chick, frog, zebra fish) and invertebrate (*Drosophila, C. elegans*) model organisms. Several transcription factors involved in early neurogenesis events have been identified that are structurally and functionally conserved among protostome and deuterostome phyla (Arendt and Nübler-Jung, 1999; Bertrand *et al.*, 2002; Reichert and Simeone, 2001). This suggests that similar transcription factors might already have been involved in neurogenesis of the common ancestor of all bilaterians. Different classes of regulatory genes involved in neurogenesis have been isolated and their expression patterns studied in the cnidarian model organism *Hydra*, and homologues of regulatory genes expressed during neurogenesis in deuterostomes and protostomes have been found.

Two homeobox genes *prdl-a* and *prdl-b* are expressed in nerve cell precursors and neurons in the body column of the *Hydra* polyp (Gauchat *et al.*, 1998, 2004; Miljkovic-Licina *et al.*, 2004). They are both related to the paired-like *aristaless* family, members of which have been shown to be important for normal forebrain development in vertebrates (Seufert *et al.*, 2005). The *COUP-TF* genes which encode orphan nuclear receptors are implicated both in neurogenesis and in CNS patterning during embryogenesis as well as in the adult nervous system of vertebrates and *Drosophila* (Gauchat *et al.*, 2004). The *Hydra* homologue *hyCOUP-TF*, was found to be expressed in a subset of neurons and in the nematocyte lineage (Miljkovic-Licina *et al.*, 2004). The bHLH transcription factor *CnASH* is related to the *achaete-scute* gene family in *Drosophila*, which has proneural activity (Grens *et al.*, 1995). *CnASH* is expressed in the differentiation of sensory neurons in the tentacles of *Hydra* (Hayakawa *et al.*, 2004). Another bHLH transcription factor *Atonal-like1* (*Atl1*), which belongs to the Atonal gene family, has been isolated in the hydrozoan *Podocoryne*. Atonal homologues are responsible for the determination of neural fate in sense organs as well as in the peripheral system and CNS of bilaterian model organisms (reviewed in Hassan and Bellen, 2000). In the medusa of *Podocoryne, Atl1* is expressed in subsets of presumed nerve cells of the tentacle and the feeding organ (Seipel *et al.*, 2004). These findings suggest that some elements of the genetic network underlying neuronal development may be conserved from cnidarians to vertebrates, implying that the

molecular genetic control of neuronal development evolved only once.

4.4.3 Genetic Control of Anteroposterior Patterning in Cnidaria and Bilateria

The bilateral symmetry of bilaterian animals is achieved by the orthogonal intersection of an anteroposterior and a dorsoventral body axis. Different genetic mechanisms are responsible for patterning each axis and the underlying gene networks are widely conserved between Protostomia and Deuterostomia. Thus, *Hox* genes play an evolutionary conserved role in patterning the anteroposterior axis of all bilaterians studied to date (Slack *et al.*, 1993). Interestingly, Hox genes are also responsible for the anteroposterior patterning of bilaterian nervous systems as has been shown in genetic experiments carried out for arthropods and vertebrate model systems. The anteroposterior expression pattern of the *Hox* genes during nervous system development largely reflects their pattern of expression in the embryonic body and corresponds to the spatial arrangement of the *Hox* genes in their chromosomal clusters (spatial colinearity). Similarly, the homeobox transcription factors of the *orthodenticle* (*otd/Otx*) and *empty spiracles* (*ems/Emx*) families have evolutionarily conserved expression domains in the anterior cephalic regions of all bilaterian animals studied to date. Moreover, both gene families are known to play an important role in the development of the most anterior part of the nervous system, the anterior brain, in arthropods and vertebrates. Mutations in these genes lead to severe brain phenotypes such as the absence of large neurogenic regions of the brains of both insects and vertebrates. Thus, bilaterian brains are universally characterized by a rostral region specified by genes of the *otd/Otx* and *ems/Emx* family and a caudal region specified by genes of the *Hox* family (Figure 8a; Shankland and Bruce, 1998; Sharman and Brand, 1998; Arendt and Nübler-Jung, 1999; Hirth and Reichert, 1999; Reichert and Simeone, 2001; Lowe *et al.*, 2003; Lichtneckert and Reichert, 2005).

Homologous genes involved in anteroposterior patterning of the body wall and nervous systems of bilaterians have been isolated from different cnidarian species. *otd/Otx* family genes have been cloned from two hydrozoans, *Hydra* (Smith *et al.*, 1999) and *Podocoryne* (Müller *et al.*, 1999). Whereas *Podocoryne Otx* is only expressed in the striated muscle of the developing medusa, which seems unrelated to *otd/Otx* function in Bilateria, *Hydra Otx* expression can be found in ectodermal epithelial cells throughout the body column. In addition, *Hydra Otx* expression has been detected in nerve cells by cell type Northern; however the *Otx*-positive neural subpopulation has not yet been identified. In gastrozooid polyps of the hydrozoan *Hydractinia symbiolongicarpus*, expression of *Emx* is detected at the oral 'head' end of the oral–aboral axis, specifically in endodermal epithelial cells of the hypostome (Mokady *et al.*, 1998). No *Emx* expression in nervous systems of cnidarians has been described so far.

The question whether true *Hox* genes are present in cnidarians is controversial (reviewed in Galliot, 2000; Ball *et al.*, 2004; Finnerty, 2003). Based on sequence analysis, several authors have argued for the presence of anterior class and posterior class *Hox* genes in cnidarians. The chromosomal linkage of these genes in clusters is still a matter of debate. The expression data from hydrozoans and anthozoans show that different *Hox* genes are expressed in specific regions along the oral–aboral body axis. Five *Hox* genes were recovered from the sea anemone *Nematostella vectensis*; their expression was studied during larval development (Finnerty *et al.*, 2004). Two cnidarian-specific gene duplications appear to have produced two pairs of sister genes *anthox1-anthox1a* which are homologous to bilaterian posterior group *Hox* genes, and *anthox7-anthox8*, which are homologous to the anterior *pb/Hox2* genes in vertebrates and flies (Figure 8b). Whereas expression of *anthox1* is restricted to the ectoderm at the aboral tip of the polyp, a nested expression of *anthox1a, anthox7*, and *anthox8* is found in the endoderm layer all along the body column. The *lab/Hox1* homologue, *anthox6* is expressed in the endodermal body layer of the pharynx, the oral-most part of the polyp. Therefore, during development *Nematosella Hox* gene expression spans nearly the entire oral–aboral axis, which is similar to the situation in the body of bilaterian animals. Whether expression of anthozoan *Hox* genes is present in the nerve cells of *Nematosella* is currently unknown. Cnidarian *Hox* gene expression has also been reported in larval development of the hydrozoan *P. carnea* (Masuda-Nakagawa *et al.*, 2000; Yanze *et al.*, 2001). Three *Hox* genes, *cnox1-Pc, cnox2-Pc*, and *cnox4-Pc* are expressed in restricted domains along the oral–aboral axis in ectodermal and endodermal germ layers of the planula larva. Although, an anteroposteriorly polarized nerve net has been described in the planula larva of *Podocoryne* (Gröger and Schmid, 2001), the presence of the *Hox* genes in the cells of this nerve net has not been investigated yet. Interestingly, comparison of orthologous *Hox* genes between *Nematostella* and *Podocoryne* reveals that their axial expression patterns in the planula are reversed.

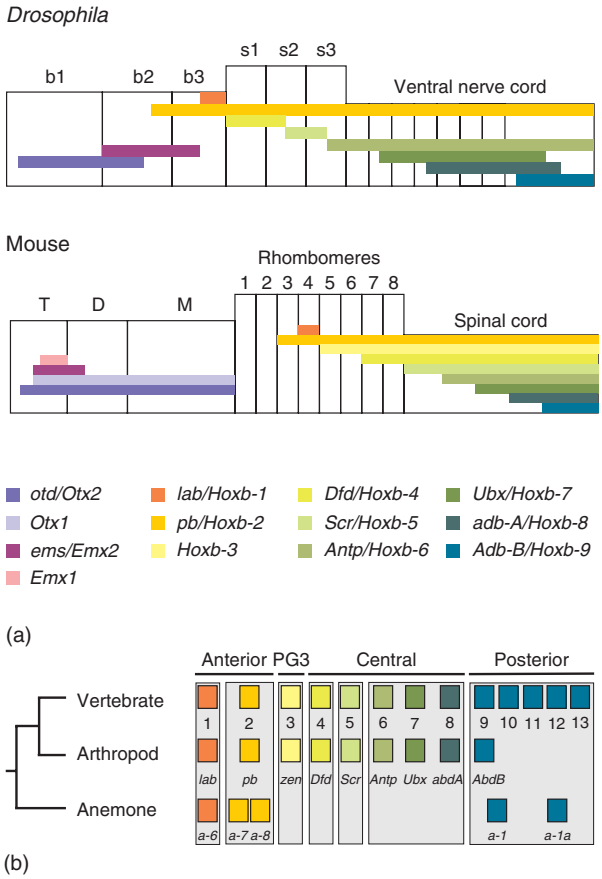

Figure 8 Conserved anteroposterior order of gene expression in embryonic CNS development of bilaterians and occurrence of Hox genes in bilaterians and anthozoans. a, Schematic of *otd/Otx, ems/Emx*, and *Hox* gene expression patterns in the developing CNS of *Drosophila* (stage 14 embryo) and mouse (stage 9.5 12.5 embryo). b, Homology of *Nematostella vectensis Hox* genes to vertebrate and arthropod orthologues based on phylogenetic analysis of homeodomains. Vertebrate *Hox* paralogues are numbered from 1 to 13. Arthropod *Hox* paralogues are named with *Drosophila* terminology (*lab, labial; pb, proboscipedia; zen, zerknüllt; Dfd, Deformed; Scr, Sex combs reduced; Antp, Antennapedia; Ubx, Ultrabithorax; abd-A, abdominal-A; Abd-B, Abdominal-B*). Paralogue groups are classified as anterior, paralogue group 3 (PG3), central, and posterior *Hox* genes. *a-1, anthox1; a-1a, anthox1a; a-6, anthox6; a-7, anthox7; a-8, anthox8;* b1 b3, segments in the *Drosophila* brain (proto-, deuto-, and trito-cerebrum, respectively); s1 s3, mandibular, maxillary, and labial segments, respectively, of the fly subesophageal ganglion; T, telencephalon; D, diencephalon; M, mesencephalon. a, Reproduced from Sharman, A. C. and Brand, M. 1998. Evolution and homology of the nervous system: Cross-phylum rescues of otd/Otx genes. *Trends Genet.* 14(6), 211 214, with permission from Elsevier. b, Reprinted with permission from Finnerty, J. R., Pang, K., Burton, P., Paulson, D., and Martindale, M. Q. 2004. Origins of bilateral symmetry: *Hox* and *dpp* expression in a sea anemone. *Science* 304, 1335 1337. Copyright 2004 AAAS.

For example, the anterior *Hox* gene, *cnox1-Pc* is expressed at the apical end of the planula in *Podocoryne*, while the *Nematostella* homologue, *anthox6* is expressed at the blastoporal end of the planula. This apparent contradiction may be attributed to a developmental reversal of spatial polarity that has been described for *Hox* expression in *Podocoryne* during metamorphosis (Masuda-Nakagawa *et al.*, 2000). Thus, while clear homologues of bilaterian anterior and posterior class *Hox* genes are present in cnidarians, the correlation between cnidarian and bilaterian *Hox* gene expression patterns remains ambiguous. Moreover, the expression and function of cnidarian *Hox* genes in nerve cells has not been explicitly investigated so far, leaving the question of their involvement in nervous system patterning unanswered.

4.4.4 Genetic Control of Dorsoventral Specification in Cnidaria and Bilateria

A hallmark of dorsoventral polarity in many bilaterians is the dorsoventral location of the CNS. Whereas in vertebrates the CNS is located dorsally, in arthropods the CNS is located ventrally. This reversal in the relative position of the CNS led Geoffroy Saint-Hilaire to propose that the dorsoventral axes of vertebrates and arthropods are inverted with respect to the position of their mouth openings (Geoffroy

Saint-Hilaire, 1822). This 'dorsoventral inversion' hypothesis has gained strong support in recent years, since homologous, but spatially inverted patterning mechanisms were found to be operating in vertebrates and insects (Holley *et al.*, 1995). The *transforming growth factor-β* (*TGF-β*) superfamily members *decapentaplegic/Bone Morphogenetic Protein 4 (dpp/BMP4)* are required for patterning the dorsal region in arthropods and for promoting ventral fates in vertebrates (Figures 9a and 9b). In both animal groups *dpp/BMP4* have strong anti-neurogenic properties, and therefore, the nerve cord can only develop where *dpp/BMP4* activity is inhibited or absent. In *Drosophila*, the ventral expression of the *dpp* antagonist *short gastrulation* (*sog*) allows the development of the ventral neuroectoderm, whereas in vertebrates, the same effect is achieved dorsally by the *sog*-related *Chordin* gene (Reichert and Simeone, 2001; Lichtneckert and Reichert, 2005).

Although textbooks usually characterize cnidarians as radially symmetrical (Brusca and Brusca, 1990; Campbell *et al.*, 2004; Johnson, 2003), it has long been recognized that many anthozoan cnidarians exhibit bilateral symmetry (Stephenson, 1926; Hyman, 1940). In many sea anemones a secondary body axis, referred to as the directive axis, crosses the pharynx orthogonally to the primary oral–aboral body axis. For example, a cross section through the sea anemone *N. vectensis* reveals that the mesenteries and their associated retractor muscle fibers exhibit a bilateral symmetry in their orientation around the pharynx. Genes involved in specifying the dorsoventral axis in Bilateria have recently been found to be expressed asymmetrically along the directive axis of anthozoans. In the gastrulating embryo of *Acropora millepora*, expression of *bmp2/4-Am* (a *dpp/BMP4* homologue) is not symmetrical about the primary body axis, which runs through the blastopore. Rather *bmp2/4-Am* mRNA is concentrated in one quadrant of the surface ectoderm next to the blastopore (Hayward *et al.*, 2002). This suggests that the *bmp2/4-Am* expression domain defines a second polarized axis, in addition to the one defined by the blastopore. A similar distribution of *dpp/BMP* mRNA has been reported during early embryogenesis of the sea anemone *N. vectensis* (Finnerty *et al.*, 2004); at later developmental stages of *Nematostella, dpp/Bmp4* is expressed in the pharynx and the mesenteries in a bilaterally symmetrical fashion relative to the directive axis (Figure 9c). Within the Cnidaria, bilateral symmetry is a characteristic of anthozoans and thus probably represents an ancestral trait of the phylum that might have been lost

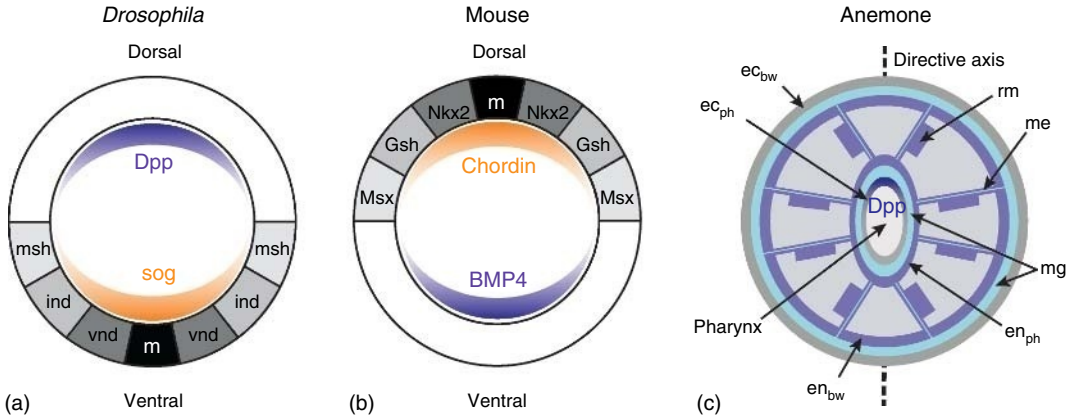

Figure 9 Asymmetric Dpp/BMP4 signaling along the dorsoventral and directive axis of bilaterians and anthozoans. a and b, The secreted products of the homologous genes *dpp/Bmp4* form a dorsoventrally inverted gradient in mouse (Deuterostomia) with respect to *Drosophila* (Protostomia). Sog/Chordin act from opposing dorsoventral poles in both insect and vertebrate embryos antagonizing the antineurogenic effect of Dpp/BMP4. The neuroepithelium is further subdivided by a set of homeobox genes into medial (*vnd/Nkx2*), intermediate (*ind/Gsh*), and lateral (*msh/Msx*) neurogenic domains in *Drosophila* and mouse. c, Cross section through the pharyngeal region of the anemone *Nematostella* reveals bilateral symmetry about the directive axis. The pharynx is attached to the outer body wall via eight endodermal mesenteries. Each mesentery bears a retractor muscle on one face. The only plane of mirror symmetry passes through the directive axis. During development, Dpp is expressed throughout the endoderm. In addition, Dpp expression is transiently found in the pharynx ectoderm in an asymmetric distribution relative to the directive axis. ec_{bw}, body wall ectoderm; ec_{ph}, pharyngeal ectoderm; en_{bw}, body wall endoderm; en_{ph}, pharyngeal endoderm; m, midline; me, mesentery; mg, mesogloea; rm, retractor muscle. a and b, Modified from Reichert, H. and Simeone, A. 2001. Developmental genetic evidence for a monophyletic origin of the bilaterian brain. *Philos. Trans. R. Soc. Lond. B* 356, 1533 1544, Copyright 2001, The Royal Society. c, Reprinted with permission from Finnerty, J. R., Pang, K., Burton, P., Paulson, D., and Martindale, M. Q. 2004. Origins of bilateral symmetry: *Hox* and *dpp* expression in a sea anemone. *Science* 304, 1335 1337. Copyright 2004 AAAS.

secondarily in medusozoans due to the emergence of a clearly radial symmetric medusoid life stage. Although at least part of the dorsoventral patterning system that has antineural function in bilaterians is present in anthozoan polyps, no morphological regionalization of the nervous system along the directive axis of polyps has yet been observed.

In arthropods and vertebrates, initial regionalization of the dorsoventral axis by *dpp/Bmp4* and their antagonists is followed by further patterning of the neuroectoderm along its dorsoventral axis by a group of conserved homeobox genes. In *Drosophila, vnd* (*ventral neuroblasts defective*), *ind* (*intermediate neuroblasts defective*), and *msh* (*muscle segment homeobox*) are involved in the dorsoventral specification of a ventral, intermediate, and lateral column of neuroblasts in the developing ventral neuroectoderm (Figures 9a and 9b). During vertebrate neurogenesis, genes closely related to *Drosophila msh* (*Msx*), *ind* (*Gsh*), and *vnd* (*Nkx2*) are expressed in domains corresponding to those in *Drosophila* along the dorsoventral axis of the developing CNS suggesting that this system was conserved throughout evolution (reviewed in Arendt and Nübler-Jung, 1999; Reichert and Simeone, 2001; Lichtneckert and Reichert, 2005).

All three of these dorsoventral patterning genes (*vnd/Nkx2, ind/Gsh, msh/Msx*) are present in cnidarians (Schummer *et al.*, 1992; Grens *et al.*, 1996; Hayward *et al.*, 2001). In the anthozoan *A. millepora, cnox-2Am*, the orthologue of the vertebrate *Gsh* gene, is expressed in scattered ectodermal cells of the larva with a restricted distribution along the oral–aboral body axis. Based on morphology, these cells have been characterized as transectodermal neurons (Hayward *et al.*, 2001). The expression of *cnox-2Am* in a subset of neurons is consistent with the restricted expression of *Gsh* orthologues in bilaterians. The presence of all three dorsoventral patterning homeobox genes in Cnidaria, together with the spatially restricted neuronal expression of *cnox-2Am* along the anteroposterior axis of the planula larva, suggests that the *msh/ind/vnd* system may have had an ancient evolutionary origin that predated the Cnidaria/Bilateria split, and thus might represent an ancient nervous system patterning process. It remains to be shown, however, if the cnidarian orthologues of *vnd/Nkx2* and *msh/Msx* are also expressed in nerve cells and if their expression specifies different neuronal subsets located on a secondary body axis, as in bilaterians.

4.4.5 Genes Involved in Eye Development in Cnidaria and Bilateria

A conserved gene regulatory network including members of the *Pax6, six, dachshund*, and *eyesabsent* families has been shown to orchestrate eye development in a wide range of bilaterian animals. *Pax6* mutations in the mouse or fly cause a reduction or absence of eyes. On the other hand, ectopic expression of *Pax6* from various bilaterian species induces ectopic eyes in *Drosophila*, implying that *Pax6* might represent a 'master control' gene for eye development (reviewed in Piatigorsky and Kozmik, 2004; Gehring, 2005). The fundamental, evolutionarily conserved role of the genetic network underlying eye development led to the suggestion of a monophyletic origin of the eye (Gehring and Ikeo, 1999). The *Pax2/5/8* family comprises one single *D-Pax2* gene in *Drosophila* (Fu and Noll, 1997), whereas in mammals three genes, *Pax2, Pax5, and Pax8*, arose by duplications at the onset of the vertebrate lineage (Pfeffer *et al.*, 1998). The *Pax2/5/8* genes play an important role in brain patterning and are also implicated in eye development.

In cnidarians, eyes are found sporadically in some hydrozoan (see Figure 7b) and cubozoan medusae, and it is not known whether other jellyfish have lost their eyes in the course of evolution or whether they never acquired them (Piatigorsky and Kozmik, 2004; Gehring, 2005). Four *Pax* genes (*PaxA, PaxB, PaxC,* and *PaxD*) have been isolated from anthozoans (Miller *et al.*, 2000) and a number of other cnidarian species (Sun *et al.*, 1997, 2001; Gröger *et al.*, 2000; Kozmik *et al.*, 2003), but none of these have a protein domain structure that corresponds of bilaterian *Pax6*. In the cubomedusa *T. cystophora, PaxB* is expressed in the lens and the retina of the complex eyes as well as in the statocyst. Interestingly, it has been shown that *PaxB* is structurally a mosaic between *Pax2* and *Pax6*. This is further supported by functional studies in *Drosophila*, where *PaxB* complements *Pax2* mutants (*sparkling*) and also induces ectopic eyes like *Pax6* (Kozmik *et al.*, 2003). Therefore, *PaxB* of *Tripedalia* might resemble an ancestral gene of the *Pax6* and *Pax2/5/8* subfamilies, which arose by duplication of the ancestral form in the bilaterian line (Kozmik *et al.*, 2003; Piatigorsky and Kozmik, 2004). Thus, the competence to regulate eye development was either inherited from the ancestral *PaxB*-like gene by cnidarian *PaxB* and bilaterian *Pax6*, which would support the monophyletic origin of eyes (Gehring and Ikeo, 1999; Gehring, 2005), or it emerged parallely during the evolution of the two *Pax* genes following the cnidarian bilaterian split (Piatigorsky and Kozmik, 2004). Interestingly, a *PaxB* orthologue has been isolated from sponges (Hoshiyama *et al.*, 1998); however, it is not known whether the expression of this gene is associated with the photoreceptive cells in sponge larva. Additional support for the monophyletic origin

of the eyes was obtained from the hydrozoan *Cladonema*. Orthologues of two *Six* family members, which are known to control eye development in vertebrates and arthropods, are expressed in the lens eyes of the hydromedusa and are involved in eye regeneration (Stierwald *et al.*, 2004). This implies that the common ancestor of Cnidaria and Bilateria may already have possessed some kind of photoreceptive organ. Moreover, it suggests that at least part of the gene regulatory network used for the development of eyes by modern species, was already used by the eumetazoan ancestor. Taken together, the presence of photosensitive cells, probably autonomous receptor–effector cells, in multicellular animals, as exemplified by certain sponge larvae, may have anticipated the emergence of a nervous system. If this were the case, then the sensory input from these photoreceptors might have had a strong influence on the early evolution of the nervous system.

4.5 Conclusions and Outlook

The origin and evolution of the first nervous system remains elusive. Over the last 150 years, the evolution of the first nervous system has been a central issue in notions about the emergence of eumetazoan animals, and a variety of theories have been proposed. The main question has been the identification of the primordial cell lineage from which nerve cells might have been derived. During the last decade, however, advances in molecular genetic techniques have focussed our interest on the genes that might have been involved in the generation of the first nervous system. In terms of comparative developmental genetics, it appears that genes involved in patterning of the anteroposterior axis in bilaterians, such as the *Hox* genes, are also expressed in restricted domains along the main body axis during cnidarian larval development as well as in the adult polyp. However, the validity of comparing gene expression patterns along the oral–aboral axis of cnidarians to those found along the anteroposterior axis of bilaterians is questionable. Moreover, in contrast to bilaterians, *Hox* gene expression in cnidarian nerve cells has not yet been unequivocally demonstrated. Similar considerations apply to most of the genes involved in dorsoventral patterning in cnidarians and bilaterians. Thus, although there is morphological and genetic evidence for bilateral symmetry with respect to the directive axis in anthozoans, no regional restriction of neurogenesis in the cnidarian body has been reported to date. Does this mean that the restriction of nervous tissue to one side of the dorsoventral body axis by early genetic pattering mechanism evolved only in bilaterian animals?

One of the most intriguing findings to emerge from preliminary expressed sequence tag (EST) projects on several cnidarian species is that the gene sets of cnidarians and, by implication, the common metazoan ancestor, are surprisingly rich and complex (Kortschak *et al.*, 2003). A long-held assumption is that fewer genes should be required to build a sea anemone than a fly, but this seems not to be true. This paradox is exemplified by the fact that, whereas anthozoan cnidarians have the simplest extant nervous systems, the *A. millepora* genome contains many of the genes known to specify and patterns the much more sophisticated nervous systems of vertebrates and insects. It has been proposed that the first major wave of gene duplications in metazoans predated the Parazoa and Eumetazoa split ~940 Mya resulting in large genomes in basal metazoans (Nikoh *et al.*, 1997; Suga *et al.*, 1999). Gene number seems to be a poor indicator of the sophistication of gene use; it is now widely accepted that alternative splicing and transcriptional regulation are generally more complex in mammals than in insects and that this difference accounts for the execution of more complex molecular programs in complex animals (Ball *et al.*, 2004).

A comparative genetic approach including Cnidaria and Ctenophora as well as different bilaterian groups may help to reconstruct different aspects of the nervous system of the last common ancestor, which might have resembled the first nervous system in evolution. Moreover, the availability of genomic data from Porifera in the near future (Leys *et al.*, 2005), should pave the way for the identification and analysis of further sponge homologues to genes involved in neurogenesis or in sensory organ development in Eumetazoa, thus providing more information about the origin and the evolution of the first nervous system.

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5 Neuronal Migration

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Glossary

cortex	Laminar neuronal structure that is formed at the surface of the central ner vous system and that includes structures such as the cerebellum and cerebral cortex.
interneuron	Local circuit neuron, sometimes also referred as a Golgi type II neuron. In a general sense, any neuron that lies between an afferent neuron and an effector neuron.
leading process	Cell extension that is located in front of the nucleus and that directs the migration of neurons.
neocortex	The six layered part of the dorsal pallium, more properly known as the isocortex.
nucleokinesis	The process of nuclear translocation in cells, including interkinetic movements during the cycle of epithelial cells or dur ing cell migration.
pallium	Roof of the telencephalon; it contains both cortical (e.g., hippocampus and neo cortex) and deep lying nuclear structures (e.g., claustrum and parts of the amyg dala). Pallium is not synonymous with cortex.
striatum	A part of the subpallium and one of the components of the striatopallidal com plex. It comprises deep (caudate nucleus, putamen, and nucleus accum bens) and superficial (olfactory tubercle) parts.
subpallium	Base of the telencephalon; it consists pri marily of the basal ganglia (e.g., striatum, globus pallidus, and parts of the septum and amygdala).

5.1 Introduction

Neurons of the central nervous system are natural migrants, as most of them originate far from the place where they will eventually perform their normal function. Indeed, the large majority of neurons are generated from precursor cells that line the walls of the ventricular system, from where they migrate until they settle at their final position. Thus, after the genesis of specific cell types through an exquisite and controlled process of patterning and regionalization, neurons of the brain are set to migrate. In some cases, new neurons migrate for relatively short distances to settle, for instance, in the ventral horn of the spinal cord, where they become somatic motor neurons. In other cases, neurons migrate for incredibly long distances, sometimes up to thousands of times their own size, to settle in remote regions of the brain, as in the case of the interneurons of the cerebral cortex or the olfactory bulb. Thus, independent of the neuronal type, location, or function, neuronal migration is always a fundamental step in brain development.

The complexity of the brain in vertebrates is proportional, to a large extent, to the elaboration of the mechanisms controlling neuronal migration. This is particularly evident in the mammalian forebrain and, more specifically, in the telencephalon, where the development of the isocortex has been accompanied by an enormous increase in the distance covered by migrating neurons from the ventricular zone to their final destination. This is in sharp

contrast with the situation found in amphibians, for example, in which neurons barely migrate away from the place they originate. Thus, as a mechanism that shapes the development of the brain, changes in neuronal migration have greatly contributed to its diversification during evolution.

In this article, we review concepts on neuronal migration through evolution, with a focus on the central nervous system (CNS). Whenever possible, we will refer to the development of the cerebral cortex as a model system for studying the cellular and molecular mechanisms controlling neuronal migration. Of note, although the general principles that control migration in the peripheral nervous system are essentially identical to those in the CNS, this subject is beyond the scope of this article. To learn more about this, the reader is referred to reviews focusing on the mechanisms controlling neural crest migration (Robinson *et al.*, 1997; Locascio and Nieto, 2001; Kalcheim and Burstyn-Cohen, 2005).

5.2 Cellular Mechanisms in Neuronal Migration

Despite prominent differences in the distance covered by distinct neuronal types until their final settlement in the brain, or even fundamental discrepancies in the primary mode of migration used by different populations of neurons (discussed in detail in the next section), migrating neurons appear to use a basic set of cellular mechanisms that is roughly similar to those used by other cell types during vertebrate morphogenesis. In that sense, neuronal migration can be considered a cyclic process, in which polarization of the cell is followed by the extension of cell protrusions and differential rearrangements in the adhesion properties of the plasma membrane leading to the movement of the neuron, including its nucleus (nucleokinesis). Moreover, because cell migration is fundamental not only during vertebrate development, but also to plants and even single-celled organisms, the molecular mechanisms underlying this process are likely to be highly preserved throughout evolution.

5.2.1 Polarization of Migrating Neurons

The initial response of an immature neuron to a migration-promoting factor is similar to that of other cell types in different organs and organisms and includes the polarization and extension of protrusions in the direction of migration. In other words, the molecular processes occurring at the front and the back of a neuron become distinct during migration, although we still do not understand the fundamentals of these differences. In migrating neurons, the polarized protrusion in the direction of movement is known as the leading process, which appears to behave similarly to extending axons during axon growth and guidance. As growing axons, migrating neurons typically have a single leading process that constitutes the compass reading structure driving directed neuronal migration. In some cases, however, such as, for example, immature cortical interneurons, two or more leading processes seem to act coordinately to direct cell movement (Marín and Rubenstein, 2001). Moreover, although the leading process in migrating neurons is typically only a few cell diameters in length, an extremely long leading process (up to more than 1 mm long) characterizes some populations of migrating neurons. This is the case for example of basilar pontine neurons, which are born in the dorsal hindbrain and migrate to the ventral midline, where they finally reside (Yee *et al.*, 1999).

Despite the close resemblance of the leading process – in particular, of long leading processes – to growing axons, marker analysis suggests that these two structures are molecularly different to a large extent, bearing strong similarities only at their more distant tip, the growth cone (Ramóny Cajal, 1911). For example, the processes of basilar pontine neurons stain with antibodies against transiently expressed axonal surface glycoprotein-1 (TAG-1), but do not express any of the common neuronal markers associated with axons, including growth-associated protein 43 (a molecule expressed by immature axons), microtubule-associated protein-2, or neurofilament 200. Thus, leading processes and growing axons seem to represent distinct cellular specializations used by neurons at different stages of development (Figure 1).

The polarization of migrating neurons (i.e., the extension of a leading process) depends on chemotactic responses to external cues, which seem to control also the orientation of the leading process and therefore the subsequent direction of movement. The molecules that influence the behavior of migrating neurons also typically control axon pathfinding, suggesting that the mechanisms underlying the polarization of both migrating neurons and axons are very similar. For example, Netrin-1, a prototypical axon guidance molecule (Serafini *et al.*, 1994), also promotes the extension of leading processes during neuronal migration and influences the direction of migration in multiple neuronal populations through the CNS. Similarly, Slit proteins prevent axon growth into undesirable regions

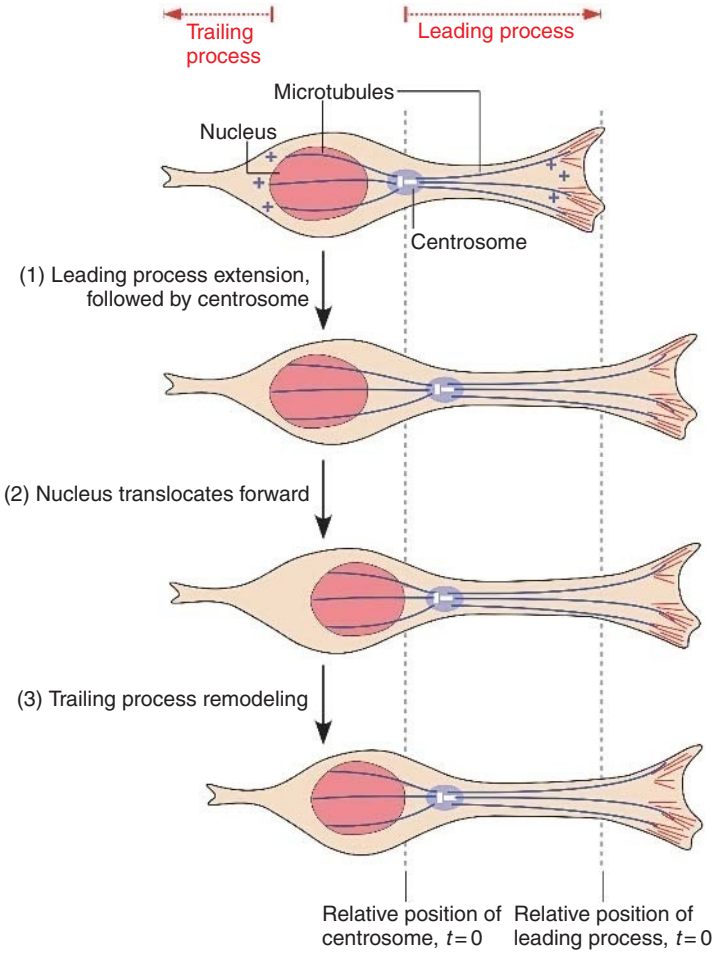

Figure 1 Steps in neuronal migration and the molecules involved. A prototypical migrating neuron contains distinct subcellular domains: the leading process, the perinuclear domain, and the trailing process. Neuronal migration involves repeated cycles of (1) polarized extension of the leading process, followed by movement of the centrosome forward, (2) a highly coordinated movement of the nucleus closer to the centrosome (nucleokinesis), and finally (3) a trailing process remodeling.

and direct neuronal migration through the CNS, acting as chemorepellent factors for both migrating axons and neurons (Brose and Tessier-Lavigne, 2000) (see also Section 5.5).

During chemotaxis, migrating cells – including neurons – appear to detect very small differences in chemical gradients and therefore it is likely that the process of polarization requires their amplification through steeper intracellular gradients that allow appropriate cellular responses (Figure 1). In *Dictyostelium* cells, this process involves the polarization of phosphoinositides (such as phosphatidylinositol-triphosphate (PIP3) and phosphatidylinositol (3,4)-biphosphate ($PI(3,4)P_2$)P_2) across the cell and is mediated by localized accumulation at the front of the cell of phosphoinositide 3-kinase (PI3K), which generates phosphoinositides, and restricted localization and activation at the rear of the phosphatase and Tensin homologue deleted on chromosome 10 (PTEN), which removes them (Funamoto *et al.*, 2002). In neurons, however, very little is known about how these molecules control directed polarization. Nevertheless, PI3K is required for the chemotaxis of neurons in response to neurotrophins (Polleux *et al.*, 2002) and perturbation of PTEN function causes abnormal neuronal migration (Li *et al.*, 2003).

Directed migration also requires the polarization of several organelles in slow-moving cells such as neurons. Specifically, the microtubule-organizing center (MTOC) and the Golgi apparatus are normally localized ahead of the nucleus and plays a role in defining the direction of movement. (This is not the case for fast-moving cells such as neurotrophils, in which the MTOC is behind the nucleus.) In other cell types, the small Rho GTPase Cdc42 is active toward the front of migrating cells during chemotactic responses and plays a role in localizing the MTOC ahead of the nucleus, although its contribution to the polarization of migrating neurons is still

unclear. Nevertheless, inactivation of Cdc42 appears to be required for Slit repulsion of migratory cells from the subventricular zone (SVZ) of the telencephalon (Wong *et al.*, 2001), suggesting that Cdc42 may normally help to polarize migrating neurons toward a chemoattractant source but is inactivated during chemorepulsion. Another Rho GTPase, Rac, is also polarized to the front of migrating cells and is involved in promoting directional extension of protrusions through a signaling loop that involves also Cdc42 and PI3K products. In neurons, the cyclin-dependent kinase 5 (Cdk5) and its neuron-specific regulator p35 localize with Rac during the extension of neurites and are part of the signaling machinery that may help neurons to engage in directional migration (Nikolic *et al.*, 1998). The direct interaction of Rac and possibly other small Rho GTPases with the cytoskeleton at the front of the cell appears to constitute the final effector mechanism that mediates the extension of migrating cells in a specific direction.

5.2.2 Nucleokinesis

One of the main differences that distinguish axon guidance from cell translocation is, obviously, the coordinated movement of the nucleus during cell migration. Thus, nucleokinesis is a fundamental step in the cycle that leads to directed cell migration and neurons are no exception to this rule. Indeed, disruption of nuclear translocation systematically leads to prominent defects in neuronal migration (Xie *et al.*, 2003; Shu *et al.*, 2004; Solecki *et al.*, 2004; Tanaka *et al.*, 2004).

Nucleokinesis in migrating neurons critically depends on the microtubule network, which plays a part in positioning the nucleus during translocation (Rivas and Hatten, 1995). As briefly mentioned in the previous section, polarization of neurons during migration includes the location of the MTOC ahead of the nucleus, an event that appears to be necessary for normal movement of the nucleus (Figure 1). This process relies on the interaction between the MTOC and the nucleus through a specialized network of perinuclear microtubules and microtubule-associated proteins, such as doublecortin (DCX) and lissencephaly-1 (LIS1). Both of these proteins bind to microtubules and appear to regulate their polymerization, bundling, and/or stabilization in migrating neurons. In humans, mutations in DCX cause an X-linked type of lissencephaly known as double cortex syndrome (also called subcortical band heterotopia), whereas mutations in the *Lis1* gene cause classic lissencephaly, the Miller–Dieker syndrome (Ross and Walsh, 2001).

As expected from their crucial function in nuclear movement, proteins involved in this process are highly conserved throughout evolution. For example, the *Lis1* homologue in the filamentous fungus *Aspergillus nidulans* is a nuclear migration gene. During development of the fungus, cells become multinucleated through several rounds of divisions and it becomes crucial that nuclei disperse uniformly within the cell for normal growth to occur. This process of nuclear migration in fungi also depends on the network of microtubules and is regulated by proteins that associate with the microtubules, such as that encoded by the *nud*F gene. (Proteins related to nuclear movement in *A. nidulans* were isolated through a screen for nuclear distribution mutants, for which they are named.) *nud*F shares 42% sequence identity with *Lis1* and both genes are considered orthologues. Analysis of other nuclear distribution mutants similar to *nud*F has helped to define the molecular mechanisms mediating the function of this protein in fungi and, by extension, in migrating neurons. For example, *nud*F closely interacts with *nud*A, a gene that encodes the heavy chain of cytoplasmic dynein and is directly involved in nuclear translocation. Another protein that appears to act as a downstream effector of *nud*F is NUDE, two homologues of which have been isolated in mammals, mNudE and NUDEL. Both of these proteins localize to the MTOC and appear to be important in controlling the movement of the nucleus through their association with other proteins, such as γ-tubulin or dynein. Indeed, Lis1, dynein, or NUDEL loss of function results in defects of centrosome–nucleus coupling during neuronal migration (Shu *et al.*, 2004; Tanaka *et al.*, 2004). In summary, these findings illustrate how the identification of homologous proteins in model systems such as *A. nidulans* is greatly contributing to the identification of the function of vertebrate proteins associated with neuronal migration and, more specifically, nucleokinesis (Feng and Walsh, 2001).

In addition to proteins that directly associate with the microtubule network encaging the nucleus during nuclear translocation, other signaling proteins appear to be crucial for normal nucleokinesis. One of these proteins is Cdk5, a serine/threonine kinase that phosphorylates proteins that maintain cytoskeletal structures and promote cell motility. Mice deficient in *Cdk5* or its activating subunits, *p35* and *p39*, exhibit prominent laminar defects in the cerebral cortex, suggesting that this signaling pathway is crucial for neuronal migration (reviewed in Dhavan and Tsai, 2001). For instance, NUDEL is a physiological substrate of Cdk5 (Niethammer *et al.*,

2000; Sasaki *et al.*, 2000). Another case is the focal adhesion kinase (FAK), which is localized in a Cdk5 phosphorylation-dependent manner to the perinuclear network of microtubules where it contributes to normal nuclear movement (Xie *et al.*, 2003). Another example is mPar6α, a protein that associates with different forms of protein kinase C and localizes to the MTOC, where it contributes to promote the polarization of the centrosome in the direction of the movement. Because movement of the centrosome precedes that of the nucleus itself, the function of proteins such as mPar6α is essential for determining the direction of nucleokinesis.

In summary, multiple components of the cellular machinery involved in nucleokinesis have been already identified and a model for understanding nucleokinesis in migrating neurons is starting to emerge (Figure 1). As in the past few years, it is expected that the discovery of other proteins involved in this process may arise through additional homology analyses, since it is clear now that the cellular mechanisms underlying nuclear migration are similar throughout evolution, from unicellular organisms to humans.

5.3 Modes of Migration in the Developing Brain

5.3.1 Two Primary Modes of Migration in the Developing CNS

As discussed in the previous section, the cellular mechanisms underlying the migration of neurons are likely to be similar to those in other cell types. Despite these molecular similarities, two different modes of migration are classically distinguished within the developing brain, radial and tangential migration. In a general sense, radial migration refers to neurons that migrate perpendicularly to the surface of the brain. In contrast, tangential migration is defined by neurons that migrate in a direction that is parallel to the surface of the brain (in either the rostrocaudal axis or the dorsoventral axis) and that is therefore perpendicular to radially migrating neurons. Although this subdivision is primarily based on the orientation of migrating neurons in relation to the neural tube coordinates, it also implicitly reflects the dependence of different classes of neurons on substantially distinct substrates for migration, as we discuss in the next section. In any case, the existence of these different modes of migration in the developing CNS does not indicate that the molecular and cellular mechanisms underlying radial and tangential migration are essentially different. In other words, independent of the mode of migration, alternating cycles of polarization and nucleokinesis are common events to any migrating neuron.

Radial migration is the principal mode of migration within the CNS. In a general sense, radial migration allows the transfer of topographic information from the ventricular zone to the underlying mantle, since neurons that are born nearby tend to occupy adjacent positions in the mantle when using radial migration to reach their final destination. This has important consequences for the organization of the brain. First, radial migration is essential to generate and maintain distinct neurogenetic compartments in the developing neural tube, which is ultimately necessary for the establishment of different cytoarchitectonic subdivisions within the brain (Figure 2). That is, different progenitor regions generate distinct structures in the CNS largely because progenitor cell dispersion is restricted in the ventricular zone (Fishell *et al.*, 1993; Lumsden and Krumlauf, 1996) and migrating neurons from different compartments do not intermingle during their migration. Second, the transfer of positional information from the ventricular zone to the mantle of the brains allows the formation of topographically organized projections, which are crucial for the proper function of the brain. For this reason, radial migration is the basic mechanism preserved throughout evolution to segregate neurons in all regions of the CNS.

Radial migration contributes to the formation of both cortical (i.e., laminar, such as the cerebral cortex, hippocampus, or cerebellum) and nuclear structures (e.g., striatum, red nucleus), although the development of laminar structures is perhaps the most remarkable example on how radial migration may contribute to the formation of complex circuits in the brain. Laminar structures are found in the brain of all vertebrates and, although the most sophisticated example is the mammalian isocortex, the optic tectum of amphibians, reptiles, or birds is a prominent antecedent of this structure. In contrast to brain nuclei, laminar structures are organized to segregate complex patterns of afferent and efferent connections. Because of this organizing principle, the formation of cortical–laminar structures requires the perfect synchronization of proliferation, cell fate, and radial migration mechanisms to determine the number of layers, as well as their cell density and arrangement.

In contrast to radial migration, which appears to play a general role in the formation of major subdivision in the brain, tangential migration is thought to increase the complexity of neuronal circuits because it allows neurons born from distinct

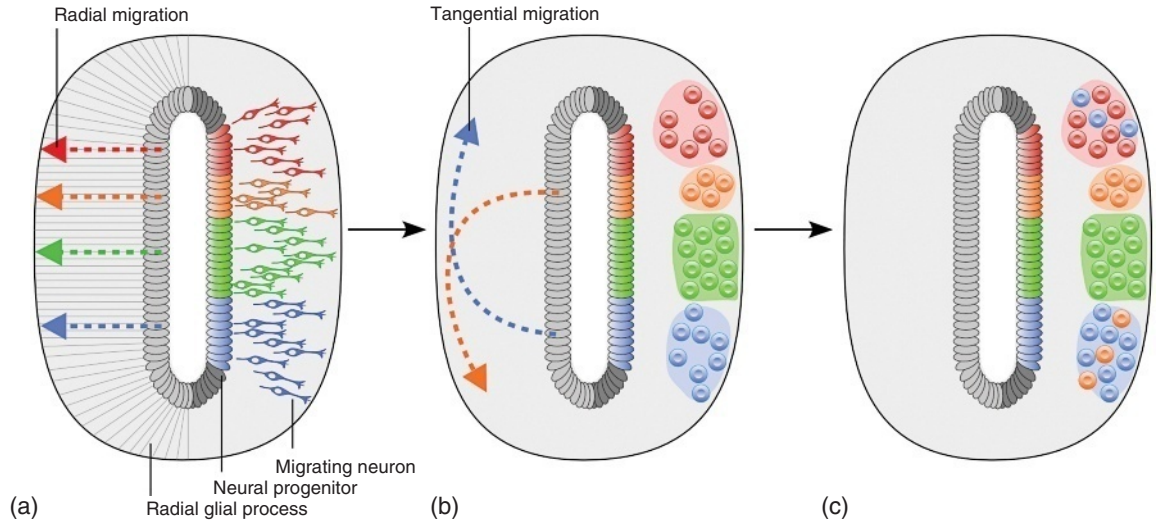

Figure 2 Radial and tangential migration in the central nervous system. a and b, Radial glial cells provide structural support for radial migration, a process that results in the generation of different nuclei that are topographically organized in relation to their place of origin. b and c, Tangential migration is independent of radial glial processes and therefore does not respect topographical references. As a result, tangential migration produces an increase in the complexity of different nuclei by providing cell types distinct from those that are locally generated.

ventricular zones to intermingle and occupy a final common destination (Marín and Rubenstein, 2001) (Figure 2). Tangential migration is likely to be a relatively modern mechanism compared to radial migration, but may have been successfully maintained during evolution because it inherently adds complexity to brain circuits through the incorporation of new cell types with those already present in each region (Figure 2).

Compared to radial migration, the existence of tangential dispersion of neurons in the developing brain has only begun to receive much attention, so it may give the impression of being a relatively contemporary discovery. During the last 30 years of the past century, the predominant view on brain development was based on the idea that radial migration was the sole mechanism allowing the movement of neurons from the progenitor regions to their final destination (Rakic, 1990). This idea was consistent with the basic notion of developmental segmentation in the brain because, as discussed earlier, radial migration contributes to the establishment of segregated cytoarchitectonic regions (Lumsden and Keynes, 1989; Puelles and Rubenstein, 1993). Nevertheless, it was clear from early studies using Golgi-stained sections or electron microscopy that some neurons within the developing brain are oriented tangentially in directions inconsistent with radial migration (Stensaas, 1967; Morest, 1970; Shoukimas and Hinds, 1978). Since then, tangential dispersion has been observed in virtually every subdivision of the developing CNS, from the spinal cord and hindbrain (Bourrat and Sotelo, 1988; Ono and Kawamura, 1989; Leber *et al.*, 1990; Marín and Puelles, 1995; Phelps *et al.*, 1996) to the telencephalon (Austin and Cepko, 1990; Halliday and Cepko, 1992; Walsh and Cepko, 1992; O'Rourke *et al.*, 1992, 1995; Tan and Breen, 1993; De Carlos *et al.*, 1996). In the case of the cerebral cortex, the most compelling experimental evidence supporting the existence of two general modes of cell dispersion, radial and tangential, came from analysis of clonally related cells using retroviral-mediated transfer or highly unbalanced chimeras (Walsh and Cepko, 1992; Tan and Breen, 1993), unequivocally demonstrated by pioneer time-lapse studies (O'Rourke *et al.*, 1992). The main conclusion from all these studies confirms a general principle in our view of brain development: the organization of distinct cytoarchitectonic regions in the CNS most frequently depends on two mechanisms of cell allocation: radial mosaicism and tangential migration.

The existence of two basic modes of migration within the CNS may lead to the erroneous conclusion that there are two major populations of neurons in the developing brain: those that migrate radially and those that use tangential migration to reach their final destination. Indeed, radial and tangential migrations are just two different mechanisms of cell dispersion that the same population of neurons may use indistinctly to reach their final position within the brain. The stereotyped behavior of the facial branchiomotor (fbm) neurons in the hindbrain perfectly illustrates this point (Figure 3). In the mouse, fbm

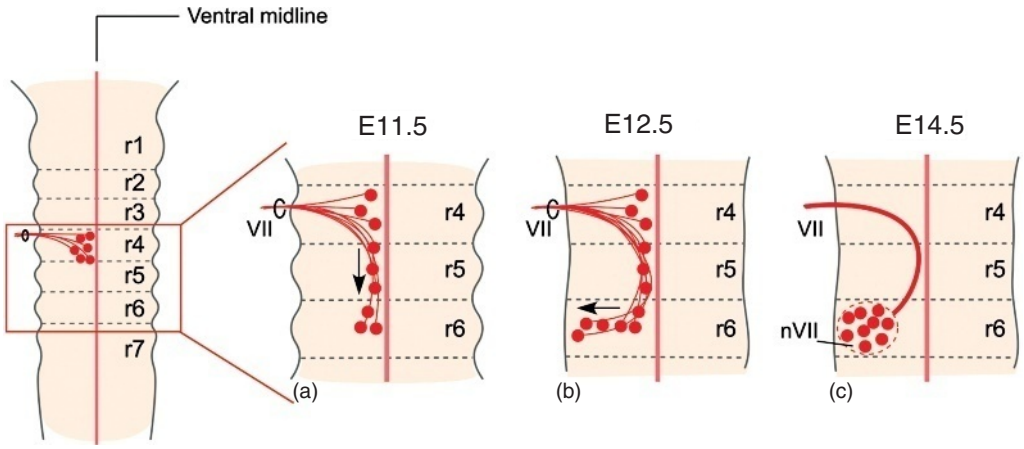

Figure 3 Facial branchiomotor (fbm) neurons adopt tangential and radial modes of migration. Schematic representation of the hindbrain showing the migration of fbm neurons (red circles) during mouse development. At embryonic (E) day 11.5 (E11.5), fbm neurons migrate tangentially (in the caudal direction) from r4, where they originated, to r6. Later, they migrate tangentially within r6, from ventral to dorsal. Finally, they adopt a radial mode of migration to finally form the facial motor nucleus laterally (nVII).

neurons are born in the basal plate of rhombomere 4 (r4), but they finally come to reside in r6. To reach their destination, fbm neurons first migrate tangentially in the caudal direction until they reach r6. Then, they turn 90° and migrate tangentially in the dorsal direction toward the alar–basal boundary. Finally, they turn 90° again and migrate radially toward the pial surface, where they settle to form the facial motor nucleus (for references, see Garel *et al.*, 2000). Similar examples of switching migratory behaviors are present throughout the CNS (cerebellar granule cells, olfactory bulb, and cerebral cortex interneurons, etc.), suggesting that this is a general trend during development. In summary, the same population of neurons may use radial and tangential migration strategies to reach their final destination, likely depending on the extracellular environment available for their dispersion.

5.3.2 Evolutionary Advantages of Different Modes of Migration

The development of the cerebral cortex nicely illustrates how the different modes of neuronal migration contribute to the formation of complex circuits in the CNS. The cortex contains two main classes of neurons, the glutamatergic pyramidal neurons and the γ-aminobutyric acid (GABA)-containing neurons. Both classes of neurons use largely different modes of migration to reach their final position in the cortex during development (reviewed in Corbin *et al.*, 2001; Marín and Rubenstein, 2001). Thus, pyramidal neurons migrate radially from the progenitor zones of the pallium to their final position in the cortex. In contrast, interneurons are largely born in progenitor regions of the subpallium and therefore have to migrate tangentially to reach the pallium. Once in the pallium, interneurons change their mode of migration from tangential to radial to reach their final destination in the cortex. Thus, projection neurons and interneurons use different modes of migration to arrive at the cerebral cortex largely because they derive from segregated progenitors within the telencephalon.

What advantage might there be in producing different classes of neurons at distant places in the CNS instead of producing all of them locally for each brain structure? This question might be answered if we consider that cell patterning and migration are intimately linked during the development of the CNS throughout evolution. In the telencephalon, for example, early dorsoventral patterning specifies distinct domains that produce neurons synthesizing different classes of neurotransmitters (reviewed in Wilson and Rubenstein, 2000; Campbell, 2003). Thus, the dorsal region of the telencephalon – the pallium – becomes patterned to produce glutamatergic neurons, whereas the subpallium is specified to generate GABAergic and cholinergic neurons. This organization is a primitive trend of the telencephalon in vertebrates, since it seems to be present in the different classes of living vertebrates (Puelles *et al.*, 2000; Frowein *et al.*, 2002; Gonzalez *et al.*, 2002; Brox *et al.*, 2003) and appears to represent an efficient way to pattern neural progenitors to produce different classes of neurons using a limited number of morphogenetic centers. Thus, patterning mechanisms that have been preserved throughout evolution appear to limit to some extent the generation of multiple classes of neurons in the exact same region of the brain, at least from the perspective of the neurotransmitter phenotype, and tangential

migration may have evolved, among other things, to overcome this limitation.

In mammals, the balance between excitatory (glutamatergic) and inhibitory (GABAergic) synaptic activity is critical for the normal functioning of the cerebral cortex. As a result, inherited disruption of this balance leads to important behavioral dysfunction in animal models (Liu *et al.*, 2000; Steinlein and Noebels, 2000; Powell *et al.*, 2003) and in severe neurological disorders in humans (Keverne, 1999; Lewis, 2000; Sanacora *et al.*, 2000; Holmes and Ben-Ari, 2001). In that context, the introduction of GABAergic interneurons from an external source to the population of cortical neurons may have played a pivotal role in shaping up neural circuits during the expansion of the cerebral cortex through evolution. A recent study by López-Bendito *et al.* (2006) has strongly suggested the convergence of these phenomena in the development of the thalamocortical system. This study has demonstrated the existence of a new tangential migration of GABAergic cells within the ventral telencephalon that mediates the navigation of thalamic axons toward their final destination in the neocortex. Specifically, tangential migration from an evolutionarily primitive intermediate target, the striatum, contributes to form a permissive bridge for the extension of thalamocortical axons through nonpermissive regions of the ventral telencephalon. In a more general sense, whereas radial migration has been preserved as the mechanism conferring regional identity to distinct structures in the CNS, tangential migration may represent a paradigm to increase the complexity of neuronal circuits during evolution. For instance, the casual incorporation of a migratory route that brings a new population of neurons into an established structure (e.g., through a mutation that induces the expression of a receptor for a guidance molecule in that specific population of neurons) may lead to a complete dysfunctional brain or, occasionally, to a modification of the normal function of the structure representing a competitive evolutionary advantage for the species. Such a mechanism may explain, for example, the differences observed in the number of GABAergic interneurons in the dorsal thalamus of primates – in particular humans – compared to other vertebrates (Letinic and Rakic, 2001). Moreover, the identification of a neocortical origin for a population of GABAergic neurons in the developing human cortex reinforces the existence of such evolutionary trend (Letinic *et al.*, 2002).

5.4 Mechanisms of Radial Migration

Radial migration has classically been known as glial-guided cell migration because during this process neurons move along the processes of specialized glial cells known as radial glia (Rakic, 1971a, 1971b, 1972; Rakic *et al.*, 1974; Edmondson and Hatten, 1987). Despite their name, however, radial glial cells do not simply function as static supportive elements. Instead, radial glial cells represent an intermediate stage in the stem cell lineage of the CNS (reviewed in Alvarez-Buylla *et al.*, 2001) and undergo mitosis to produce new neurons (Noctor *et al.*, 2001). In addition, radial glial cells have a process that spans the wall of the neural tube and reaches the pial surface (Bergman glial cells being one exception to this rule), where it is anchored to the basal membrane. This process establishes a point-to-point relation between the ventricular zone and the surface of the brain, supporting neuronal movement during radial migration. Genetic defects affecting the development of radial glia cells lead to abnormal neuronal migration in the CNS (reviewed in Ross and Walsh, 2001; Marín and Rubenstein, 2003), suggesting that radial glia integrity is fundamental for radial migration.

Although radial glia integrity is largely essential for radial migration, there seem to be exceptions to the rule described above. During early stages of corticogenesis, for example, new neurons undergo radial migration through a process known as somal translocation (described as perikaryal translocation by Morest, 1970), which appears to be largely independent of radial glial cells (reviewed in Nadarajah and Parnavelas, 2002). During somal translocation, the leading process of migrating cells terminates at the pial surface and it becomes progressively shorter as the cells approach their final position. This is also observed in cells moving through glial-guided radial migration as they approach the pial surface. Thus, for some cell types or specific developmental periods, radial migration may not directly depend on radial glial cells.

Radial migration has been preferentially studied during the development of the cerebral cortex and the cerebellum and thus most of our knowledge on the mechanisms that control radial migration derives from the analysis of these structures. *In vitro* and *in vivo* studies of radial cell migration have identified a number of molecules that mediate this mode of migration. These molecules belong to multiple categories, including motogenic factors (i.e., factors that promote migration), cell adhesion molecules, receptors, and secreted factors, some of which are described below. We have excluded from this list molecules controlling those aspects of migration that are likely to be common to any type of neuronal migration (e.g., LIS1, DCX; see Section 5.2), even though they have been classically

associated with radial migration defects. In that context, it is worth noting that neuronal migration abnormalities are likely to be more easily identified in laminar than in nuclear structures; this does not exclude, however, a role for these molecules in other types of migration (see, for example, McManus *et al.*, 2004b; Pancoast *et al.*, 2005).

Several classes of molecules have been described to stimulate radial migration. In the cerebral cortex, for example, members of the neurotrophin family such as brain-derived neurotrophic factor (BDNF) and neurotrophin-4 (NT-4) promote the motility of cortical cells through their high-affinity receptor tyrosine kinase B (TrkB) (Behar *et al.*, 1997; Brunstrom *et al.*, 1997). Other factors, such as (GABA) and glutamate, also appear to promote the migration of cortical neurons *in vitro*. These neurotransmitters are released independently of the conventional soluble N-ethylmaleimide sensitive fusion protein attachment protein receptor (SNARE)-dependent mode of secretion – probably through a paracrine mechanism – and mediate their effects primarily through the activation of $GABA_A$ and N-methyl-D-aspartate (NMDA) receptors (Komuro and Rakic, 1993; Behar *et al.*, 1996, 1999, 2001; Manent *et al.*, 2005).

To a large extent, most factors directly involved in controlling radial migration are molecules that regulate the interaction between migrating neurons and radial glial. This is the case of Astrotactin-1 (Astn1), which was first identified as an activity mediating the interaction of neurons and radial glial processes in cerebellar cultures (Edmondson *et al.*, 1988). Astn1 is a glycoprotein expressed by migrating neurons both in the cerebellum and in the cerebral cortex and it is required for normal migration of neuroblasts along glial processes (reviewed in Hatten, 2002). Integrins constitute another family of factors implicated in the association between migrating neurons and radial glia. Thus, function-blocking antibodies against α3, αv, and β1 integrins perturb the interaction between neurons and radial glial cells *in vitro* (Anton *et al.*, 1999; Dulabon *et al.*, 2000). Moreover, radial migration is altered in the cerebral cortex of α3, α6, or β1 integrin mutant mice (Georges-Labouesse *et al.*, 1998; Anton *et al.*, 1999; Graus-Porta *et al.*, 2001), although the precise function of integrins during *in vivo* radial migration remains unsettled. In the case of α3 integrin, however, it has been suggested that signaling through this receptor may directly control actin dynamics and consequently influence the ability of migrating neurons to search and respond to guidance cues in the developing cortex (Schmid *et al.*, 2004).

The interaction between migrating neurons and radial glial fibers may also be controlled through intracellular signaling cascades. For example, correct apposition of neurons to radial fibers may largely depend on the morphology of migrating neurons. In the cerebral cortex, migrating neurons are largely bipolar, which possibly facilitates their interaction with radial glial processes. In the absence of p35, a regulatory activator of Cdk5 that controls the function of many proteins associated with the cytoskeleton, the leading process of radially migrating neurons is branched, and this associates with an impaired neuronal–glia interaction and perturbed migration (Gupta *et al.*, 2003). Thus, the morphological organization of migrating neurons might be an important factor in determining their mode of migration.

The interaction between migrating neurons and radial glial processes is important not only for the initiation and maintenance of radial migration, but also for the control of its finalization. The precise termination of radial migration is crucial for the normal organization of brain structures. This is more evident in cortical structures, in which the pattern of radial migration termination determines the establishment of the laminar organization. In the case of the isocortex, birth-dating studies have shown that layers in the cortical plate (future cortical layers 2–6) are established according to an inside–outside pattern, where the deeper layers contain cells that become postmitotic earlier than the cells in more superficial layers (Angevine and Sidman, 1961; Rakic, 1974). During development, new neurons migrate radially toward the surface of the cortex, passing through cohorts of previously born neurons, and detach from radial glia as they approach the marginal zone. Analysis of mutations in mice and humans has revealed that the interaction between migrating neurons and Cajal–Retzius cells, a specialized cell type present in the embryonic marginal zone, is essential for controlling the detachment of migrating neurons from radial glia and, subsequently, the normal laminar organization of the cortex (reviewed in Gupta *et al.*, 2002; Marín and Rubenstein, 2003).

The interaction between Cajal–Retzius cells and radially migrating neurons is mediated, at least in part, by Reelin, a large glycoprotein secreted by Cajal–Retzius cells during early stages of the development of the cortex. Reelin is expressed in many regions of the developing brain and in many species of vertebrates, but its function has been most extensively studied in the developing cortex. Reelin is a high-affinity ligand for two members of the LDL family of lipoprotein receptors, the very low-density lipoprotein receptor (VLDLR) and the low-density

lipoprotein receptor-related protein 8 (LRP8, also known as ApoER2), which are expressed by radially migrating cortical neurons (D'Arcangelo *et al.*, 1999; Hiesberger *et al.*, 1999). Signaling through VLDLR/LRP8 mediates tyrosine phosphorylation of the mouse homologue of the *Drosophila* protein Disabled (DAB1). DAB1 is a cytoplasmic adapter protein that interacts with the cytoplasmic tails of VLDLR and LRP8 and is linked to events related to the reorganization of the cytoskeleton.

Although many aspects of the function of the Reelin–VLDLR/ApoER2–Dab1 pathway in radial migration remain unsettled, it is clear that Reelin signaling is involved in the final events that lead to the detachment of migrating neurons from radial glia. Loss of Dab1 function, for example, results in an impairment of the adhesive properties of radially migrating neurons, which fail to detach normally from the glial fiber in the later stage of migration (Sanada *et al.*, 2004). Importantly, the influence of Reelin on the adhesive properties of radially migrating neurons may be the result of its interaction with other proteins, such as $\alpha 3\beta 1$ integrin receptors, which are expressed in radially migrating neurons (Dulabon *et al.*, 2000). It should be noted, however, that Reelin function is likely not restricted to controlling the interaction between migrating neurons and glial fibers.

It is likely that the Reelin–VLDLR/ApoER2–Dab1 pathway is just one of many signaling routes controlling neuronal detachment from radial glia and movement termination in the cerebral cortex. Thus, other proteins that are specifically expressed in radial glial processes at the level of the cortical plate are also candidates for the regulation of this process. One of these proteins is secreted protein acidic and rich in cysteine-like 1 (SPARC-like 1), which appears to function in ending neuronal migration by reducing the adhesiveness of neurons to glial fibers in the cortical plate (Gongidi *et al.*, 2004).

5.5 Mechanisms of Tangential Migration

Tangential migration, defined as a nonradial mode of migration, includes distinct types of cell movement that differ in the type of substrate used by migrating cells. Regardless of the substrate employed, tangentially migrating cells share an important common feature: they do not respect regional forebrain boundaries. Thus, cell populations engaged in tangential migration normally move over long distances and follow complex trajectories before reaching their final destination. These migrations usually involve multiple changes in the direction of the movement, which depend on changes in the environment and/or the responses of migrating neurons. In the past few years, many studies have demonstrated the existence of environmental cues that can act as contact or diffusible attractants or repellents that provide directional information to tangentially migrating neurons through interactions with cell-surface receptors (see Table 1). Here, the cellular and molecular mechanisms controlling tangential migration are reviewed using as examples two well-characterized tangential migratory populations, cortical interneurons and facial branchiomotor neurons.

5.5.1 Migration of Cortical Interneurons

The tangential migration of cortical interneurons to the cortex is, most likely, one of the most intensively studied cell populations of the developing brain since the seminal discovery of their subpallial origin in mammals (Anderson *et al.*, 1997). Since then, several other studies have shown that a subpallial origin of cortical interneurons is a common feature to, at least, tetrapod vertebrates (Cobos *et al.*, 2001; Gonzalez *et al.*, 2002; Brox *et al.*, 2003), suggesting that this is a highly conserved trait in cortical evolution.

Cells migrating tangentially to the cortex have multiple origins within the subpallium (reviewed in Corbin *et al.*, 2001; Marín and Rubenstein, 2001), although most GABAergic interneurons seem to derive from the medial ganglionic eminence (MGE). Interestingly, the MGE is also the source of interneurons for other forebrain structures, such as the striatum (Marín *et al.*, 2000; Wichterle *et al.*, 2001). Consequently, most of our knowledge on the mechanisms controlling the migration of cortical interneurons refers to MGE-derived cells and it is likely that different molecules may control the migration of interneurons generated in other subpallial structures, such as the caudal ganglionic eminence (Nery *et al.*, 2002).

There are several key decision points affecting the migration MGE-derived cortical interneurons. First, interneurons initiate their migration in response to factors that stimulate their movement. Second, interneurons refrain from migrating in ventral and ventromedial regions – thus avoiding the preoptic area and the septum – directing instead their movement in a dorsal direction. Third, cortical interneurons actively avoid entering the developing striatum, a target for other classes of MGE-derived interneurons. Early during development, interneurons tend to migrate superficial to the striatal mantle. However, as development proceeds and the dorsal striatum becomes a large structure in the basal ganglia, cortical interneurons migrate preferentially deep

Table 1 Guidance factors and neuronal migration in the CNS

Gene	*Function*	*Neuronal population*	*Refs.*
BDNF, NT4	Growth factor; motogenic; promotes neuronal migration	Cortical interneurons, cortical projection neurons	*a*
SDF1	Chemokine; chemoattractant	Cerebellar granule cells, dentate granule cells, cortical interneurons	*b*
EphrinB2, EphrinB3	Guidance molecules; chemorepellents	Rostral migratory stream	*c*
GABA	Neurotransmitter; chemoattractant	Cortical interneurons, cortical projection neurons	*d*
Glutamate	Neurotransmitter; promotes neuronal migration	Cortical projection neurons	*e*
Gdnf	Growth factor; motogenic; promotes neuronal migration	Cortical interneurons	*f*
Hgf	Growth factor; motogenic; promotes scattering of neurons	Cortical interneurons	*g*
Netrin-1	Guidance molecule; chemoattractant and chemorepellent	Basilar pontine neurons, precerebellar nuclei, cerebellar granule cells, striatal projection neurons	*h*
Nrg1	Guidance molecule; chemoattractant and permissive factor	Cortical interneurons, rostral migratory stream	*i*
Sema3A, Sema3F	Guidance molecules; chemorepellents	Cortical interneurons	*j*
Somatostatin	Motogenic; movement promotion and termination	Cerebellar granule cells	*k*
Slit1, Slit2	Guidance molecules; chemorepellents	Rostral migratory stream, different classes of neurons derived from the subpallium	*l*

[a]Brunstrom *et al.* (1997); Polleux *et al.* (2002).
[b]Zou *et al.* (1998); Bagri *et al.* (2002); Stumm *et al.* (2003).
[c]Conover *et al.* (2000).
[d]Behar *et al.* (1996, 2000); López-Bendito *et al.* (2003); Luján *et al.* (2005).
[e]Hirai *et al.* (1999).
[f]Pozas and Ibañez (2005).
[g]Powell *et al.* (2001).
[h]Bloch-Gallego *et al.* (1999); Yee *et al.* (1999); Alcántara *et al.* (2000); Hamasaki *et al.* (2001).
[i]Anton *et al.* (2004); Flames *et al.* (2004).
[j]Marín *et al.* (2001); Tamamaki *et al.* (2003).
[k]Yacubova and Komuro (2002).
[l]Hu (1999); Wu *et al.* (1999); Zhu *et al.* (1999); Gilthorpe *et al.* (2002); Marín *et al.* (2003).

to the striatal mantle (i.e., through the interface between the SVZ of the lateral ganglionic eminence and the striatal mantle). Fourth, interneurons cross the subpallial–pallial boundary, invading the pallium through highly stereotyped routes of migrating, which include the marginal zone, the subplate, and the cortical SVZ. And fifth, interneurons invade the cortical plate and integrate in their appropriate layer according to their birth date. Thus, the migration of cortical interneurons is a complex and well-orchestrated event in the developing forebrain. So, what are the molecular cues that regulate each of these decisions?

Cortical interneurons initiate their movement and engage in long-distance migration probably because they respond to motogenic/scatter factors along their pathway. Several such factors have been identified in the past few years, all of which have in common their ability to also influence the maturation and final differentiation of cortical interneurons. Thus, the neurotrophins BDNF and neurotrophin-4, the scattered factor/hepatocyte growth factor, and the glial-derived neurotrophic factor (GDNF) all have the ability to promote interneuron migration to the cortex (Powell *et al.*, 2001; Polleux *et al.*, 2002; Pozas and Ibañez, 2005).

Cortical interneuron migration to the cortex is strongly influenced by molecular activities that prevent interneuron invasion of unsolicited regions. This is the case in the preoptic area and the septum, where the molecular nature of the repulsive activity preventing the migration of interneurons is still unknown (Marín *et al.*, 2003). The striatum constitutes a nonpermissive territory for the migration of cortical interneurons because it expresses class 3 semaphorins (Sema3A and Sema3F) and cortical interneurons express neuropilin receptors for these repellent cues (Marín *et al.*, 2001; Tamamaki *et al.*, 2003).

Cortical interneuron migration is also controlled by permissive and attractive factors that direct

interneurons in a dorsal direction from the MGE to the cortex (Marín *et al.*, 2003; Wichterle *et al.*, 2003). However, for the first time, a chemoattractive effect on cortical interneurons by a molecule expressed at the cortex has been described. This cue is Neuregulin-1 (NRG1), a member of the neuregulin family of proteins. Of note, different isoforms of NRG1 are differentially expressed in the developing telencephalon, thus controlling distinct aspects of the migration of cortical interneurons (Flames *et al.*, 2004). Thus, membrane-bound forms of NRG1, Cystein-rich domain (CRD)-NRG1, are expressed in the route of interneuron migration from the MGE to the pallial–subpallial boundary, and it seems to create a permissive corridor for interneuron migration toward the cortex. In addition, diffusible forms of NRG1, Ig-NRG1, are specifically expressed in the cortex, from where they appear to attract interneuron migration. Other factors are likely to attract interneuron migration to the cortex. For example, GDNF also acts as an attractive cue for interneuron migration *in vitro* (Pozas and Ibañez, 2005), although its wide distribution in the telencephalon suggests that it may rather act as motogenic factor *in vivo*.

It has been suggested that cortical interneurons may use corticofugal axons as a substrate for their migration to the cortex. Axons have been proposed as substrates for other tangentially migrating cell populations, such as gonadotropin-releasing hormone neurons (Wray, 2002), and *in vitro* evidence suggests that axons may serve as substrates for the migration of cortical interneurons (McManus *et al.*, 2004a). Moreover, molecules specifically expressed in corticofugal axons appear to influence interneuron migration *in vitro* (Denaxa *et al.*, 2001). At the peak of interneuron migration, however, most cells migrate through axon-poor regions such as the cortical SVZ, suggesting that axons may influence primarily early stages of interneuron migration to the cortex.

The guidance of cortical interneurons may also be influenced by neuronal activity. In agreement with this hypothesis, several studies have described the early expression of GABA and glutamate receptors at the cerebral cortex before the formation of synapses (Métin *et al.*, 2000; López-Bendito *et al.*, 2002a, 2002b; Luján *et al.*, 2005). The function of some of these receptors has been tested *in vitro*. For example, stimulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in slice cultures induces GABA release in tangentially migrating cells (Poluch and Konig, 2002). In contrast, *in vitro* blockade of $GABA_B$ receptors leads to a derailment of GABAergic interneurons within the neocortex (López-Bendito *et al.*, 2003). Others neurotransmitter receptors, such as NMDA, AMPA/Kainate, and $GABA_A$, are also functional on tangentially migratory interneurons (Métin *et al.*, 2000; Soria and Valdeolmillos, 2002), suggesting that they also influence the migration cortical interneurons through a yet unknown mechanism.

Once interneurons reach the cortex, they invade the cortical plate and distribute through the different cortical layers. Interestingly, invasion of the cortical plate does not occur automatically as interneurons reach the cortex, but rather seems to be a highly stereotyped process designed to allow the homogeneous dispersion of cortical interneurons throughout the whole rostrocaudal and mediolateral extent of the cerebral cortex (G. López-Bendito and O. Marín, unpublished observations). In addition, invasion of the cortical plate by interneurons may depend on radial glia (Ang *et al.*, 2003; López-Bendito *et al.*, 2004; Tanaka *et al.*, 2003) and thus on the mechanisms described for cortical pyramidal cells (see Section 5.4). Nevertheless, some of the molecules that influence migration of projection neurons, such as Cdk5, do not seem to influence the tangential migration of cortical interneurons or their subsequent movement into the cortical plate (Gilmore and Herrup, 2001).

5.5.2 Migration of Facial Branchiomotor Neurons

Tangential cell movements during CNS development are not restricted to forebrain. Indeed, tangential migration is present at all rostrocaudal levels of the neural axis. In the hindbrain, for example, the facial (nVII) branchiomotor neurons of several vertebrates, including fish and mammals, follow a large stereotyped migration that includes tangential migration from their origin in r4 to caudal r6 or r7 (reviewed in Chandrasekhar, 2004). Tangentially migrating fbm neurons use a mode of migration very similar to the somal translocation described in the cortex (Book and Morest, 1990), in which the nucleus moves along a large leading extension as the migrating cell leaves behind an axonal process that reflects the migratory path.

It has been shown that environmental cues present in r5 and r6 mediate fbm neuronal tangential migration. Interestingly, chick fbm neurons undergo limited caudal migration naturally; however, transplantation studies have demonstrated that these cells have the ability to migrate caudally when transplanted into mouse r4, demonstrating that the cues necessary for the initiation, and perhaps maintenance, of caudal migration are absent in the chick

hindbrain (Studer, 2001). Additional evidence for environmental cues regulating fbm migration comes from genetic and molecular studies in zebra fish. In the zebra fish mutant *trilobite* (*tri*), fbm neurons fail to migrate tangentially into r5–7 (Bingham *et al.*, 2002). This phenotype, however, can be rescued when *tri* mutant fbm neurons are transplanted into a wild-type environment, whereas wild-type fbm neurons fail to migrate caudally in a mutant context. What molecules are responsible for this behavior? Tangentially migrating fbm neurons regulate the expression of genes encoding the cell membrane proteins, such as TAG-1, Ret, and Cadherin-8, and this regulation is dependent on their location at r4, r5, or r6 (Garel *et al.*, 2000). Interestingly, in embryos deficient for *Ebf1* or *Nkx6-1*, fbm neurons either fail to migrate or undergo an incomplete caudal migration, prematurely expressing an abnormal combination of markers (Garel *et al.*, 2000). These data suggest that fbm neurons adapt to their changing environment by switching on and off specific genes.

Finally, studies have shown that tangential migration of fbm neurons is controlled by neuropilin receptors, as is the case for cortical interneurons. Thus, loss of Neuropilin-1 (Nrp1) in the mouse compromises the tangential migration of fbm neurons, causing the formation of misshapen and malpositioned facial motor nuclei. In contrast to cortical interneurons, however, which rely on class 3 semaphorins for their guidance, soma migration of fbm neurons relies on the presence of a structurally unrelated Nrp1 ligand, an isoform of vascular endothelial growth factor (VEGF) termed VEGF164 (Schwarz *et al.*, 2004).

5.6 Migration in the Postnatal Brain

Neuronal precursor cells persist in the adult vertebrate forebrain and thus new neurons are continuously added to restricted regions, such as the olfactory bulb and hippocampus in mammals. Consequently, neuronal migration is not restricted to the embryonic milieu but also exists in an adult brain environment. Because the latter is thought to be largely a nonpermissive territory for cell movement – with the obvious exception of cancer cells – migration is restricted to very specific permissive pathways within the brain.

Perhaps the best-known example of adult neurogenesis and neuronal migration is among the first discovered, that of the adult songbird forebrain (Goldman and Nottebohm, 1983). Songbirds display widespread neurogenesis and migration during adulthood, most remarkably in an area of the telencephalon involved in song learning, the higher vocal center (HVC). Studies carried out by Alvarez-Buylla and colleagues (Alvarez-Buylla and Nottebohm, 1988) showed that neurons originating in the SVZ migrate to the cortex when new neurons are added to the songbird hippocampus and HVC, in a process involving the guidance of radial fibers. Moreover, both diffusible and substrate-bound molecules control this migration through a set of hormonally regulated short-distance cell–cell interactions.

In contrast to the relatively widespread neurogenesis found in songbirds, the adult mammalian forebrain utilizes progenitors to generate new neurons destined for very few regions. Specifically, the SVZ of the lateral ventricle and the dentate gyrus subgranular zone (SGZ) of the hippocampus are the regions where adult neurogenesis has been demonstrated (reviewed in Gage, 2000; Alvarez-Buylla and Lim, 2004). The adult SVZ produces new GABAergic interneurons for the olfactory bulb, whereas the SGZ gives rise to granule cells of the hippocampus. Despite the hostile territory that the adult brain represents for migration, new neurons in the hippocampus have a relatively easy path to their final destination, because they are very close to their final location. A different case is the migration of olfactory interneurons, which need to navigate through an extremely long distance from the SVZ to the olfactory bulb.

In contrast to the findings regarding neurogenesis and neuronal migration in songbirds, radial glia cells do not guide the postnatal migration of newly born cells. Instead, olfactory interneurons migrate using a cellular process called chain migration, which involves homotypic interactions between the migrating cells and tubular structures formed by specialized astrocytes (Lois *et al.*, 1996). This migration occurs through a highly restricted route termed the rostral migratory stream (RMS). Like other cell populations in the embryonic brain, tangentially migrating olfactory interneuron precursors change the direction of movement on arriving in the olfactory bulb, migrating radially into specific layers.

Defining the diffusible or membrane-bound factors that guide the tangential migration of new interneurons from the adult SVZ to the olfactory bulb is a very active field in developmental neurobiology. A polysialated glycoprotein neuronal cell adhesion molecule (PSA–N-CAM) is highly expressed on the surface of olfactory migrating neurons and it has been shown that deletion of the gene for N-CAM or enzymatic removal of PSA results in deficits in the migration of olfactory interneurons and a reduction in the size of the olfactory bulb

(Cremer *et al.*, 1994; Ono *et al.*, 1994). Evidence suggests that PSA and/or N-CAM may not be essential for chain formation but, without them, there are several alterations in the nature of the chains that may inhibit the migration of neuronal precursors (Hu *et al.*, 1996). Several additional adhesion molecules have been identified in the migratory route of olfactory interneuron precursors. For example, Tenascin-C, a ligand for αvβ3 and αvβ6 integrins, is strongly expressed in the astrocytes that form the tubes through which olfactory precursors migrate in the RMS (Jankovski and Sotelo, 1996), and αv-, β3-, and β6-integrin subunits are also present in the postnatal RMS. Nevertheless, the lack of abnormalities in the olfactory bulb of mice with individual mutations for some of these molecules prevents a more definitive evaluation of the function of these proteins *in vivo*.

The molecular mechanisms guiding the highly directed migration of olfactory interneurons in the RMS are still unclear, although both attractive and repulsive guidance cues have been proposed to mediate this process. Among the repellents, Slit proteins have been shown to repel SVZ-derived cells *in vitro* (Hu, 1999; Wu *et al.*, 1999). In addition, evidence demonstrates that the activation of the receptor tyrosine kinase ErbB4 is essential for regulating the organization of neural chains in the RMS and therefore their migration (Anton *et al.*, 2004). It seems evident that other molecules are likely to be involved in this process – future experiments will determine their molecular nature.

5.7 Conclusions

Studies on the cell biology of neuronal migration suggest that migrating neurons share many common mechanisms with other migrating cell types in the vertebrate body, although additional experiments are required to comprehensively decipher the cellular and molecular components of the migratory machinery in neurons. Regardless of the cell biological mechanisms, distinct modes of migration exist in the embryonic and adult brains, which seem to be adapted to fulfill different functions during evolution. Thus, whereas radial migration may have evolved as a mechanism to preserve the identity of different regions in the developing brain, tangential migration may have provided a means to increase the complexity of neural circuits during evolution.

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6 The Role of Transient Connections in Brain Evolution

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Glossary

connection In this article, connection is used to refer to situations for which the existence of synapses was demonstrated or appears very probable on grounds of electron micro scopic data or light microscopic data with sufficient resolution.

projection This refers to situations where the demon stration is missing, for example due to the use of retrogradely transported tract tra cers. Notice that the distinction is justified for the developing brain, although it is often unclear whether a certain projection is actu ally forming synapses. In most of this article, the terms are synonymous.

6.1 Introduction

Developmental mechanisms, some of which have needed retuning while others have been maintained, have both permitted and constrained evolution. It is therefore legitimate to ask which developmental mechanisms were modified and which were maintained in the evolution of the cerebral cortex. Comparative studies suggest that two processes dominated the evolution of cerebral cortex: tangential expansion, and increased regional differentiation. The most commonly proposed scenario, accounting for the increased tangential expansion of the cerebral cortex, is an increased period of symmetrical divisions in the proliferative ventricular and subventricular zones. Regional differentiation, however, is multifaceted and the emergence of a new cortical area in the course of evolution must have required the coordination of several different developmental processes.

A primary cause of regional differences in the cortical mantle is probably the differential expressions of genes along tangential gradients. It is unclear, however, how the regionalization of the cortical mantle, presumably caused by gene expression, corresponds to the parcellation of cerebral cortex into structurally and functionally distinct areas, that is, into its arealization. Indeed, the identity of a cortical area is defined by a large set of morphological and functional criteria. The morphological criteria include not only cytoarchitectonics, myeloarchitectonics, and sometimes molecular differences, but also, most importantly, differences in connectivity with thalamic nuclei and with other cortical regions. The functional criteria include the sensory, motor, or cognitive consequences of lesions, the response properties of individual neurons, and the patterns of activation during specific tasks. Assuming that the patterns of genetic expression might be the primary determinants of cortical regionalization, the question is: which other changes in developmental processes were required to achieve the full set of local differentiations that characterize a cortical area?

As mentioned above, connectivity is a central feature in the definition of a cortical area. In addition, however, it also determines some of the other criteria that define a cortical area, in particular its architectonics, the response properties of its neurons, and their participation in specific functional neuronal assemblies and/or processing streams. Thus, the appearance of a new cortical area in

evolution required adjustments of cortical connectivity. These adjustments, in turn, were a major factor in determining arealization.

This article illustrates how the development of cortical connectivity is based on the following mechanisms: (1) exuberant development, i.e., initial distribution of cortical axons to territories wider than in the adult; (2) selection, based on specific axon/pathway and axon/target recognition mechanisms, as well as on axoaxonal interactions; and (3) selection/validation of the connections by activity.

I claim that the algorithms of connectional development listed above, while maintaining a coherent Bauplan across the mammalian radiation, provided the degree of flexibility required to accommodate genetically based regionalization of the cerebral cortex and thus played a major role in the emergence of new cortical areas. The article is mainly restricted to data and concepts that appeared within the last 10–15 years, since the older literature has been reviewed previously (Innocenti, 1991; O'Leary, 1992).

6.2 Macroscopic versus Microscopic Exuberance in the Development of Connections

Structural exuberance in development includes the overproduction of neurons and non-neuronal cells, as well as that of cellular components, in particular axons and/or axon collaterals, synaptic boutons, dendritic branches, and spines. The common theme is that a part or all of the juvenile structures are eliminated at later stages of development.

Leaving overproduction of neurons aside, two kinds of developmental exuberance have been described over the last 30 years. Both are involved in the construction of neural circuits.

Macroscopic exuberance refers to the formation of transient projections (and/or connections) between macroscopic partitions of the brain; it includes transient afferent and efferent projections between a cortical site and other macroscopic subdivisions of the brain, such as cerebellum, subcortical nuclei, spinal cord, or cortical areas. Microscopic exuberance refers to the formation, within a restricted cortical territory, of transient structures involved in the communication between neurons; it includes the formation of transient axonal and dendritic branches and/or synapses. Some of the transient structures, but not all, are formed within layers and/or columns where they are no longer found in the adult.

The distinction between the two types of exuberance is not always sharp, and it is essentially based on the methods used. In particular, in studies of synaptic counts, performed at the electron microscopic level, the origin of the supernumerary synapses could not be determined. In some systems of connection there is a smooth transition from macroscopic to microscopic exuberance: the production of exuberant structures becomes progressively more topographically circumscribed, as if the target was reached by progressively refined approximations.

6.3 Methodological Issues

Neuronal connections are usually assessed by tracers that are either actively transported or diffuse along axons driven by concentration gradients. Nevertheless, the usage of most tracers in the developing brain can be problematic. Uptake, transport, and diffusion of tracers can vary with age. This is particularly true with the lipophilic tracers of the DiI-DiO family, which tend to mark better the young unmyelinated axons and much less, or not at all, the older and myelinated ones. The existence of tracer-permeable gap junctions and leaky membranes in the young brain raises the possibility of transneuronal diffusion of tracers, producing false-positive results. Finally, tracers tend to be less effectively taken up and/or transported in the young nerve tissue, therefore failing to visualize connections which, if more mature, can be readily visualized. In some of the studies summarized below, particularly for the callosal connections of the cat, the same projections were studied at different ages with different tracers (horseradish peroxidase (HRP), wheat germ agglutinin (WGA)-HRP, fluorescent tracers, including fast blue and diamidino yellow, fluorescent beads, lipophilic tracers, and biocytin). Unfortunately this is not the case for other projections. The possibility that some juvenile connections may have been missed due to insufficiently sensitive tracing conditions must be kept in mind in the interpretation of negative results.

Another difficulty in using tracers in the developing brain is that certain target structures can undergo complex reshaping due to displacement of neuronal populations. Thus, what might appear to be a transient projection could in fact be a projection to the appropriate target, but which has not yet reached its final location. A protection against the latter type of artifact is provided by tracers that can remain in the neurons for a long time, without being metabolized or eliminated. Tracers of this kind, e.g., fast blue and fluorescent beads, allow us to take snapshots of the state of the same connection at different developmental stages (Innocenti, 1991;

O'Leary, 1992). They also permit the differentiation of neuronal death from axonal elimination in the deletion of the transient connections.

While the magnitude of exuberance and elimination can be difficult to estimate, it is clear that some projections are fully eliminated. This is the case, for example, in the corticofugal projections to the spinal cord or to the cerebellum. The most satisfactory quantification, however, comes from electron microscopic studies. These studies have shown a loss of 70% of the callosal axons in both cat and monkey, although this figure might still underestimate the real loss (below).

6.4 Exuberant Projections/Connections in Cortical Development

The first demonstration of macroscopic exuberance in development, i.e., of the formation of long/transient projections, came from the study of connections between the visual areas of the two hemispheres in the cat (Innocenti *et al.*, 1977; reviewed in Innocenti, 1991; O'Leary, 1992). Parts of areas 17 and 18 devoid of callosal connections in the adult exhibited transient projections to the contralateral hemisphere at birth. The elimination of the projections was fast, and was mostly terminated by postnatal day 21, although some elimination might have continued until day 30 and beyond. The elimination was due to selective loss of axons and the neurons, giving rise to the transient callosal projections, forming permanent connections in the ipsilateral hemisphere, by selection of collaterals. These findings were confirmed and extended by the demonstration of exuberant callosal connections between the somatosensory areas, and of projections from cerebral cortex to the spinal cord, cerebellum, and to other cortical areas. Particularly striking among the latter was the discovery of transient projections from the auditory to the visual cortex in both hemispheres in the cat (reviewed in Innocenti, 1991). Equally striking was the more recent demonstration of exuberant projections from the temporal cortex of the monkey into limbic structures (Webster *et al.*, 1991a, 1991b). Finally, the work of several groups focused attention on the microscopic aspects of exuberant connections by showing how elimination of axon collaterals of pyramidal neurons leads to the formation of local, clustered connections. Exuberant intra-areal axons were also described: within sublayers, in the monkey area 17, and as long tangential axons spanning the white matter under area 17 in the cat. In the cat, callosal axons, both those that are maintained and those that are later eliminated, initially form transient branches, first in the subplate and then in the gray matter (Aggoun-Zouaoui *et al.*, 1996; Bressoud and Innocenti, 1999). In addition, the number of synaptic boutons produced in the gray matter overshoots the adult number (Aggoun-Zouaoui *et al.*, 1996; Bressoud and Innocenti, 1999). As mentioned above, it appears that the production of exuberant axonal structures becomes progressively more topographically circumscribed, as if the target were reached by correspondingly more refined approximations. Similar events were described in the striate and extrastriate areas of the cat (Bressoud and Innocenti, 1999).

6.5 Exuberance and Selection versus Connectional Specificity

Developmental exuberance in no way excludes the existence of selectivity and order in the formation of the juvenile projections/connections. Both might be the expression of cellular specificities responsible for guiding growing axons along given pathways and determining their choice of a target. Most of the evidence has been gathered in studies of visual callosal connections in the cat.

First, the juvenile corticocortical connections, including the exuberant ones, are topographically organized from their early stages. Thus, injections of tracers spaced in the anteroposterior direction in one hemisphere label correspondingly spaced territories in the other hemisphere. Second, the cortical projections exhibit laminar specificity from the earliest stages in development. Corticocortical axons mainly originate from layers 3 and 6, although the relative contribution of the two layers to a given projection can change in development, due to the elimination of exuberant projections from layer 3. Third, from the earliest stages of their development, cortical axons can be classified into different types based on their pattern of projection to areas in the contralateral hemisphere. Interestingly, this targeting specificity includes axons that establish transient projections. This suggests that the whole projection, including both the transient and the permanent fractions, consists of a mosaic of cell types with different growth/targeting specificities (Bressoud and Innocenti, 1999). Finally, origin-to-target selectivity is expressed at the time axons grow near, and into their terminal sites. Irrespective of their final fate, both callosal and intrahemispheric axons reach the white matter/gray matter border,

which contains a largely transient neuronal population, the subplate, where they branch profusely. Then, the axons to be maintained invade the gray matter, where they develop terminal arbors and synapses, while the transient axons remain mainly in the white matter and are subsequently eliminated.

After entering the gray matter, axons exhibit further specific growth. Axonal branching and the formation of synapses are progressively focused on the sites of adult termination, although transient branches and synapses are also formed (Bressoud and Innocenti, 1999). The overproduction and elimination of synapses occur without noticeable changes in the topography of the connections, although presumably it modifies the strength of the connections.

6.6 Testing the Role of Exuberance in the Evolution of Cerebral Cortex

The hypothesis that exuberant development of connections could provide a permissive mechanism favoring cortical evolution can be tested against a number of potentially invalidating conditions. Exuberant development of connections should be found across species and systems. The fate of the juvenile connections, whether maintenance or elimination, should be modulated by factors that could have operated during the evolution. Finally, one might expect a greater developmental plasticity of cortical connections in areas that underwent the most massive evolution.

6.6.1 Exuberant Development Is Found across Phylogenetically Distant Species

Transient, exuberant projections/connections occur across all the mammalian species that have been studied (Table 1). Most of the studies have involved rodents, carnivores, and primates. However, exuberant corticocortical and/or corticofugal projections have also been demonstrated in rabbit and opossum. These findings can be mapped on to the evolutionary trees of the mammalian radiation (Figure 1). The fact that exuberant projections in development span widely across the mammalian radiation suggests that they were indeed present in the ancestors of most or all the extant mammals, as required by the hypothesis that they played a role in evolution.

6.6.2 Exuberant Development Is Found across Systems of Cortical Connections

Exuberant development occurs in several different types of cortical connections, including interhemispheric, intrahemispheric, and local connections, as well as the corticofugal connections. Sensory, motor, and association areas are involved. This is not to say that the magnitude of exuberance/elimination is the same for the different species, systems, and types of connection (Barone *et al.*, 1996), although cross-species comparisons must be made prudently, given the above-mentioned difficulties in the quantification of the transient projections. Furthermore, cross-species comparisons can be complicated by different speeds of axonal development. Interestingly, electron microscopic counts of callosal axons have provided similar estimates of elimination in cat and monkey (reviewed in Innocenti, 1991). However, this similarity might be fortuitous since both studies lack an estimate of the life span of individual axons. Obviously, if the life span of the transient axons differed in the two species, the quantitative estimates of axonal exuberance/elimination would have to be corrected.

The occurrence of developmental exuberance in the different kinds of cortical connections suggests that the newly emerging areas are able to establish an adequate complement of connections in evolution. However, different cortical territories might differ with respect to the type and/or amount of transient, exuberant projections they send and/or receive in development. These hypothetical differences might have favored the emergence of new cortical areas at some specific locations. Thus, precise estimates of the exuberant connectivity of the different cortical territories in a given species might hint at their evolutionary potential.

6.6.3 Multiple Factors Regulate the Maintenance/Elimination of Exuberant Connections

Transient connections can be maintained or eliminated by experimental manipulations of the developing cortex.

Although these conditions do not necessarily mimic the evolutionary history of the cerebral cortex, they highlight the mechanisms whose alteration in evolution could have affected the development of corticocortical connections. Particularly important is the evidence linking maintenance/elimination of the juvenile connections to information coming from the sensory periphery, since in evolution changes in body and brain had to be coordinated. Strong evidence has accumulated that maintenance and elimination of callosal connections may be under the control of thalamocortical input, conveying information originating more peripherally, in the retina, in the case of the visual system. Studies in the cat and in the

Table 1 Transient (exuberant) axonal projections in development cerebral cortex (1976 2004)

Species	*Thalamocortical*		*Callosal*		*Intrahemispheric/area*		*Corticofugals*	
	Macro	*Micro*	*Macro*	*Micro*	*Macro*	*Micro*	*Macro*	*Micro*
Rodents (rat, hamster, mouse)	38, 48, 51	61	30, 31, 38, 49, 52		38		1, 5, 14, 15, 29, 50, 54, 62	32, 47
Carnivores (cat, ferret)	7	33, 34, 35, 39	2, 6, 22, 24, 25, 26, 27, 28	2, 3, 6	4, 17, 19, 25, 56, 53	10, 11, 41, 42, 58	8, 40, 46, 63, 64	
Primates (rhesus)	16, 45	59	12, 18, 37		36, 65	60	23	
Others (rabbit, opossum)			13				9, 20, 21	

Macroexuberance refers to situations where the projection is probably due to long axons.
Microexuberance refers to situations where the projection is due to local branches or synapses. In some cases, however (e.g., in the case of electron microscopic synaptic counts or anterograde transport data), the two conditions cannot be easily differentiated.

Dehay *et al.* (1988a) reported exuberant callosal projections from area 18 of the rhesus monkey but not from area 17.

References: (1) Adams *et al.* (1983); (2) Aggoun-Zouaoui and Innocenti (1994); (3) Aggoun-Zouaoui *et al.* (1996); (4) Assal and Innocenti (1993); (5) Bates and Killackey (1984); (6) Bressoud and Innocenti (1999); (7) Bruce and Stein (1988); (8) Bruce (1993); (9) Cabana and Martin (1984); (10) Callaway and Katz (1990); (11) Callaway (1998); (12) Chalupa and Killackey (1989); (13) Chow *et al.* (1981); (14) Curfs *et al.* (1994); (15) D'Amato and Hicks (1978); (16) Darian-Smith and Darian-Smith (1993); (17) Dehay *et al.* (1984); (18) Dehay *et al.* (1988a); (19) Dehay *et al.* (1988b); (20) Del Caño *et al.* (1997); (21) Distel and Holländer (1980); (22) Feng and Brugge (1983); (23) Galea and Darian-Smith (1995); (24) Innocenti and Caminiti (1980); (25) Innocenti and Clarke (1984); (26) Innocenti and Clarke (1984); (27) Innocenti *et al.* (1977); (28) Innocenti (1981); (29) Iriki *et al.* (1988); (30) Ivy *et al.* (1979); (31) Ivy and Killackey (1981); (32) Joosten and Van Eden (1989); (33) Kato *et al.* (1983); (34) Kato *et al.* (1984); (35) Kato *et al.* (1986); (36) Kennedy *et al.* (1989); (37) Killackey and Chalupa (1986); (38) Kolb *et al.* (1994); (39) LeVay *et al.* (1978); (40) Leonard and Goldberger (1987); (41) Luhmann *et al.* (1986); (42) Luhmann *et al.* (1990); (43) Manger *et al.* (2002a); (44) Manger *et al.* (2002b); (45) Meissirel *et al.* (1990); (46) Meissirel *et al.* (1993); (47) Mihailoff *et al.* (1984); (48) Minciacchi and Granato (1989); (49) Mooney *et al.* (1984); (50) Murakami *et al.* (1993); (51) Nicolelis *et al.* (1991); (52) Olavarria and Van Sluyters (1985); (53) Olavarria (2001); (54) O'Leary and Stanfield (1986); (55) Payne and Siwek (1991); (56) Price (1986); (57) Price and Blakemore (1985); (58) Price and Zumbroich (1989); (59) Rakic (1976); (60) Rakic *et al.* (1986); (61) Rios and Villalobos (2004); (62) Stanfield *et al.* (1982); (63) Tolbert and Panneton (1983); (64) Tolbert *et al.* (1984); (65) Webster *et al.* (1991a); (66) Webster *et al.* (1991b).

rat have shown that callosal as well as corticocortical connections require retinal input for their maintenance (reviewed in Innocenti, 1991; Zufferey *et al.*, 1999). The first evidence of the role of peripheral input in shaping callosal connections came from the work of Shatz (1977), demonstrating abnormal callosal connections in the Siamese cat, as a consequence of the abnormal crossing of retinal axons in this species. The finding that visual callosal axons are either lost or altered in animals binocularly deprived of vision by eyelid suture or eye enucleation (reviewed in Innocenti, 1991) stressed the role of the periphery, mediated in part by activity, in selection of the juvenile axons. The fact that the axons surviving the binocular deprivation are stunted brought further support to the notion that the periphery controls the development of the connections (Zufferey *et al.*, 1999).

Perhaps the strongest argument indicating that evolution did indeed operate through a periphery-driven selection of exuberant connections in development is provided by the analysis of callosal connections at the border between visual areas 17 and 18 in different species. As reviewed by Olavarria (2001), the width of the callosally connected region near the 17/18 border varies across mammals. The callosally connected region near the 17/18 border represents portions of the visual field close to the representation of the visual field midline. It follows that in different species, different fractions of these areas, and consequently different extents of the visual field representations, ought to be callosally connected. Indeed, at comparable elevations, the portion of the visual field represented in the callosally connected portion of the visual areas appears to be wider in the ferret, where it includes azimuths well beyond 25° (Manger *et al.*, 2002a) than in the cat, where it seems not to exceed 15° (Payne and Siwek, 1991). Since in all mammals (including the ferret; Innocenti, unpublished observations) the visual callosal connections develop by exuberance, one can safely infer that some of the projections that are normally transient in one species are maintained in another.

6.6.4 More Developmental Plasticity in Cortical Areas Which Evolved More?

Any hint of developmental processes capable of channeling evolution might reveal a source of

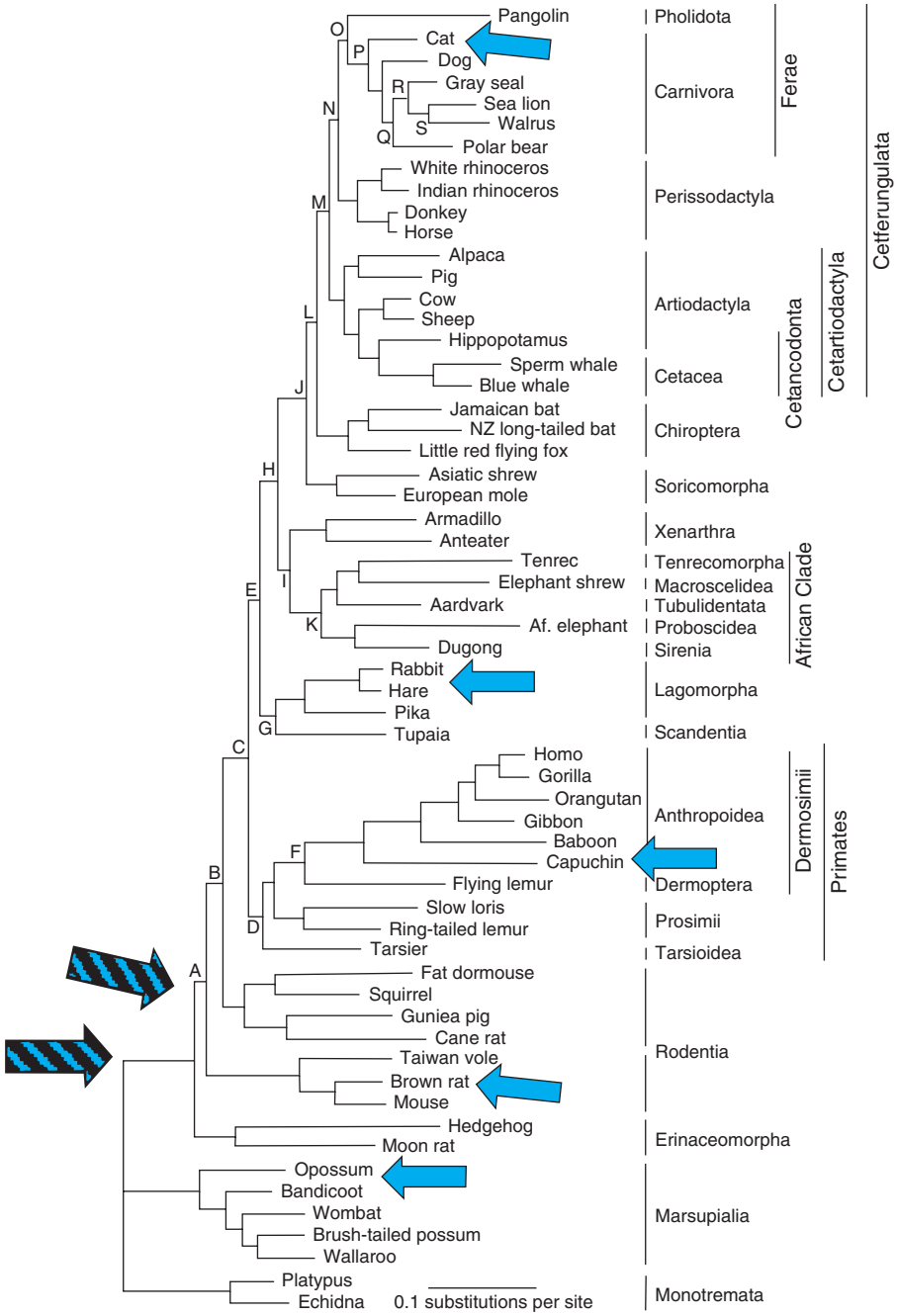

Figure 1 Exuberant connections in development were reported in a number of species (small arrows). This suggests that this mode of development appeared in the earliest ancestors of mammalian radiation (large hatched arrows) and was preserved in evolution. Adapted from Arnason, U., Adegoke, J. A., Bodin, K., *et al.* 2002. Mammalian mitogenomic relationships and the root of the eutherian tree. *Proc. Natl. Acad. Sci. USA* 99, 8151 8156. Copyright (2002) National Academy of Sciences, USA, with permission.

directedness in the otherwise haphazard emergence of the phenotype by trial and error, which is the legacy of classical Darwinism. Some hypotheses on directedness in the evolution of cortical areas can be derived from what has been discussed above. First, the emergence of cortical areas might have been favored at certain specific locations within the cortical surface. These locations should be those with the richest complement of afferent and efferent exuberant projections in development. Second, areas at these locations might have evolved to a large extent under the pressure of information coming from the body periphery. Third, these newly emerged areas

might also be endowed with the highest degree of developmental plasticity.

Large differences exist in the number of cortical areas across species, but they are probably greatest in the parietal, temporal, and prefrontal cortex. It is unknown whether developmental exuberance is largest in these areas, as would be required by the first of the hypotheses discussed above, although this would only be an approximation of the developmental differences in the ancestors. Consistently with the second hypothesis proposed above, only two areas seem to exist in the posterior parietal cortex of the ferret (Manger *et al.*, 2002b). This is far from the complexity found in the monkey, even taking into account the possibility that some areas might have been missed in the ferret. These differences can be tentatively ascribed to the much more complex repertoire of hand and eye movements, and eye–hand coordination in the monkey compared to the ferret.

Consistently with the third hypothesis mentioned above, experiments with early lesions have revealed an important reorganization of connections in the parietal cortex of the ferret (Restrepo *et al.*, 2003). Similarly, lesions in the temporal cortex of the newborn monkey led to the stabilization of otherwise transient connections (Webster *et al.*, 1991a, 1991b).

6.7 Conclusions

Language-related areas are among the most recent acquisitions of the mammalian brain. The integration of these areas into the cortical network, and more generally the functional and structural lateralization of the human brain, would not have been possible without a massive reorganization of corticocortical connectivity, in particular of callosal connections. In this article, I have developed some arguments in favor of the view that developmental exuberance, an interesting blend of directedness and groping around of axons in the formation of cortical connections, appeared early in evolution and was maintained through phyologenesis. Indeed, the exuberant juvenile axonal projections/connections provided the substrate from which new connections could be selected. At the same time, the rules underlying the selection, including information coming from the periphery, channeled evolution. Perhaps the maintenance of rule-driven but flexible developmental syntaxes might be the best key to apprehending how evolution, unlike human-directed mutagenesis, generated the multitude of viable brain architectures we know.

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7 Neural Wiring Optimization

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Glossary

adjacency rule	"If components are connected, then they are adjacent." A wire saving heuristic for laying out a system; also a simple wire cost measure for such layouts.
component placement optimization	The positioning of a system of interconnected components to minimize total connection cost.
network optimization theory	The characterization of minimized use of limited connection resources (e.g., wire length) in a system.
NP hard	A set of problems, each conjectured to require computation time typically on the order of a brute force search of all possible solutions, and often therefore intractable.
size law	For some optimized systems, the smaller a subset, the poorer its optimization.
Steiner tree	A minimum cost arbor connecting a set of terminal loci, which may include branch junctions not at terminals.

Long-range connections in the brain are a critically constrained resource, hence there may be strong selective pressure to finely optimize their deployment. The formalism of scarcity of interconnections is network optimization theory, which characterizes the efficient use of limited connection resources. The field matured in the 1970s for microcircuit design, typically to minimize the total length of wire needed to make a given set of connections among components. When this simple 'Save wire' idea is treated as a generative principle for nervous system organization, it turns out to have some applicability: to an extent, 'instant brain structure – just add wire minimization'. The main caveat is that, in general, network optimization problems are easy to state, but enormously computationally costly to solve exactly; those reviewed here are NP-hard. We focus on the Steiner tree concept and on component placement optimization, with emphasis on the latter.

7.1 Neuron Arbor Optimization

The basic concept of an optimal tree is given a set of loci in 3-space, find the minimum-cost tree that interconnects them, e.g., the set of interconnections of least total volume. If branches are permitted to join at sites other than the given terminal loci (the leaves and root), the minimum tree is of the cheapest type, a Steiner tree. If the synapse sites and origin of a dendrite or axon are treated in this way, the optimization of the dendrite or axon can be evaluated. Approximately planar arbors in 2-space are easier to study. The most important feature of naturally occurring arbors – neuronal, vascular, plant, water drainage networks, etc. – is that, unlike much manufactured circuitry, for each internodal junction, trunk costs (e.g., diameter) are higher than the two branch costs. When such Y junctions are examined in isolation, positioning of the junction sites shows minimization of total volume cost to within approximately 5% of optimal (Cherniak, 1992). Furthermore, the relation of branch diameters to trunk diameter fits a simple fluid-dynamical model for minimization of wall drag of internal laminar flow: neuron arbors act like flowing water.

This Y-tree cost minimization constitutes local optimization. Only one interconnection pattern or topology is involved. Such small-scale optimization does not entail larger-scale optimization, where local trade-offs are often required. When more complex portions of a total arbor are analyzed, optimization becomes a global problem, with an exponentially exploding number of alternative possible interconnection topologies. For example, a nine-terminal tree already has 135 135 alternative

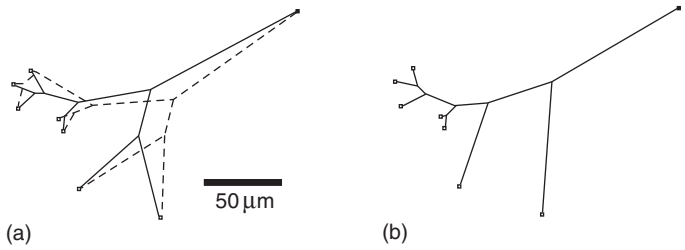

Figure 1 Actual vs. optimal neuron arbors, mouse thalamus extrinsic axon, ascending reticular formation (from data of Scheibel and Scheibel, 1966). The arbor best fits a minimized-volume model. a, Wire-frame representation of an eight-terminal subtree of an observed arbor. Actual tree, with actual topology in its actual embedding, appears as dashed lines. Optimal embedding with respect to volume minimization of the actual topology is superimposed as solid lines. The cost in volume of the actual arbor exceeds that of the optimized embedding of its topology by 2.20%. b, "Best of all possible topologies" connecting the given terminal loci: the optimal topology with respect to volume, optimally embedded. The volume cost of the actual arbor exceeds that of the optimal topology by 2.47%. Only 10 of the 10 395 possible alternative topologies here (approximately 0.14%) have lower total volume costs, when optimally embedded, than the actual topology (Cherniak *et al.*, 1999).

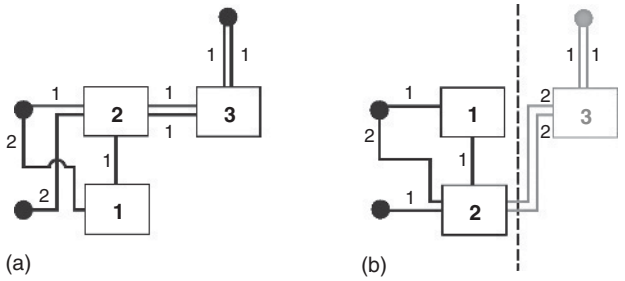

Figure 2 Simple illustration of component placement optimization: minimization of total length of connections. The complete system here consists of a 2-D array of movable components (1, 2, and 3) with given interconnections. All connections are of equal cost per unit length. Component 1 connects to a fixed edge terminal and also to 2; component 2 connects to two fixed edge terminals, and to 1, and also twice to 3; component 3 also connects twice to a fixed edge terminal. a, A globally optimal layout of the three components (cost: 10); cost includes a decussation (connection crossing). b, A complete layout that lacks the decussation, but now is suboptimal (cost: 11). Note also that if the system subset is restricted to only components 1 and 2, including connections to edge terminals, then their layout in (b) is cheaper than their layout in (a), with total connection length reduced from 6 to 5. Hence, these layouts also illustrate global optimization (a) at the trade-off expense of a locally suboptimal cost (b); connection minimization of a total system does not entail connection minimization of its subsets. (A similar pattern holds here also for the simpler connection cost measure of adjacency-rule violations explained in text (Cherniak *et al.*, 2004).)

topologies, each of which must be generated and costed to verify the best solution (see Figure 1). Neuron arbor samples, each with three internodal Y junctions, minimize their volume to within approximately 5% of optimal (Cherniak *et al.*, 1999). This optimality performance is consistent for dendrites (rabbit retina ganglion and amacrine cells, and cat retina ganglion cells) and also for some types of axons (intrinsic and extrinsic mouse thalamus).

7.2 Component Placement Optimization

Another key problem in microcircuit design is component placement optimization (also characterized as a quadratic assignment problem): given a set of interconnected components, find the placement of the components on a two-dimensional (2-D) surface that minimizes the total cost of connections (e.g., wire length). Again, this concept seems to account for aspects of neuroanatomy at multiple hierarchical levels.

"Why the brain is in the head" is a one-component placement problem. That is, given the positions of receptors and muscles, positioning the brain as far forward in the body axis as possible minimizes total nerve connection costs to and from the brain, because more sensory and motor connections go to the anterior than to the posterior of the body. This seems to hold for the vertebrate series (e.g., humans) and also for invertebrates with sufficient cephalization to possess a main nervous system concentration (e.g., nematodes).

Multiple-component problems again generally require exponentially exploding costs for exact solutions; for an n-component system, $n!$ alternative layouts must be searched (see Figure 2). One neural wiring optimization result is for placement of the 11 ganglionic components of the nervous system of the roundworm *Caenorhabditis elegans*, with ~1000

interconnections. This nervous system is the first to be completely mapped (Wood, 1988), which enables fair approximation of wire lengths of connections. When all 39 916 800 alternative possible ganglion layouts are generated, the actual layout turns out in fact to be the minimum wire-length layout (Cherniak, 1994a). Some optimization mechanisms provide convergent support for this finding: a simple genetic algorithm, with wire cost as fitness measure, will rapidly and robustly converge on the actual optimal layout (Cherniak *et al.*, 2002). Also, a force-directed placement (mesh of springs) algorithm, with each connection approximated as a microspring acting between ganglion components, attains the actual layout as a minimum-energy state, without much trapping in local minima (Cherniak *et al.*, 2002).

There is statistical evidence that this "brain as microchip" framework also applies in the worm down to the level of clustering of individual neurons into ganglionic groups and to soma positioning within ganglia to reduce connection costs (Cherniak, 1994a).

Finally, the wiring-minimization approach can be applied to placement of functional areas of the mammalian cerebral cortex. Since wire lengths of intrinsic cortical connections are difficult to derive, one strategy is to explore a simpler measure of connection cost, conformance of a layout to an adjacency rule: if components *a* and *b* are connected, then *a* and *b* are adjacent. An exhaustive search of all possible layouts is still required to identify the cheapest one(s). One promising calibration is that the actual layout of the nematode ganglia is among the top layouts with fewest violations of this adjacency rule. For 17 core visual areas of macaque cortex, the actual layout of this subsystem ranks in the top 10^{7} layouts best fitting this adjacency costing; for 15 visual areas of cat cortex, the actual layout ranks in the top 10^{6} of all layouts (Cherniak *et al.*, 2004; see Figure 3), (See The Role of Vision in the Origin and Evolution of Primates, Primate Brain Evolution, Captured in the Net of Space and Time: Understanding Cortical Field Evolution, The Evolution of Visual Cortex and Visual Systems.)

In general, a Size Law seems to apply to cases with such local–global trade-offs: the larger proportion of a total system the evaluated subsystem is, the better its optimization (see Figure 4). Similar findings have also been reported for rat olfactory cortex and for rat amygdala (Rodriguez-Esteban and Cherniak, 2005). For the largest systems studied (visual, auditory, and somatosensory areas of cat cortex), there is evidence of optimization approaching limits of current detectability by brute-force sampling techniques. A similar Size Law pattern also appears to hold for Steiner tree optimization of neuron arbor topologies (see Figure 1). The picture then is of limited connections deployed very well, a predictive success story. The significance of

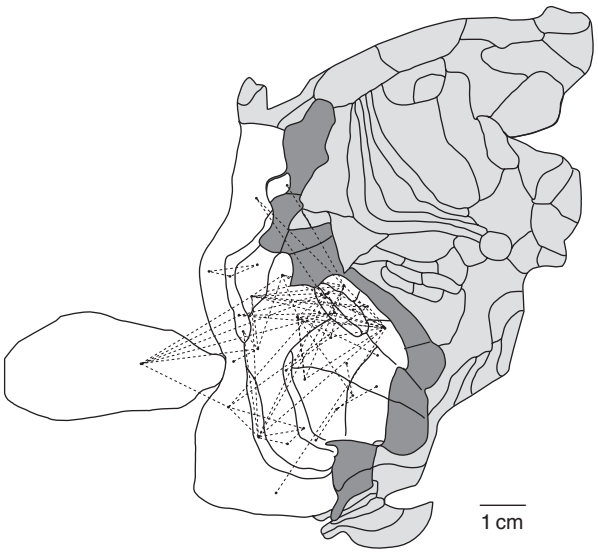

Figure 3 Parcellation of functional areas of macaque cerebral cortex (after Felleman and Van Essen, 1991). Component placement optimization analysis of a layout of 17 core areas (white) of visual cortex, along with immediately contiguous edge areas (dark gray). Reported interconnections among core areas are indicated by dotted lines. Rostral is to the right. In a connection cost analysis, this actual layout of the core visual system ranks in the top one-millionth of all alternative layouts (Cherniak *et al.*, 2004).

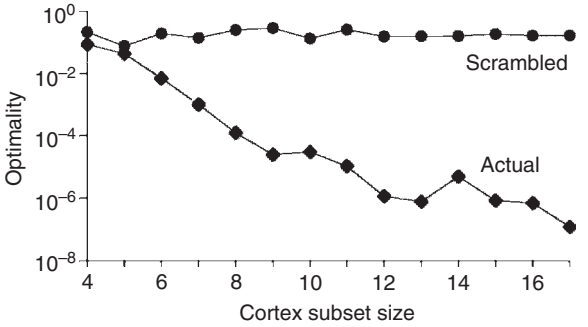

Figure 4 Size Law for macaque visual cortex areas. The system of components here consists of 17 contiguous visual areas of macaque cortex, as in Figure 3. A layout is scored in terms of its violations of the adjacency rule. A series of nested compact subsets of the set of visual areas was generated; each subset was compared with all possible alternative layouts of that subset for adjacency-rule optimality. As subset size increases, optimality ranking of the actual layout consistently improves (with two exceptions, $p < 0.02$). For comparison, the corresponding analysis for a layout of the 17 visual areas with their adjacencies randomly shuffled shows no trend toward improving optimality. Note that this analysis includes only 17 of the total 73 areas of macaque cortex.

ultrafine neural optimization remains an open question. Levels of connection optimization in the nervous system seem unlike levels of optimization elsewhere in organisms.

7.3 Optimization: Mechanisms and Functional Roles

Mechanisms of neural optimization are best understood against the background that the key problems of network optimization theory are NP-complete, hence exact solutions in general are computationally intractable. For example, blind trial and error exhaustive search for the minimum-wiring layout of a 50-component system (such as all areas of a mammalian cerebral cortex), even at a physically unrealistic rate of one layout per picosecond, would still require more than the age of the Universe (Cherniak, 1994b). Instead, even evolution must exploit quick and dirty approximation/probabilistic heuristics.

One such possible strategy discernible above is optimization for free, directly from physics. That is, as some structures develop, physical principles cause them automatically to be optimized. We reviewed above some evidence for arbor optimization via fluid dynamics, and for roundworm ganglion layout optimization via mesh of springs force-directed placement simulation. Although neuron arbors appear to optimize on an embryological timescale, component placement optimization appears to proceed much more slowly, on an evolutionary timescale. For component placement optimization, there is the chicken–egg question of whether components begin in particular loci and make connections, or instead start with their interconnections and then adjust their positions, or some mix of both causal directions. It is worth noting that both a force-directed placement algorithm for ganglion layout and genetic algorithms for layout of ganglia and of cortex areas suggest that simple 'connections → placement' optimization processes can suffice.

Wiring optimization is, of course, subject to many basic constraints and so cannot be ubiquitous in the nervous system; the question is where it does in fact occur and how good it is. Trade-offs of local optimality for better cost minimization of a total system (as Figure 2 illustrates) are one way in which global optimization can be obscured.

If the brain had unbounded connection resources, there would be no need or pressure to refine employment of wiring. Thus, to begin with, the very fact of neural resource limitations appears to drive 'Save wire' fine-grained minimization of connections. Another part of the functional role of such optimization may be the picture here of 'physics → optimization → neuroanatomy'. Perhaps such an economical means of self-organizing complex structure generation eases transmissibility through the information bottleneck of the genome. This constitutes a thesis of nongenomic nativism, that some innate complex biological structure is not encoded in DNA, but instead derives from basic physical principles (Cherniak, 2005).

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8 Principles of Brain Scaling

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Glossary

allometry A relationship between the sizes of two biological structures or other quantities that follows a power law. For example, suppose that L is the average length of an animal's life (a biological quantity) for a particular species, and W is the average weight for adults of that species (a measure of total animal size). Further suppose that, across species in some taxonomic group, the length of life is related to body weight by the equation $L = aW^b$ for some quantities a and b that are independent of both L and W. This relationship would constitute an allometry, and lifetime would be said to have an allometric relation to animal size. The constant a is called the scale factor and b is known as the allometric constant for the two quantities L and W.

grade shift If an allometric relationship holds for two different taxa with the same allometric constant but different scale factors, it is said that a grade shift has occurred. For example, there is an allometric relationship between brain size and body weight for both monkeys and teleost fish, but monkeys have larger brains; that is, a monkey always has a larger brain than a fish of the same weight, although a tiny monkey might have a smaller brain than a giant fish.

isometry An isometry is a special case of an allometry for which the allometric constant is unity. That is, two biological quantities are said to be isometric when they are related by a simple proportionality.

map Brain areas, like the thalamus or visual cortex, are said to have a map when the neurons are arranged in a way that preserves the neighbor relationships present in some reference structure. For example, the visual world is projected onto the retina, and the neighbor relations in the visual world are preserved in the projection of the retina to the visual thalamus, and from the visual thalamus to the visual cortex. Thus, both the visual thalamus and the visual cortex have a map of the visual world (the reference structure). Although a map must preserve neighbor relations, in general, other features may not be preserved: ordinary two dimensional maps of the world keep things on the globe next to each other, but they change the size and shape of the continents. It is generally believed that all brain structures have maps but as for language cortex or the olfactory cortex, for example we do not know the reference structure for the map.

scalable architecture A term from computer science that refers to designs for computing circuits that can be made more powerful that is, can carry out the computation for which they were intended more quickly or accurately by simply increasing the size of the circuit (by, for example, increasing the number of computing elements) while keeping the same design. Familiar digital computers do not have a scalable architecture, which means that they must be redesigned each time their power is increased.

self similar function A function that depends on parameters which change its size on a graph without changing its shape. A Gaussian is an example of a self similar function because a plot of it still has the familiar bell shape even when its parameters (its mean and standard deviation) are changed.

8.1 Introduction

The extent to which the brains of all mammals share a common design is striking: even though the brains of mammalian species vary in size by more than three orders of magnitude, the essential features of their design are unchanging (Butler and Hodos, 1996; Striedter, 2005; see Primate Brain Evolution, The Evolution of Parallel Pathways in the Brains of Primates). This observation means, for example, that rodent, feline, and nonhuman primate brains can serve as model systems for studying principles of structure and function that apply to the human nervous system. From a computational perspective, then, one of the most remarkable features of the mammalian brain is that it has a scalable architecture (Comer, 2005). That is, the computational power of a brain can be increased continuously and gracefully by adding more components while adhering to a single basic design.

Understanding the scalable architecture of the mammalian brain is clearly important for determining how neuronal circuits are designed and how they compute. And to elucidate scalability, one must learn the rules followed when brain size and computing power are increased. An important quantitative tool for investigating scalability is allometry (Huxley, 1932; Schmidt-Nielsen, 1984), the study of how the size of one part of the brain scales up with the size of another part as the entire brain is enlarged. The rules that describe the relationship between the sizes of two different structures in brains of different sizes are called allometries, or allometric relations, or scaling relations. Two examples of allometric (or, equivalently, scaling) relations are illustrated in Figure 1, where the size of primary visual cortex (V1) – the first cortical processing center for visual information – in various primates is compared with the size of the lateral geniculate nucleus (LGN), the immediate source of the cortex's visual information. Two different measures of size are used here. In Figure 1a, the volume of V1 for a range of primate species is plotted, on double logarithmic axes, as a function of the volume of the LGN (Stephan *et al.*, 1981; Frahm *et al.*, 1984). The relationship is linear on this double logarithmic plot with a slope of 1.125. The sizes of the same two structures are compared again (for the same primate species) in Figure 1b, this time with size being measured by the number of neurons present in each structure rather than by volume; that is, Figure 1b presents the logarithm of the number of cortical neurons processing visual information (the number of neurons in V1) as a function of the logarithm of the number of neurons providing that information (the number of neurons in the LGN) (Stevens, 2001). As before, the data points fall along a straight line, but this time the slope of the line is $3/2 = 1.5$, not 1.125. Here, then, are two scaling laws – allometric relationships – that describe aspects of visual system scalability, and any theory of how the visual system processes information must account for these scaling relationships.

8.2 The Interpretation of Scaling Laws

The relationships in Figure 1 have a particular functional form; they are power laws

$$S = as^b,$$

with S being the size of the cortex, s the size of the LGN, and a and b constants ($b = 1.125$ in Figure 1a and 1.5 in Figure 1b). Taking the logarithm of this equation, one finds that

$$\log S = b \log s + \log a,$$

an equation with the form

$$y = bx + c$$

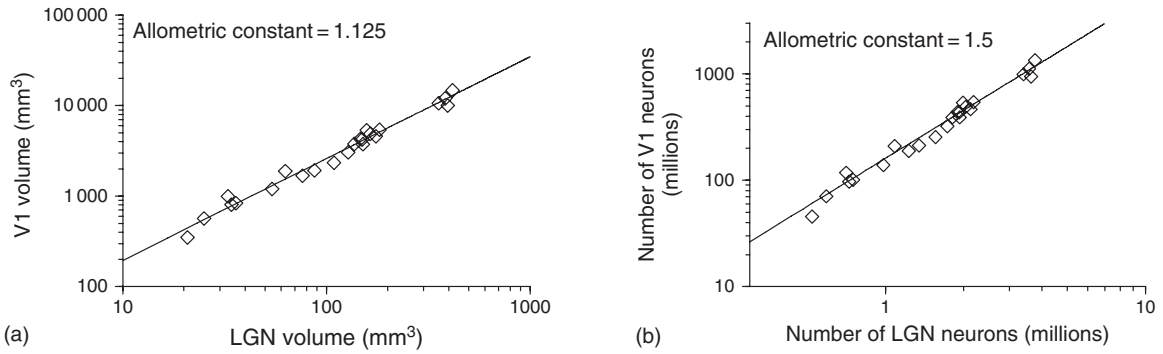

Figure 1 Relationship between the size of primary visual cortex (V1) and the lateral geniculate nucleus (LGN) for 23 primates (haplorhines). a, Size of the two structures is measured as volume (mm^3). The allometric constant is 1.125. b, Size of the two structures is measured in numbers of neurons in each. The allometric constant is 1.5.

if one defines $y = \log S$, $x = \log s$, and $c = \log a$. This means that, on a double logarithmic plot like the ones in Figure 1, a power law results in a straight line with slope b and intercept of $c = \log a$. The constant a is called the scale factor, and the constant b is known as the exponent or the allometric constant. Note that if the exponent $b = 1$, the allometric relationship becomes a simple proportionality

$$S = as.$$

Clearly, although power laws result with either measure of structure size (structure volume in Figure 1a as opposed to number of neurons in Figure 1b), the values of the allometric constants differ (1.125 vs. 1.5 in Figure 1) according to how size is measured.

The relationship displayed in Figure 1 is a power law, but the functional form might, in general, be some other type of function. For example, one could imagine that there might be some pair of variables R and r, representing the sizes of two hypothetical structures, for which the relationship is

$$R = a(1 - \mathrm{e}^{\ br}),$$

with constants a and b; this is an exponential rather than a power law. Although this exponential equation does relate the sizes of two structures, it would not count as an allometric relationship because the term allometry is reserved for just those pairs of structures whose sizes are related by a power law. Power laws arise in many situations, and their study has been particularly important in various areas of physics (Barenblatt, 1996).

Empirically, many pairs of brain structures have been found to follow power laws with various different allometric constants (Huxley, 1932; Schmidt-Nielsen, 1984; Striedter, 2005). In some of these cases, the actual functional form of the relationship is indeed a power law and, in other cases, the functional form is not really a power law but can be approximated as one over some restricted range of sizes. In general, just as almost any equation can be approximated over a limited range by a linear equation, so can almost any relationship describing the sizes of a pair of structures be approximated, on a double logarithmic plot, as a straight line (which is equivalent to a power law). I am concerned here with those situations that produce actual power laws – that is, true allometric relationships – and I will not consider (except for one case) why functional forms other than power law might occur.

In the following, I give three different ways that true allometric relations can arise. The first is the original interpretation, due to Julian Huxley, of differential growth; Huxley's view was that allometries arose when one structure grew faster than another (Huxley, 1932). If, on the other hand, the growth rate of two structures were always exactly the same, then the relative size of the structures would be constant and they would be said to have an isometric (as opposed to allometric) relationship to one another; this is a special case of the allometric relationship with the allometric constant $b = 1$.

A second way in which allometric relations can be generated is through the preservation of the form of structures. Often, one has the idea that structure and function are intimately related so that form must be evolutionarily preserved in order to preserve function. For example, the hand is a structure beautifully designed for fine manipulations of elements in the environment, and a single species keeps the same form of the hand for small and large individuals by changing hand size and also by altering some dimensions more than others (long, thin fingers as opposed to thick, stubby fingers). Allometric relations can be generated when the basic form of structures is maintained but is stretched in some directions more than in others as the organism is made larger. The production of allometric relationships in this way differs from Huxley's because it does not consider growth of structures but just properties of the final products. This idea is made more precise in an example below by considering what are called self-similar functions (Barenblatt, 1996).

A third possibility for generating allometric relations depends on the unfamiliar notion of changing dimensions in going from one structure to another (Stevens, 2001). For an example of how dimensionality can be changed by an operation, imagine illuminating a three-dimensional object from one direction so that it casts a shadow on a screen. This object (a three-dimensional first structure) casts a two-dimensional shadow on the screen (the second structure) and so one can say that, in some sense, a shadow forms a two-dimensional representation on the screen of a three-dimensional object in space as this object is made smaller and larger. The size of the shadow is related to the size of the object casting the shadow: for example, the area of the shadow will scale as the 2/3 power of the volume of the object casting the shadow; the size of the two-dimensional shadow, then, will bear an allometric relationship to the size of the original three-dimensional object. As will be described below, certain types of computation can change the dimensions of the space used to represent the input and output of a computation, and this change in dimension can lead to allometric relations between the numbers of neurons in one structure and another.

8.3 Huxley's Allometry

How are the scaling laws in Figure 1 to be interpreted? As noted above, the linear relationships of the double logarithmic plots are equivalent to power laws. That is, if S is the size of V1 and s is the size of the LGN, these quantities are related by the power law equation

$$S = as^b,$$

where a and b are constants.

Huxley (1932) discovered a number of scaling relations – it was Huxley who coined the name allometric relation – and attributed them to the differential growth of the two structures being compared. To see how a power law can result from differential growth rates, suppose that one structure (for example, V1), whose size is $S(t)$, grows exponentially with a rate m for a growth duration t. At the end of the growth period, the cortical size would be

$$S = S_0 e^{mt},$$

where S_0 is the size of the structure at the start of the exponential period of growth. The actual final size of S would, of course, depend on the length of the growth period t. Suppose further that another structure (LGN, for example) with size $s(t)$ follows the same exponential growth law for the same growth duration t except this structure has a growth rate n and an initial size s_0; the size s of this second structure, then, is

$$s = s_0 e^{nt}$$

and it also depends on the growth duration t. If one takes the natural logarithm of these two equations and combines them by eliminating the growth duration t (which is assumed to have the same value in both equations), the result is

$$\log S = (m/n)\log s + [\log S_0 - (m/n)\log s_0].$$

That is, S is related to s by a power law whose exponent (or allometric constant) is $b = (m/n)$ and for which the scale factor a above is given by

$$\log a = [\log S_0 - (m/n)\log s_0].$$

For each growth duration, then, different sizes S and s will result, and these sizes are related by a power law. Thus, differential growth rates can lead naturally to scaling relations that are power laws.

8.3.1 Detail

The example just given assumed that the growth rate of the structures is constant throughout development and that structure size therefore increases exponentially. A power law results, however, from less restrictive assumptions. Specifically, the growth rate can vary with time during development for two structures and a power law will still result if the two growth rates vary the same way so one is always proportional to the other one.

Let $S_1(t)$ be the size of structure 1 at time t during development, and $S_2(t)$ be the size of a second structure at that time. These structures grow according to the equations

$$dS_1/dt = g(t)S_1(t) \quad \text{or} \quad dS_1/S_1 = g(t)\,dt$$

and

$$dS_2/dt = h(t)S_2(t) \quad \text{or} \quad dS_2/S_2 = h(t)\,dt,$$

where $g(t)$ and $h(t)$ are the growth rates at time t during development; note that the growth rate can vary over the course of development. Eliminate dt between these equations to give a growth equation for the pair of structures:

$$dS_1/S_1 = [g(t)/h(t)]\,dS_2/S_2.$$

If we suppose that the ratio $g(t)/h(t) = b$, where b is a constant – that is, if we suppose that $g(t)$ is proportional to $h(t)$ at all times with the proportionality constant b, even if the growth rates vary over time during development – then the growth equation becomes

$$dS_1/S_1 = b\,dS_2/S_2$$

and integrating this equation one finds that

$$\log(S_1) = b\log(S_2) + c$$

where c is a constant of integration. Thus, even if the growth rates change during development and the growth is not exponential (because growth rate is not constant), as long as the growth rates remain proportional to one another through the time of development, a power law relates the sizes of the two structures (S_1 and S_2) generated by different growth periods.

The problem with this differential-growth explanation for scaling is that one does not know why the growth rates should be different or by how much. A more complete explanation for the scaling relation, then, would require either some notion of the mechanisms that lead to differential growth rates for two structures or for a reason why having differential growth rates suits the structures to their function. Two alternative explanations for allometric relations are considered below.

8.4 When Form Follows Function

Often in biology, the function of a structure and its form are intimately related. If the function is to be preserved as the size of the structure is changed, then

the form must also be preserved in order to maintain the form/function connection. This sort of situation can give rise to allometric relations.

To illustrate how preserving form can yield scaling laws, picture a hypothetical axon, much like a retinal axon in the tectum, whose terminal branches form a flat, round disk-like arbor. For this illustration I imagine that all axons in a particular species have arbors of the same size. Suppose we wish to describe how this type of axon distributes synapses in the target structure (the tectum, for example) in a range of comparable species with different brain sizes. The job of this arbor is to distribute information over a map in the target brain area, and the way synapses are distributed should be preserved as the size of the arbor is increased. For example, the computations carried out by the circuit might require that an arbor produce an approximately Gaussian density of synapses over space, and so the form must always be one that will give a Gaussian distribution of synapses for any arbor size (Figure 2). I will denote the size of the target for a particular species by the variable s; this might be, for example, the average lateral length of the tectum, or its average surface area for that species, and I pick some particular species as a reference, for which I choose $s = 1$. The size of target structure, then, is measured relative to the size in our reference species, so that, if $s = 3$, the target structure in that species would be three times the size in the reference species.

The average spatial distribution of synapses provided by the arbor will be given by the function $f(r,s)$, where r is the radial distance from the center of the arbor, s is the target size, and the value of the function f gives the average density of synapses in a circular annulus at a distance r from the arbor center. For example, $f(r,s)$ might be a Gaussian whose variance depends on s, as shown by the pair of functions in Figure 2. What does it mean to preserve the form of this arbor? The usual idea for maintaining form is to have the function f be self-similar, which means that its shape is unchanged when s is varied (Barenblatt, 1996); that is, the function might be stretched or compressed in the vertical (y) and horizontal (r) directions, and the amount of the stretching or compression would depend on s. But even when a Gaussian function is stretched in this way, it still is a Gaussian. For example, the function (dotted line) in Figure 2 results when the function presented in a solid line in Figure 2 is stretched vertically by 2 and horizontally by 1/4.

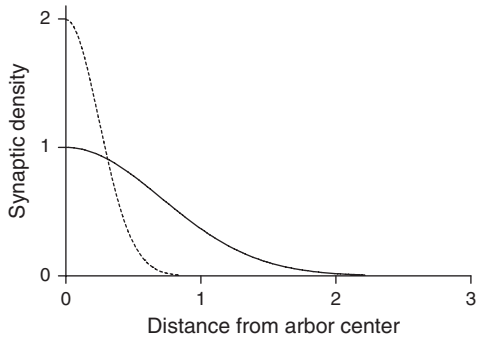

Figure 2 Hypothetical density of synapses formed by an axonal arbor as a function of the distance from the center of the arbor. Two different-sized arbors are depicted, a larger one (solid line) and a smaller, more compact one (dotted line). Both functions are Gaussians.

Now suppose we wish to compare the distribution of synapses in the target structure (tectum, for example) across a range of different species. If we take $f(r,1)$ to describe the density of synapses for an axon arbor in the target structure in our reference species (see Figure 2, for example) and if we want to preserve the shape of the function f, then the spatial distribution of synapses for the general target of size s is given by the relation

$$f(r,s) = u(s)f(r/v(s),1).$$

What this means is that, when the target size is s, you can find the spatial distribution of synapses by vertically stretching the distribution of synapses for the reference target by the amount $u(s)$ ($u(s) = 2$ in going from the solid to dotted function in Figure 2), and stretching horizontally by the amount $v(s)$ ($v(s) = 1/4$ in going from the solid to dotted function in Figure 2).

It is easy to see that the vertical stretch is specified by $u(s)$, but perhaps a little harder to understand how $v(s)$ determines the horizontal stretch (in the r-direction). The key is to observe that the value of a function (f in our example) depends on whatever is inside the parentheses $f(\ )$. When r is divided by $v(s)$, the function f decides its value based on the ratio $r/v(s)$ (not just on r), and if $v(s) > 1$, r has to be larger to get the same value of f as when $v(s) = 1$; this stretches the function out along the r-axis.

A remarkable feature of self-similar functions, which has been shown by mathematicians (Aczel, 1969), is that both the vertical and horizontal stretches must have a power law dependence on the size s. Specifically, $u(s)$ and $v(s)$ must both have the form

$$u(s) = as^b$$

and

$$v(s) = As^B$$

for some constants a, b, A, and B if the shape of the function $f(r,s)$ does not change with brain size and if

$u(s)$ and $v(s)$ depend smoothly on the size s. Now go back to the synaptic distribution $f(r,s)$, make use of the power law form of $u(s)$ and $v(s)$, and add up the number of synapses in the arbor (integrate $f(r,s)$ over all values of r) to give the total number of synapses $n(s)$ made by each arbor in a target structure of size s. The result can be shown to be the scaling law for synapses per arbor as a function of target size given by

$$n(s) = n(1)s^k$$

for some constant k; here, $n(s)$ is the number of synapses made by an arbor in a brain with target size s and $n(1)$ is the number of synapses made by the reference arbor (whose target size is taken as 1). Thus, preserving the shape of an arbor's distribution of synapses as the target structure is increased or decreased in size results in an allometric relation; often, it is possible to interpret allometric relations in this way. Here I supposed for simplicity that there is only a single variable (r above) and a single parameter (s above for the size of the target structure), but the same sort of argument can be used when more variables are involved.

8.5 Changing the Number of Dimensions from One Map to the Next

In addition to differential growth and preserving the form of structures to maintain form/function relations, power laws can also result from certain types of computations and mappings that change the dimension of what is being represented. For example, a two-dimensional map can be transformed into a three-dimensional map, and this can lead to a 3/2 power-scaling law, as will be described below; this happens in the mammalian visual system between the LGN (with a two-dimensional map) and the V1 (which contains a three-dimensional map) (Stevens, 2001).

How can the dimension change in going from one map to another? Describing a curve in a plane – like a circle drawn on a piece of paper – requires two dimensions with an x- and y-coordinate for each point on the curve; Figure 3 (dashed line) illustrates, in perspective, a circle in the x–y plane. This two-dimensional circle can be mapped into three dimensions in a natural way by appending a third number to the (x, y) pair for each point on the circle. For example, each (x, y) pair can be made into an (x, y, z) triplet by setting z equal to the slope of a tangent to the curve at point (x, y). This is seen in Figure 3 (solid line), in which the circle has been 'lifted' into a three-dimensional curve by plotting the orientation of the tangent to the circle at each (x, y) point. Thus, the two-dimensional circle on a plane (dotted line in Figure 3) is transformed into a curve (solid line in Figure 3) in three-dimensional space. The three-dimensional curve can be changed back into the two-dimensional circle by projecting the three-dimensional curve on a plane (for example, by casting its shadow with illumination from above). The three-dimensional space depicted in Figure 3 is called the tangent space of the x–y plane because it not only specifies the position of the curve in the plane (from the (x, y) in each (x, y, z) triplet), but it also gives the slope of the tangent to each point along the curve (the z in the (x, y, z) triplet).

When it comes to the brain, it may be slightly difficult to understand what one means by the dimension of maps – say a map of the visual world – represented in some brain region. For example, the visual cortex is essentially a two-dimensional sheet, so how can it contain a representation of the world that is other than two-dimensional? To explain this, I need to consider the essential idea behind the dimensionality of something. The critical notion is: the dimensionality of a space is determined by how many numbers are necessary to specify a point in that space. For example, because a pair of numbers (x, y) is required to determine the position of any point in a plane, a plane is two-dimensional. Similarly, three numbers (x, y, z) are needed to determine the location of any point in a three-dimensional volume. In the same way, one can imagine (although not picture) a four-dimensional space in which four numbers are required to characterize a point. In physics, it is common to talk about a four-dimensional space, called spacetime, in which the position of a person, for example, is specified by four numbers, three (x, y, z) to define the person's location in space

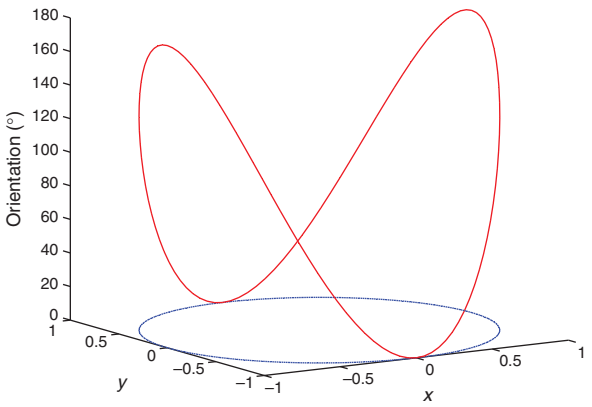

Figure 3 Illustration of how a two-dimensional figure, the circle (dotted blue line) in the x y plane, might be extended into a three-dimensional volume (solid red line). The vertical axis is the orientation of a tangent to each point on the circle.

and a fourth number (t) to specify the time at which the person occupied that location. Of course, abstract spaces of any dimension can be constructed.

To understand how a three-dimensional space is represented in the brain, it is convenient to consider a specific example provided by V1. Neurons in V1 respond preferentially to lines or edges, but the response of the neurons depends not only on the location of the line or edge in space but also on its orientation (Hubel and Wiesel, 1959). Thus, if an animal looks at a house at the center of a picture, a particular V1 cell would respond only to a line that is in some specific place in that picture (for example, the line that defines the edge of a tree to the right of the house). But this cell would not respond to just any line there, but only to a line that is, say, vertically rather than horizontally oriented. For each location on the retina, then, there is a population of neurons in V1 that respond only to lines whose images cross that retinal location and, among cells in this population, there are distinct V1 neurons that respond best to each possible direction of the line. This means that each neuron in V1 requires three numbers to characterize its behavior: two numbers to specify the (x, y) position on the retina to which the V1 neuron is assigned, and a third number to give the orientation (from 0° to 180°) of a line that the neuron prefers. Because three numbers (x, y, and orientation) are needed to determine if a particular neuron in V1 is responding, the V1 can be said to have a three-dimensional representation of the visual scene. And because only a pair of numbers are needed to characterize the response of retinal cells, the dimensionality of the retinal representation of the visual scene is two. The visual cortex, then, carries out computations to map a two-dimensional image on the retina into a three-dimensional space in V1. In a similar way, the dimensionality of the representation in any brain area can be described: the dimensionality is determined by how many numbers are needed to characterize the response properties of neurons in that region. As an aside, I should note that the representation of maps in cortical areas is actually greater than three because more than three parameters are needed to characterize completely the response properties of cortical neurons.

How would a scaling law result from such a two-into three-dimensional transformation? To answer this question, one has to recognize that neural representations are grainy because only a relatively small number of neurons are available. Just as the pixel size – and, therefore, the resolution – of a digital camera is determined by the number of pixels available, so is the resolution of a neural map, like the retina or V1, determined by the number of neurons available. If the number of neurons in a hypothetical retina were increased from $1000 \times 1000 = 1$ million to $2000 \times 2000 = 4$ million (a 1-megapixel retina upgraded to a 4-megapixel one), the linear resolution of the retinal image would be doubled because the number of neurons (pixels) would be doubled in each direction. Going from the retina to the LGN (where the image is also represented as two-dimensional), the number of neurons in each direction would also have to be doubled for the LGN to keep up with the resolution available from the retina; there would be no point in making a larger eye, and a larger retina with more neurons, if the increased resolution were just thrown away at the next stage. Going from the LGN to V1, the number of neurons in each direction would also have to be doubled if the resolution in the cortical representation is to keep pace with that available from the eye. The cortex, however, not only has x- and y-directions, each of which must have the number of neurons doubled, but also a z-direction (line orientation) which would also have to double its neurons to make use of the improved resolution available in the image from the larger eye.

To see what happens in general, suppose that the linear resolution in the retina is increased a-fold (doubled in each direction in the example above) so that the total number of retinal neurons is increased by $a \times a = a^2$. For the cortex resolution to keep pace with the resolution available from the retina, the number of cortical neurons would have to be increased a-fold in the x-, y-, and z-directions, so the number of cortical neurons would become $a \times a \times a = a^3$ larger. If n_0 is the initial number of retinal neurons, and n is the number after the increase in size, n would be $n = n_0 a^2$. And if N_0 is the initial number of cortical neurons, and N is the number after the increase in size, N would be $N = N_0 a^3$. Now eliminate a between these two equations to give the result

$$N = (N_0/n_0^{3/2})n^{3/2}.$$

Thus, the number of cortical neurons (N) is related to the number of retinal ganglion cells (n) by a power law whose exponent is the ratio of the number of dimensions in the visual cortical representation (3) to the number of dimensions in the retinal representation (2). Such a power law will result whenever the number of dimensions changes in going from one area to another and the resolution in each dimension is increased in parallel.

Certain kinds of computation do not change dimensions whereas others do (Stevens, 2004).

For example, if an image is simply filtered – by blurring or sharpening edges, for example – the number of dimensions in the original representation of the image and its filtered versions is the same. This is the sort of operation performed in going, for example, from retina to LGN. On the other hand, some operations do change the number of dimensions. For example, a sonogram of a birdsong takes a one-dimensional function (the sound pressure produced by the bird as a function of time; time is the single independent variable) and displays it as sound intensity of pitches produced as a function of time, a two-dimensional representation of the bird song (the two independent variables are pitch and time). The common image compression schemes, like the modern jpeg format used by digital cameras, also increase the number of dimensions but save space by limiting the resolution.

A widely used class of computations, known as wavelet transforms, always change the dimension of a map. For example, a two-dimensional image becomes four-dimensional after it is wavelet-transformed and it has been argued that V1 carries out such a transform with the firing rate of each neuron representing the magnitude of one coefficient of the transformed image (Stevens, 2004).

In summary, then, allometric relations can arise in different ways and can potentially give information about various underlying mechanisms. According to the original Huxley interpretation, the allometric constant tells you the relative growth rates of two structures. The interpretation of the allometric constant when the allometry arises from self-similarity of structures is more complex, and depends on the details of the situation. Finally, when an allometric relation arises because a computation alters the dimension of the neural representation of a map, the allometric constant specifies the ratio of the number of dimensions in the original representation to the number of dimensions needed for the result. For example, going from a two- to three-dimensional map, the allometric constant would be 3/2.

8.6 Some Limitations

Although allometric relations are very important as a tool for describing how brain structures change in size as the brain is made smaller or larger, drawing meaningful conclusions from them requires care. Here I indicate three potential problem areas.

8.6.1 Selecting the Appropriate Measures of Size

Figure 1 presents allometric relations between the size of the LGN and V1 with two different measures of size that give two different allometric constants (1.125 and 1.5). Which allometric constant is correct? Both may be correct but not necessarily equally easy to interpret. To decide on the appropriate measure for a structure's size, one must decide, based on the function of the structure, what measure is most natural. For example, number of neurons is an appropriate size measure if one believes that neurons are the computational units of the brain, and volume would be a less natural measure because the essential function does not depend on the volume of the structure (unlike, for example, the liver, where volume is presumably the relevant variable) but rather on the number of computational units.

The volume of a brain structure is related to the number of neurons it contains by the neuronal density (number of neurons per cubic millimeter), and neuronal density is known to decrease as the volume of the structure increases. For the LGN and V1, for example, the neuronal density is related to the structure's volume by power laws (Stevens, 2001), and so the power law for cell numbers, together with the power laws for neuronal densities, dictates that a power law should also result when structure sizes are measured by volumes. To account for the Figure 1a allometric relation, then, one would have to combine the Figure 1b allometric relation and the allometries that relate structure volumes to neuronal densities. The determination of what size measures are relevant for allometric relations depends, then, on one's ideas about how the structures function.

8.6.2 Internal Consistency

Figure 4 (open circles) shows an allometric relation between the neocortical volume and the subcortical gray-matter volume for 24 primates (Stephan *et al.*, 1981; Frahm *et al.*, 1982); the allometric constant is 0.73. The subcortical gray matter comprises a number of nuclei, like the thalamus, hypothalamus, and striatum, and each of these structures also obeys a scaling law. For example, Figure 4 (open squares) plots, on a double logarithmic scale, the volume of the striatum as a function of the neocortical volume for the same primate species, and a power law with the allometric constant = 0.9 appears to hold for this pair of structures. The remainder of the subcortical gray-matter volume – including everything except the striatum – is plotted as a function of neocortical volume in Figure 4 as open triangles and the least-squares fitted power function gives an allometric constant of 0.65. Thus, two

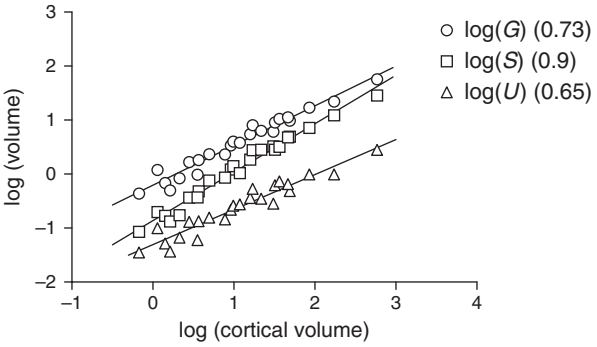

Figure 4 Double logarithmic plots of the volume (mm^3) of subcortical gray-matter structures as a function of the volume of the cortical gray-matter volume for 24 primates. Open circles represent the total volume of the subcortical gray matter, open squares the volume of the striatum, and open triangles the volume of the total subcortical gray matter except for the striatum. Note that the open triangles have been shifted vertically down 10-fold (1 log unit) for clarity.

components whose volume adds together to give the entire subcortical gray-matter volume (striatum and all subcortical structures other than the striatum) each are related to the neocortical volume by power laws with different allometric constants (0.65 and 0.9), and the entire subcortical gray-matter volume is itself related to the neocortical volume by a third power law with an allometric constant of 0.73.

The problem with these allometric relations is that they are internally inconsistent and cannot all be right. The reason is that the sum of power laws with different exponents is not again a power law; for example, there is no value of the constant a that makes $x^2 + x^3$ equal to x^a. Thus, if the striatial volume and the nonstriatal subcortical gray matter are both proportional to different powers of the cortical volume, the total subcortical gray-matter volume (= striatal + nonstriatal) cannot also be proportional to a power of the neocortical volume, as it appears to be from the graph. The sum of power laws with different exponents often can, over some range of values, be approximated by a power law (as illustrated by the data in Figure 4), but the allometric constant for this approximate allometry is not simply related to the allometric constants of the two constituent allometries. This means that the allometric constant for the combined case is difficult or impossible to interpret.

In summary, then, what appears on a double logarithmic plot to be an allometric relation may not, in fact, be one but only an approximation. If it is an approximation that consists of the sum of power laws with different exponents, the allometric constants are not easily interpreted, and may not be useful except as descriptive parameters.

8.6.3 Grade Shifts

Allometric relations usually do not hold across all vertebrates or all mammals but rather are restricted to specific taxa. An example is shown in Figure 5a for hominoids (family Hominindae, the great apes and humans) and for monkeys (families Platyrrhini and Ceercopithecoidea), where the volume of the cerebellar hemispheres is plotted, double logarithmically, as a function of the volume of the cerebellar vermis (MacLeod *et al.*, 2003); the allometric constants (the slopes of the lines on the plots) for these hominoids and monkeys are the same (1.4), but the scale factors (determined from the intercepts) are about threefold different (the hominoid curve is displaced vertically from the monkey curve). Allometric relations that differ by their scale factors for different taxa (here, hominoids versus monkeys) are known as grade shifts, and they are very common (Pagel and Harvey, 1989).

The points plotted in Figure 5a are derived from measurements on several specimens from each species, but frequently one uses data that are averaged across all specimens from a given species (for example, the values for all human cerebella are averaged together). The family Hominindae contains only five species, and one generally wishes to seek allometric relations across a larger sample than this. In Figure 5b, data points that appear in Figure 5a have been averaged across species, and the hominoids and monkeys have been plotted together (one point for each species; i.e., one human point, one chimp point, etc.). In Figure 5b, the cerebellar hemisphere volume appears to be related to the vermis volume by a power law with an allometric constant of 1.9 but, from Figure 5a, we know this value for the allometric constant is an artifact that arises from combining allometric data with different scale factors. Ignoring grade shifts in determining allometric constants can therefore lead to incorrect values, and consequently to misinterpretations of the meaning of scaling laws.

In summary, allometric relations reveal orderly rules used by brains when their sizes are increased. These scaling laws can be interpreted in different ways – three have been given here – but these interpretations are the start, not the end, of the job of understanding the scalable architecture of the brain. For each allometric relation, one must determine the mechanisms that generated it and understand why evolution has chosen a particular value for an allometric constant.

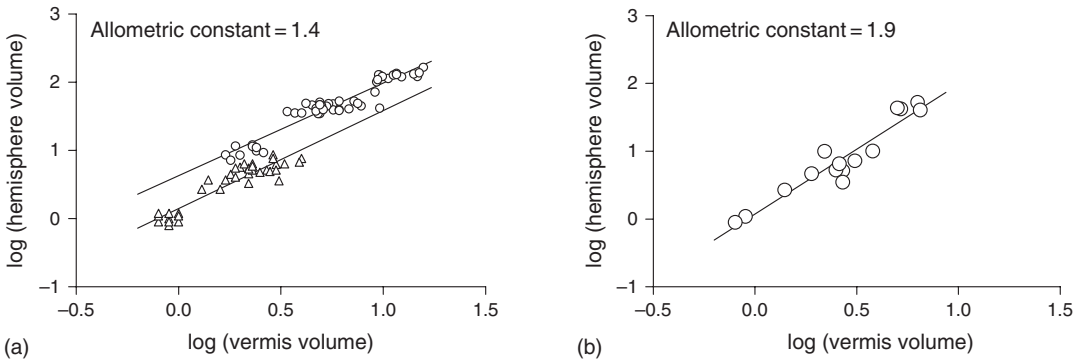

Figure 5 Double logarithmic plot of volume (mm^3) of the cerebellar hemispheres as a function of the volume of the vermis of the cerebellum for five hominoids and 10 species of monkey. a, Each data point represents an individual specimen. b, Each data point is the average of all of the specimens for a particular species.

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THE EVOLUTION OF BRAINS IN EARLY VERTEBRATES, FISHES, AMPHIBIANS, REPTILES AND BIRDS

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9 Structure of Brains of Primitive Vertebrates (tunicates, amphioxus, lampreys) and the Basic Features of the Vertebrate Brain

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Glossary

acrania A chordate with no obvious head (such as the lancelet, Amphioxus).

axon Neuronal process typically involved in emitting output.

BF1 (Foxg1) Brain forkhead gene 1 (forkhead gene g1).

bHLH Basic helix loop helix gene (tran scription factors such as Atoh1, Neurog1).

BMP Bone morphogenic protein.

cephalochordate Animals in which the notochord extends throughout the body, including the most rostral of head part (see acrania).

chordate Deuterostome animals with a notochord.

coelenterate Radial symmetric, diploblastic ani mals (jellyfish, corrals, cnidarians).

craniate A chordate with fully dev eloped head (cyclostomes and gnathostomes).

cyclostomes Jawlesss with a circular mouth (lam preys and hagfish).

dendrite Neuronal process(es) typically in volved in receiving input.

deuterostomes Animals in which the invaginating gastroporus becomes the anus.

Dmbx Diencephalon/mesencephalon homeobox 1.

ecdysozoans Animals that use ecdyson for molting.

En Engrailed gene.

FGF Fibroblast growth factor.

GABA Gamma amino butyric acid.

Gbx Gastrulation brain homeobox gene.

genotype The sequence of all or of specific genes.

gnathostomes Jawed vertebrates (sharks, bony fish, tetrapods).

hemichordates Animals with a notochord equivalent.

hodology Connections between a complex set of neuronal entities.

Hox Homeobox gene.

lophotrochozoans Animals that develop larvae with a lophotroch.

MHB Midbrain/hindbrain boundary.

nerve Bundle of nerve fibers in the PNS.

Otx Orthodenticle homologue gene.

Pax Paired box gene.

phenotype The endproduct of all genes.

progenitor(s) An incompletely committed cell or population of cells.

protostomes Animals in which the invaginating gastroporus becomes the mouth.

rhombomere Compartment of the hindbrain.

topology The position of structures such as nerve centers in a defined spatial relationship.

tract Bundle of nerve fibers running in the CNS.

transcription factors Proteins that bind to DNA to regu late gene transcription.

urochordates Animals in which a notochord exists only in the tail (tunicates, ascidians).

Wnt Wingless type MMTV integration site family member.

9.1 Introduction

Multicellular animals other than sponges, coelenterates and some other basic taxa have a sizable portion of their nervous system grouped into a more or less continuous tissue aggregate, referred to as the central nervous system (or CNS: comprising the brain and spinal cord in vertebrates, the preoral and postoral chain of ganglia in invertebrates). Evolution of this centralized nervous system from a diffuse epidermal nerve network such as is present in more primitive taxa requires a reorganization or elaboration at two basic levels. First, patterning of the developing embryo must establish the position and size of the CNS anlage within the ectoderm, possibly by co-opting existing genes into a new developmental module. Second, the specification of neuronal phenotypes (which in taxa without a CNS is limited predominantly to simple neurosensory and neuromuscular cells whose connections provide limited integrative capacity) must provide molecular diversity to develop an expanded interneuron population that permits the sophisticated sensorimotor processing and integration necessary to govern the more complex motor repertoires exhibited by animals with a CNS.

Although some rudiments of the relevant molecular developmental modules are traceable to taxa without a CNS (Martindale *et al.*, 2004), the evolutionary elaboration has reached paramount status in animals in which the two basic levels have become interdependent features of an integrated process of neural patterning. In these animals, the developmental process that creates the neuronal phenotype diversity has become integrated into the patterning process to generate specific neurons in the correct places, to specify their migratory pathways and their dendritic and axonal growth patterns (in an interaction with environmental influences) to form a characteristic pattern of tracts and nerves, and to regulate proliferation and survival so that the appropriate adult number of neurons is established and maintained (Ghysen, 2003). The evolutionary transformation of a distributed subectodermal nerve net into a highly patterned CNS with numerous compartments, each containing a rich variety of characteristic neuronal types, must be explained as a well-coordinated progression of cell specification at the global and local levels. Moreover, evolution of the structure of the CNS also has to be integrated with the evolution of more sophisticated sensory apparatuses. Combination of the two would facilitate the extraction of more specific sensory information and its utilization to govern more graded and detailed motor responses, thus endowing the bearer of a given modification with enhanced survival.

A narrative of brain evolution can be assembled through a deconstruction process in which various nervous systems of extant animals are compared and a common set of principal features, or Bauplan is deduced. The validity but also the limitations of this approach are discussed in recent reviews (Butler and Hodos, 1996; Nieuwenhuys *et al.*, 1998). While successful at the level of the CNS of craniate vertebrates, it has only limited analytic power at the level of animals without a CNS such as coelenterates and basic bilaterians. Ultimately, the insights gained by this approach have to be related to the developmental patterning mechanisms that regionalize the neural anlage and specify the various populations of neurons within it (Puelles and Rubenstein, 2003). At the core of any model of brain evolution must therefore be the evolution of the transcription factors and intercellular signaling molecules that govern the patterning events necessary for CNS development (Ghysen, 2003; Holland, 2003; Lowe *et al.*, 2003; Meinhardt, 2004) and cell type-specifying transcription factors that govern the evolution of unique cell fates in specific locations (Bermingham *et al.*, 2001; Ghysen, 2003).

In this review we will outline first the basic brain organization of a cyclostome to highlight the differences from the epithelial nerve net found in hemichordate taxa (Figure 1), an outgroup of chordates among deuterostomes. We then briefly compare the major organizational differences in the CNS of adult chordates and other deuterostomes, and highlight what we consider to be the minimal steps in development necessary to generate a craniate nervous system. We then provide an overview of our understanding of the developmental patterning events that underlie CNS regionalization in major deuterostome phyla and then highlight the molecular interactions between regional patterning and neuronal phenotype determination. Finally, to underscore the importance of functional context in brain evolution, we briefly outline how the evolution of the CNS might be related to evolution of the peripheral nervous system (PNS), in particular the evolution of major sensory systems.

We hope that this review, as incomplete a snapshot of our current insight and ignorance as it is, will nevertheless provide a basis for critical and fruitful discussion of the hypotheses and ideas presented.

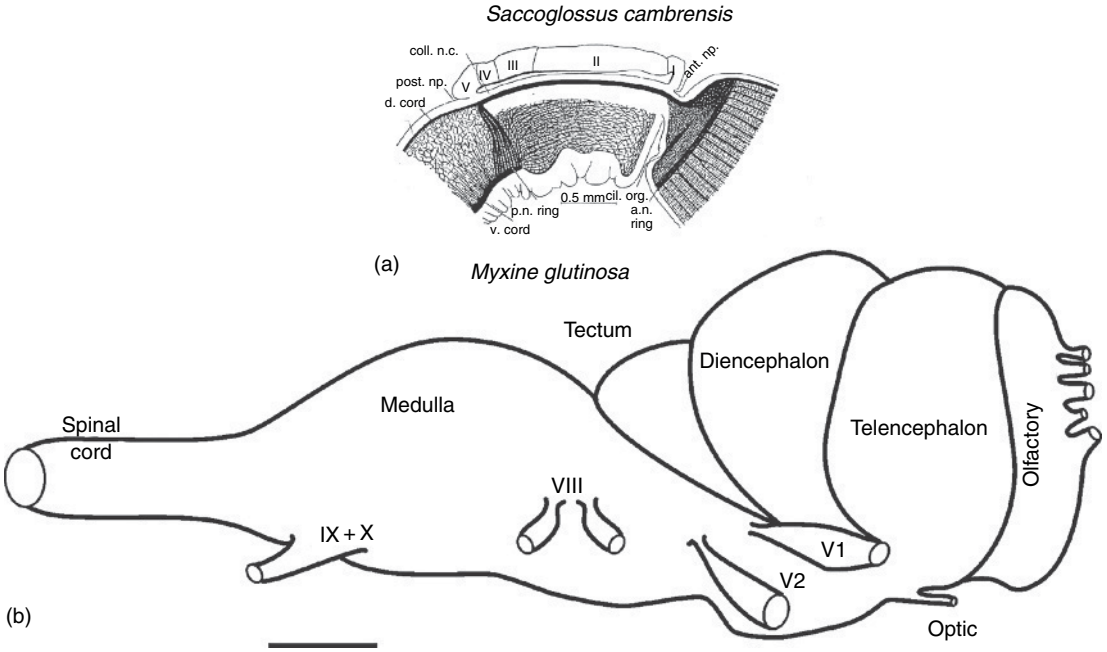

Figure 1 A hemichordate (a) and craniate (b) CNS compared at the same scale. Enteropneust hemichordates have an epidermal nerve network that shows condensations in certain areas. At the base of the proboscis is an anterior nerve ring (a.n. ring) that is next to the ciliary organ (cil. org), which is adjacent to the oral opening (org). The collar region has a collar nerve cord (coll. n.c.), an invaginated part of the epidermis with anterior and posterior neuropores (ant. np., post. np.) that lies dorsal to the buccal cavity. At the third body division, the metasome, the collar nerve cord becomes confluent with the dorsal nerve cord (d. cord) and, through the posterior nerve ring (p.n. ring), with the larger ventral cord (v. cord). Neither true nerves nor major sensory organs are apparent in this simple epithelial nerve net. In contrast, craniates (here shown is a hagfish) have a typical craniate brain that develops from invaginated ectoderm that becomes completely transformed into nervous tissue but remains confined within the former epithelial basement membrane. Only numerous distinct nerves pass through the basement membrane to connect the brain with various multisensory organs that provide chemical (olfaction and taste), mechanical (touch and vestibular sense), and visual (eyes) input for the brain to integrate into a motor output that is elicited via the brainstem and spinal cord. Adapted from Bullock and Horridge (1965) and Nieuwenhuys *et al.* (1998).

9.2 Cladistic Analysis of Major Differences in the Deuterostome Metazoan CNS

Analysis of CNS morphology has to be rooted in an independently corroborated cladistic analysis of the taxa to be investigated, for example, based on gene sequence data (Figure 2). Such analysis exists for only some genes, including the 18s ribosomal DNA, and relationships are likely to change as more data are considered. It should be stressed that the most recent molecular analysis has strongly supported the grouping of all extant jawless craniates into a single taxon, the cyclostomes (Winchell *et al.*, 2002; Takezaki *et al.*, 2003). This analysis also supported the sister taxon relationships of hemichordates and echinoderms and of cephalochordates and craniates. However, it only weakly supported a coherent chordate taxon, indicating that the apparent morphological similarities among chordates are imposed on deep divisions among extant deuterostome taxa (see Evolution of the Amphibian Nervous System).

Obviously, characters should be grouped to fit to such cladograms with minimal additional assumptions. Given that these separations in deuterostome phyla are approximately 600 million years old, it is to be expected that none of the crown taxa will in actuality reflect the ancestral features but rather will represent each its own idiosyncratic mix of characters retained in nearly ancestral states, characters transformed, and characters evolved anew. Such assumptions are warranted as genetic analysis has shown that about 30% of the genes of mammals are not shared with insects and likely arose after the split of protostomes from deuterostomes (Venter *et al.*, 2001). This split also led to an increase in the number of genes including generation of multiple orthologues (Wada *et al.*, 1998; Meinertzhagen *et al.*, 2004).

9.2.1 Principles of Comparative Neuroanatomy

Chordate brains are basically two-dimensional sheets of epithelial cells that have been folded during

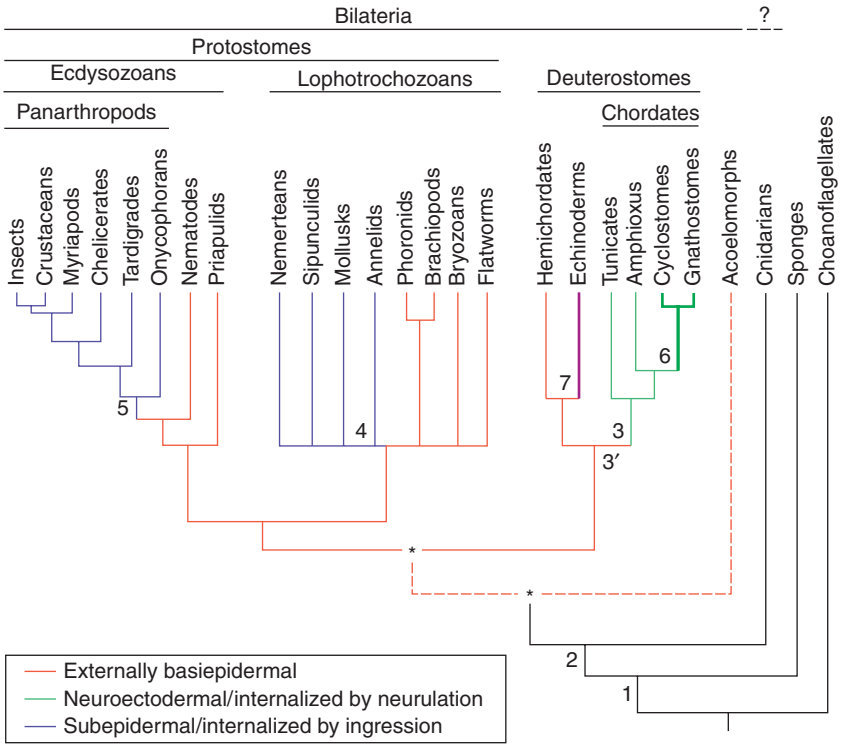

Figure 2 This cladogram of animal relationships shows major evolutionary steps in the generation of the nervous system. Outgroup comparison suggests that ancestral deuterostomes had an epidermal nerve plexus. Formation of a dorsal hollow nerve tube characterizes chordates (3), but a hollow epithelium with an epithelial nerve plexus is also found in hemichordates, suggesting that invagination of ectoderm may be primitive for deuterostomes (3′). Transforming the neural tube into a brain coincides with the formation of all craniate sensory systems, the eyes, ears, olfaction, taste, and lateral line (6). Clearly, under any scenario, the pentameric nervous system of echinoderms is considered as secondarily derived (7). Other major steps in evolution of the brain were the formation of interneurons (1) and the formation of a basiepithelial nerve net (2). Internalized nervous systems evolved independently in lophotrochozoans (4) and ecdysozoans (5). Asterisk indicates uncertainty for the position of the common ancestor of deuterostomes and protostomes. Note that some data question the coherence of the taxon chordates (Winchell *et al.*, 2002). Adapted from Holland, N. D. 2003. Early central nervous system evolution: An era of skin brains? *Nat. Rev. Neurosci.* 4, 617 627.

development in various ways, have translocated below the epidermis and have differentially increased in thickness. Within this tissue sheet, connections are established that provide highways for information flow from sensory inputs, such as the eye, to motor outputs, such as spinal motoneurons. Comparative neuroanatomy tries to unravel the basic organizational principles of neuron populations and fiber tracts, whereas comparative embryology tries to relate species differences in this organization to developmental modifications, and ultimately to alterations in genes or gene expression (Fritzsch, 1998; Nieuwenhuys *et al.*, 1998; Nieuwenhuys, 2002). Essential for this approach is that the sameness of a given structure has to be established to verify homology. Two principal criteria have been used to identify homologous structures in the brain: topology and hodology. Topological analysis compares the relative positions of structures and neuron groups, preferably tracing them back to their origins in the proliferative centers at the ventricles (Bayer *et al.*, 1993). This approach can lead directly to the definition of a blueprint of clonal origin of neurons that can be related to transcription factor expression at the start of brain development and can therefore be integrated with known alterations in the sequences (both coding and regulatory sequences) and expression patterns of genes (Shubin *et al.*, 1997; Puelles and Rubenstein, 2003). Once completed, this approach will allow for establishing homology of a given neural structure across phyla using both topological and genetic criteria (Holland *et al.*, 1992; Takahashi and Holland, 2004). Such a phylogenetic definition of homology for the nervous system will have to face issues of homoplasy, deep homology, and serial homology, all problems already raised for the evolution of other systems (Figure 3).

A second approach to generating a blueprint of vertebrate and invertebrate brains is through hodological analysis of connections (Herrick, 1948; Butler and Hodos, 1996). Ideally, connections

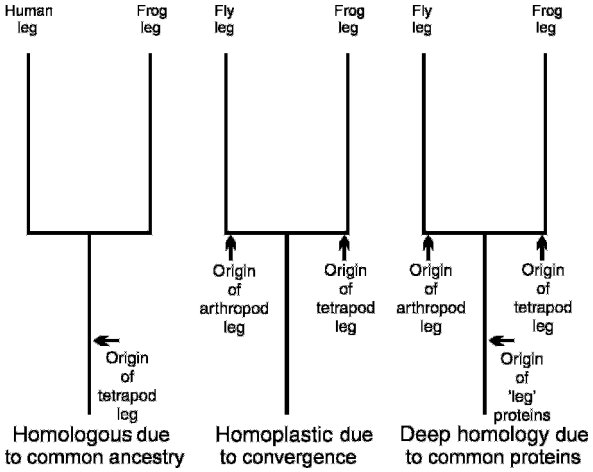

Figure 3 The basic problem of evolutionary homology is depicted. Homologous structures, such as various tetrapod appendages, are considered homologous because they all derive from the ancestral tetrapod limb. In contrast, homoplastic characters are independently derived from an ancestor that had no legs. Deep homology is rooted in molecular mechanisms as well as morphological similarity. For example, recent data shows that the proteins governing leg development are conserved across phyla, indicating that the molecular evolution predated the morphological evolution. Cases for which deep homology (also known as homocracy; Nielsen and Martinez, 2003) has been argued include the legs of arthropods and vertebrates, and the eyes and ears of multiple phyla. Whether this is also the case for brains remains more controversial (Arendt and Nubler-Jung, 1999; Lowe *et al.*, 2003; Meinhardt, 2004). Modified from Arthur (1997), Fritzsch and Beisel (2004), Kozmik *et al.* (2003), and Shubin *et al.* (1997).

between topologically identifiable neuron populations should exhibit a substantial degree of conservation through evolution, because the molecular basis for the homology of neuron populations is also likely to be involved in guiding axon pathway selection by the neuron populations that are specified. Indeed, numerous pathway selection genes appear to be conserved across phyla, suggesting that pathway selection molecules arose early in metazoan evolution (Ghysen, 2003). Nevertheless, the only existing direct analysis that compares cytoarchitectonic and hodologic approaches shows limited congruence between the two blueprints (Diaz *et al.*, 2003). In addition, despite the absence in animals such as salamanders of recognizable neuronal condensations that can be identified cytoarchitectonically, these brains nevertheless exhibit distinct fiber projections that allow for hodological comparisons across phyla in adults (Herrick, 1948; Nieuwenhuys *et al.*, 1998) and during development (Rettig *et al.*, 1981). Hodology may therefore provide a stronger basis for comparison than cytoarchitectonics, even if it does not derive from cytoarchectonically distinct groups of neurons, the so-called nuclei.

The analysis provided below will follow both of these approaches (topological and hodological), keeping in mind that topological relationships can be complicated through postnatal migration, modifications of input and output relationships, and alterations in absolute positions owing to intercalation of different cellular masses (Glover, 2001).

9.2.2 Comparative Appearances of Brains, Spinal Cords, and Nerves

9.2.2.1 Craniates (cyclostomes and gnathostomes) The jawless cyclostomes, lampreys and hagfish, constitute a basic clade of craniates that shows the major features of brain organization also found in gnathostomes (jawed vertebrates). Externally, the brain of extant cyclostomes consists of a bilaterally symmetric rostral enlargement, the forebrain (telencephalon). This is composed of an olfactory bulb and a cerebral hemisphere (Figure 4). Each hemisphere is connected to the single, bipartite diencephalon. The diencephalon gives rise to a number of neural appendages. Ventrolaterally are located the paired optic nerves leading to the lateral eyes. Dorsally lies a single enlargement, the habenula, that in lampreys, but not hagfish, has an attached pineal organ (Pombal *et al.*, 1999). Ventrally is located the pituitary gland. The central ventricle of the diencephalon is continuous with the ventricle in the midbrain, the hindbrain, and the central canal of the spinal cord, all of which are greatly reduced in hagfish (Nieuwenhuys *et al.*, 1998). Lampreys have a dorsal central opening in the midbrain that is covered by a choroid plexus, a uniquely derived feature of lampreys not shared by other craniates. Lampreys have an oculomotor nerve leaving the midbrain ventrally, which hagfish do not possess. Likewise, the next more caudal nerve found in lampreys, the trochlear nerve, exiting lateral to the cerebellum, is not found in hagfish. Caudal to the small cerebellum in lampreys is the rhombencephalon (hindbrain) with a large choroid plexus covering the IVth ventricle. A small ventricle is also found in hagfish but there is no trace of a choroid plexus and the presence of a cerebellum has been questioned (Nieuwenhuys *et al.*, 1998). Whether the absence of a choroid plexus in hagfish is primitive and related to the unusual isotonicity of hagfish to seawater (Griffith, 1987) remains unclear.

As discussed previously (Fritzsch and Northcutt, 1993), lampreys have all the hindbrain nerves found in gnathostomes (trigeminal, abducens, facial, otic or statoacoustic, glossopharyngeus, and vagus), with the possible exception of the hypoglossal

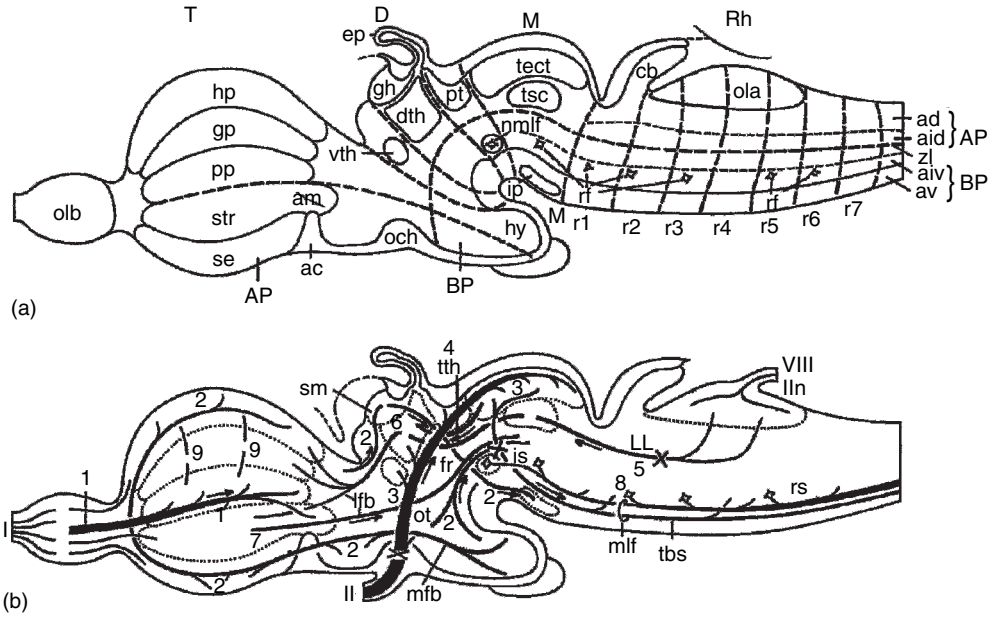

Figure 4 Basic organization (a) of a craniate brain (T, telencephalon; D, diencephalons; M, mesencephalon; Rh, rhombencephalon) and fiber tracts (b) are shown. Longitudinal divisions are the alar plate (AP) and basal plate (BP), which are separated by the sulcus limitans (zl). Each of these plates is subdivided into two areas (ad, area dorsalis; aid, area intermediodorsalis; aiv, area intermedioventralis; av, area ventralis). The rhombencephalon has approximately seven rhombomeres (r1 r7) with motoneurons and the reticular formation (rf) deriving from the basal plate and the octavolateral area (ola) and cerebellum (cb) from the alar plate. The midbrain has the tectum (tect), torus semicircularis (tsc), and interpeduncular (ip). Several prosomeres are shown in the diencephalon that extend from the hypothalamus (hy) to the pallium (hp, hippocampal pallium; gp, general pallium; pp, piriform pallium) and basal telencephalon (str, striatum; am, amygdale; se, septum). Several neuronal masses differentiate in the diencephalon (gh, habenula; pt, pretectum; nmlf, nucleus of the medial longitudinal fascicle; vth, ventral thalamus). The olfactory tract (1) originates from the olfactory bulb (olb) that receives the olfactory input (I). Secondary olfactory fibers reach various areas of the telencephalon, including the dorsal (sm, stria medullaris) and ventral thalamus (2). The fasciculus retroflexus (fr) relays information to the ip. Retinal axons (II) project through the optic chiasme (och) to the dorsal thalamus and optic tectum (3). The midbrain receives fibers from the octavolateral area via the lateral lemniscus (LL, 5), which projects (4) to the dorsal thalamus (6), which in turn projects to various parts of the telencephalon. Fibers descend via the lateral forebrain bundle (lfb) to the midbrain. Fibers from the interstitial nucleus of the medial longitudinal fascicle (mlf) (is), the tectum (tbs) and the reticular formation form together the mlf (mlf, 8) as well as the reticulospinal (rs) and tectobulbospinal (tbs) tracts. Association fibers (9) interconnect pallial areas. Modified from Nieuwenhuys, R. 2002. Deuterostome brains: Synopsis and commentary. *Brain Res. Bull.* 57, 257 270.

nerve (but see Kuratani *et al.*, 2002), the relative positions and fiber compositions of these nerves are very similar but not identical in cyclostomes and gnathostomes. For example, the abducent nerve root is almost integrated into the trigeminal nerve root in lampreys, whereas it is always a separate ventral nerve root at a more caudal level in gnathostomes. Moreover, gnathostomes have three distinct motoneuron populations in the brainstem, each innervating a different type of peripheral target: the somatic motoneurons that innervate somitomere-derived musculature, the branchial motoneurons that innervate branchial arch-derived musculature, and the visceral motoneurons that innervate neural crest-derived parasympathetic ganglia of the head and body. In contrast, cyclostomes as a group lack the somatic motoneurons of the hypoglossal nucleus and have no visceral motoneurons as no cranial parasympathetic ganglia are known to exist (Fritzsch and Northcutt, 1993; Nieuwenhuys *et al.*, 1998). To emphasize differences between gnathostomes and cyclostomes, the hagfish hindbrain, while recognizable as such, is unusually shaped, which relates to differences in its internal organization (Nieuwenhuys *et al.*, 1998). The organization of cranial nerves in hagfish, other than the apparent absence of the entire extraocular muscle-related nerves, shows a number of deviations from gnathostome vertebrates. Hagfish have three completely segregated parts of the trigeminal nerve, two otic (statoacoustic) nerves, no recognizable vagal ganglion, and a facial nerve that exits dorsal to the otic nerves (Figure 1). The composition and evolution of cranial nerves will be discussed below and compared with other deuterostomes (see Section 9.2.3).

The spinal cord in craniates is a continuous extension of the neural tissue of the hindbrain. Adult

cyclostomes have unusually shaped spinal cords that are dorsolaterally flattened and have ventral and dorsal nerve roots. Lampreys have separated dorsal and ventral roots that do not form mixed spinal nerves and are asymmetric between the left and right side. Similar organizations of spinal nerves are noted for cephalochordates (Bone, 1960) and have been suggested to be primitive for chordates as this pattern is also found in hagfish and some gnathostomes (Fritzsch and Northcutt, 1993). Hagfish have, like gnathostomes, fused dorsal and ventral nerve roots except in the tail. It is believed that having fused dorsal and ventral roots is a derived feature of gnathostomes and that the superficial similarity of this feature in hagfish is independently derived (Bone, 1963; Nieuwenhuys *et al.*, 1998). As we will see below, the overall fiber composition of spinal nerves is highly variable among cyclostomes and fits neither the cephalochordate nor the basal gnathostome composition. Consequently, the analysis of character polarity of such basic issues as cranial and spinal nerves, their composition and their relationship to nerves of cephalochordates and urochordates is problematic (Fritzsch and Northcutt, 1993). We will revisit this issue after the internal organization of deuterostome nervous systems and nerves has been described (see Section 9.2.3).

9.2.2.2 Cephalochordates The central nervous system of cephalochordates is a simple tube that does not show any obvious enlargement at the rostral pole that can be compared with the brain of craniates (Figure 5), hence the alternate name acrania for this taxon. The hollow tube has a central canal that shows a vesicular enlargement at the anterior pole. The open neural canal of this vesicle has processes of the frontal eye sensory cells extending into it (Lacalli, 2004). Like craniates, cephalochordates and urochordates have a unique structure that extends throughout the central canal, Reissner's fiber (Figure 5). However, cephalochordates show a number of obvious topological differences from craniates with respect to apparently similar structures. For example, in craniates the notochord ends at the level of the hindbrain, whereas the notochord extends in cephalochordates beyond the rostral aspect of the neural tube. The notochord is now known to have a major inductive influence on brain formation through diffusible proteins such as sonic hedgehog (Shubin *et al.*, 1997; Litingtung and Chiang, 2000) and thus this influence would likely be different in cephalochordates and craniates.

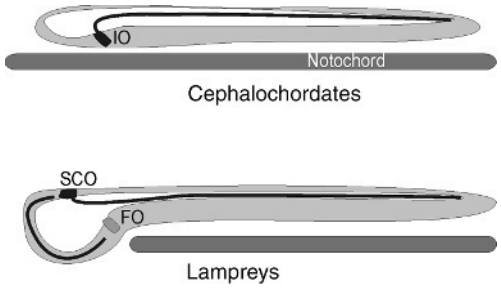

Figure 5 The different position of the origin of Reissner's fiber can be partially reconciled through developmental switches, as described in a single species of bony fish (Olsson, 1993). In principle there are three solutions: (1) Reissner's fiber is not homologous. (2) Despite the topographical differences of the organs that produce it, Reissner's fiber is homologous and is related to the apparently homoplastic structures through a deep homology for which no molecular evidence exists at the moment. (3) The infundibular (IO) and the subcommisural organ (SCO) represent a split of a single original cell population, intercalating a novel population (black line). If this is accepted, than all the area between the subcommisural and flexural organ (FO) of craniates and the infundibular organ of cephalochordates could be viewed as a craniate neomorph, that is the entire telencephalon and large areas of the thalamus of craniates have no equivalent in cephalochordates, as previously suggested (Takacs *et al.*, 2002). Modified from Fritzsch and Northcutt (1993) and Olsson (1993).

Turning now to the cranial and spinal nerves of cephalochordates, it is obvious that they are difficult to compare with those of craniates. To begin with, they are distributed too far rostral (Northcutt, 2001), leaving virtually no space for what is typically considered the brain. There are no true ventral roots in the sense of bundles of motoneuron axons leaving the spinal cord (Bone, 1960) and the notochord is composed of muscle fibers that form a unique synaptic contact with the ventral part of the spinal cord (Holland, 1996). Moreover, there are no dorsal root ganglia anywhere along the neuraxis (Fritzsch and Northcutt, 1993; Lacalli, 2004). Lastly, there is only one true ventral nerve, the first nerve. Starting with the second nerve, all dorsal nerves are more and more caudal on the right than on the left side, an asymmetry that by far exceeds anything found in other chordates (Fritzsch and Northcutt, 1993; Nieuwenhuys *et al.*, 1998). Other features of the cephalochordate anterior neuraxis are the conspicuous lamellar body (present only in larvae) and the Joseph cells. More caudally, one can find individual ocelli that extend throughout the spinal cord (Nieuwenhuys *et al.*, 1998; Lacalli, 2004). How these features relate to hindbrain and spinal cord divisions is unclear, leaving the questions of the caudal boundary of the brain and of the existence of brain subdivisions open (Northcutt, 2003). Paired sensory organs are

conspicuously absent in cephalochordates. However, certain primordia of mechanosensors and chemosensors may be present among the numerous single or multicellular organs (Fritzsch, 1996; Lacalli, 2004; Mazet *et al.*, 2004; Holland, 2005).

9.2.2.3 Urochordates In contrast to the rather uniform appearance of the neuraxis of cephalochordates, the developing urochordate CNS can be easily divided into a tripartite structure (Figure 6) that has long been recognized (Bone and Mackie, 1982). These divisions are (1) a rostral ganglion (totaling roughly 215 cells in *Ciona* and approximately 75 cells in *Oikopleura*), which contains sensory receptor structures, (an ocellus and/or an otolith), followed by (2) a caudal ganglion (containing approximately 45 cells in *Ciona* and approximately 25 neurons in *Oikopleura*), from which extends a caudal nerve cord (roughly 65 cells, mostly ependymal, in *Ciona*, and roughly 30 neurons and 25 support cells in *Oikopleura* (Meinertzhagen *et al.*, 2004; Søviknes *et al.*, 2005)). In addition, a slender neck region containing six cells lies between the two ganglia in *Ciona*. Each of these anteroposterior subdivisions of the ascidian CNS is itself patterned along the dorsoventral axis, a patterning that is revealed by the expression of specific marker genes. Other urochordate taxa seem to have primarily variations in size, not in structure (Meinertzhagen *et al.*, 2004). The ganglia of adult urochordates have the organization of an invertebrate ganglion, with cell bodies at the periphery and the neuropil in the center (Bullock and Horridge, 1965). Several nerves that vary considerably between species have been traced from adult ganglia, reaching up to 75 nerves in certain salps. These nerves appear to be mixed sensory and motor nerves and are asymmetric in several species, potentially related to the overall body asymmetry. The organization of motor projections varies among urochordates. In the *Ciona* larva, all the motoneurons are located in the caudal (visceral) ganglion and project along the aneural nerve cord and on to the peripheral muscle (Katz, 1983). In *Oikopleura*, the nerve cord contains motoneurons, which project directly laterally to the peripheral muscle (Bone, 1992). It is noteworthy here that comparisons between urochordates and cephalochordates claim similarities in the internal sensory organs, in particular the infundibular sensory cells and otolith (Nieuwenhuys, 2002; Lacalli, 2004). However, the interpretation of various sensory cells and organs outside the CNS is controversial with respect to vertebrate homology (Burighel *et al.*, 2003; Lacalli, 2004; Mackie and Singla, 2004; Holland, 2005). Some adult urochordates have fairly complex eyes attached to the cerebral ganglion. Modern tracing studies to unravel the details of neuronal connections within urochordate ganglia have yet to be conducted (Bullock and Horridge, 1965; Meinertzhagen *et al.*, 2004), but some are on their way.

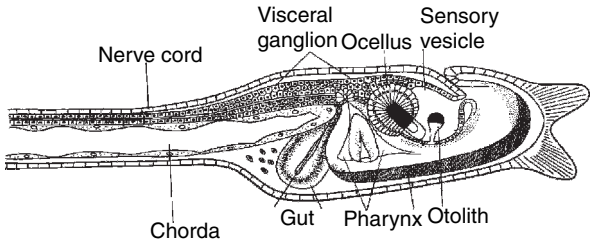

Figure 6 Simplified scheme of the urochordate *Ciona* larva. The nerve cord of *Ciona* consists only of epithelial cells (whereas in the appendicularian *Oikopleura* it contains neurons). In *Ciona*, the visceral (caudal) ganglion contains the motoneurons and is separated by a small neck from the sensory vesicle (rostral ganglion) that contains a rostral otolith and a caudal ocellus. The pharynx has gill slits.

9.2.2.4 Hemichordates Whether the simple nervous system of hemichordates should be referred to as a CNS is unclear (Bullock and Horridge, 1965). The overall organization is that of a basiepithelial plexus that shows regional concentrations in the three parts of the body, the protosome (the preoral proboscis), the mesosome (the postoral collar), and the metasome (or trunk, with rostral gill slits). The intraepithelial nerve plexus is well developed on the basement membrane but remains epithelial even in the invaginated collar region, which is hollow and opens through two neuropores (Figure 1). Concentrations of longitudinal strands of cells and fibers exist also on the trunk, where they form a dorsal and a larger ventral cord. The proboscis has a well-developed nerve plexus and numerous sense cells. A gut diverticle, the stomochord (which shares anatomic features with the notochord; (Welsch and Storch, 1970) but is different in its molecular organization (Shubin *et al.*, 1997)), extends into the proboscis. Except for the preoral ciliary organ with its abundance of sensory cells, there are no specialized sense organs. Concerning nerves, it appears that muscles are supplied by nerve fibers that cross the basement membrane singly and diffusely without forming obvious peripheral nerves.

9.2.2.5 Echinoderms The nervous system of echinoderms is interesting in its own right (Bullock and Horridge, 1965), but is likely of limited significance for chordates, as this would require the transformation of a pentameric organization into the dorsal

hollow nerve chord. While this cannot be ruled out, we assume for the sake of simplicity that echinoderms are derived and not directly related to the chordate ancestor. It needs to be stressed, however, that tremendous progress has been made in the study of sea urchin embryogenesis and these insights have recently been extended to nervous system development (Poustka *et al.*, 2004). However, the most detailed analysis seems to be concentrated on endoderm, ectoderm, and mesoderm formation (Davidson *et al.*, 2002). This analysis has revealed a complex network of gene interactions, many of which are conserved across phyla and are thus likely to be at least equally complex in other deuterostomes. It is important to understand that these data show that patterning genes function in networks and their function needs to be understood in the context of other genes with which they are co-expressed.

In summary, the CNS of deuterostomes shows a variety of forms, from a hardly specialized basiepithelial nerve plexus (hemichordates), to a few small ganglia with a tail nerve cord (urochordates; or a tailless head in adult sessile urochordates), to a swimming spinal cord with a hardly recognizable cerebral vesicle (cephalochordates), to a fully developed brain and spinal cord (craniates). Despite these overall differences, similarities related to a certain degree of rostrocaudal and dorsoventral patterning are present. These may be directly related to the overall rostrocaudal patterning of the body and to the fact that the neural tube evolved only once in ancestral chordates (Meinhardt, 2004) and has maintained a molecularly identical dorsoventral patterning scheme (Wada and Satoh, 2001). These issues will be revisited in a later section once we have introduced the transcription regulating genes that are involved in such patterning.

9.2.3 Organization of Identified Neuron Populations and Projections

Cytoarchitectonic specializations akin to cortical layers and aggregations of neurons into nuclei have not been observed in any deuterostome animal below the level of craniates. However, neuron populations can be identified throughout the animal kingdom on the basis of unusual size or through the use of specific markers.

9.2.3.1 Large neurons Particularly large neurons are a common feature of both invertebrate and vertebrate nervous systems. In many chordates, large neurons with descending axons have been identified in the rostral region of the neuraxis. Using cytological criteria, one can identify certain large reticulospinal neurons in the rhombencephalon of lampreys (the Muller and Mauthner cells) that have long descending axons that extend the length of the spinal cord (Nieuwenhuys *et al.*, 1998). Similar neurons are found in hagfish, but Mauthner cells cannot be identified among them and whether the other large cells are homologous to Muller cells is unclear. In the rostral neuraxis of juvenile cephalochordates, two pairs of larger interneurons with descending axons have been identified and termed ventral giant cells of the primary motor center. Similar large neurons have been serially reconstructed from electron micrographs in larval cephalochordates (Lacalli, 1996). These are potentially homologous to the reticulospinal neurons of cyclostomes. The axons of cephalochordate motoneurons also descend along the cord for unknown distance, however, and thus could be mistaken for reticulospinal neurons. Assessment of neurotransmitter phenotype could resolve this question, as motoneurons are expected to be cholinergic (see Section 9.2.3.2).

Cephalochordates are well known for another system of large neurons, the Rhode cells (Bone, 1960; Nieuwenhuys *et al.*, 1998). These cells are situated dorsally in the spinal cord starting at nerve VI in juveniles and somewhat more rostrally in adults (Ekhart *et al.*, 2003). Neurons with somewhat similar characteristics have been described in various craniates (Harper and Roberts, 1993) and may be a common feature of cephalochordates and craniates (Fritzsch, 1996).

No large neuronal elements have been described in urochordates (Bullock and Horridge, 1965), but large neurons have been described in hemichordates, clustered in the caudal part of the collar cord and also scattered in more rostral and caudal areas. Some of these neurons have uncrossed or crossed axons that extend toward the ventrolateral longitudinal muscles and therefore may be motoneurons (see below). Others have been compared to the Mauthner and Muller cells of craniates (Bullock and Horridge, 1965). However, independent confirmation of similarities with other deuterostome neurons needs to be established using immunocytochemistry or *in situ* hybridization for molecular markers, as in recent analysis of the urochordate ocellus (Sun *et al.*, 2003). At the moment, all the above anatomical similarities are tentative and more work combining tract tracing with assessment of gene expression is needed in more deuterostomes to substantiate potential homologies.

9.2.3.2 Motoneurons Motoneurons can be compared easily across deuterostomes for the following reasons. First, motoneurons are cholinergic in echinoderms, urochordates, cephalochordates, and craniates, and this may constitute a conserved feature of deuterostomes (Holland, 1996). Second, all motoneurons constitute efferent populations that target structures outside the CNS. However, these targets may be mesoderm-derived muscle fibers, neural crest-derived autonomic ganglia, or placode-derived hair cells (Fritzsch, 1999).

In hemichordates, motoneurons may be among the identified giant neurons, but details are unclear and no data on the cholinergic nature of these neurons is available (Bullock and Horridge, 1965). In some urochordates (such as *Ciona*), motoneurons are found only in the visceral ganglion where three to five pairs of cells have been recognized. The axons of these cells extend down the nerve cord and then exit to innervate adjacent muscle fibers (Katz, 1983). In other urochordates, such as *Oikopleura*, motoneurons are additionally found in the nerve cord itself (Bone *et al.*, 1996).

Cephalochordates have three different types of contacts with muscle fibers (Bone, 1960; Bone *et al.*, 1996; Holland, 1996). Two of these contacts are from muscle fibers to the spinal cord forming ventral roots and medioventral roots with the axial musculature and the muscles of the notochord. The motoneurons supplying these muscle fibers have only been tentatively identified for axial musculature and appear to project axons for at least three segments rostral or caudal before exiting (Figure 7).

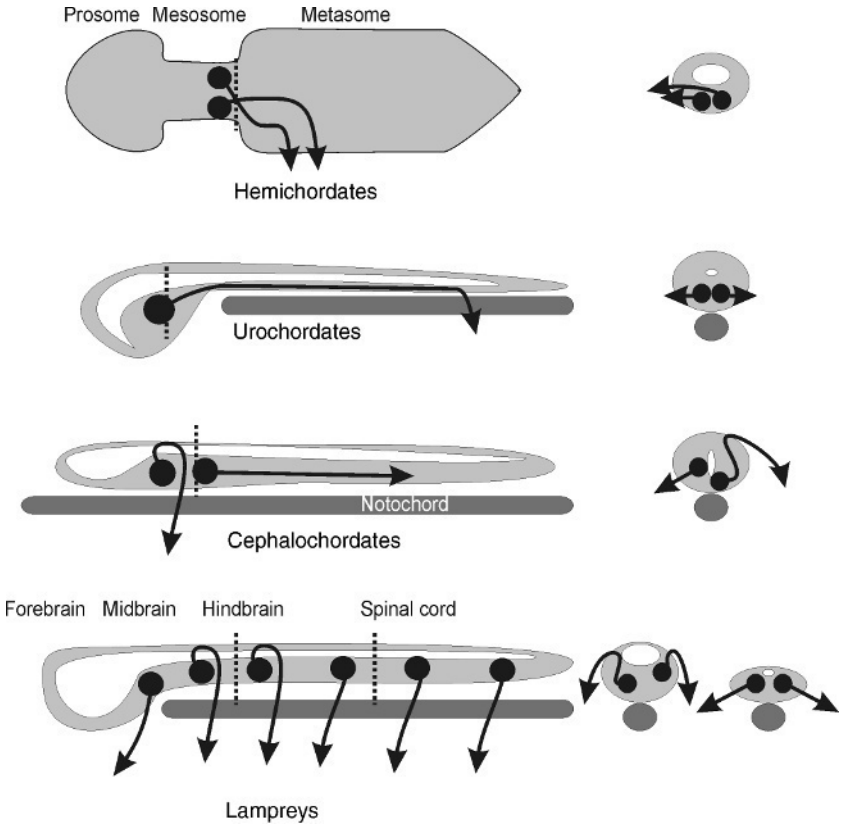

Figure 7 This scheme shows the distribution of motoneurons viewed from the dorsal aspect (Hemichordates) or lateral aspect (others) as well as in coronal sections at the levels indicated by the dotted lines. Motoneurons in hemichordates are predominantly located in the caudal half of the collar cord and their axons typically cross the basal membrane individually to reach muscle fibers. In some urochordate larvae, all motoneurons are concentrated in the caudal ganglion and project through the nerve cord to reach muscle fibers of the tail. In other urochordates, motoneurons are additionally present in the nerve cord and project directly to adjacent muscles (not shown). Cephalochordates have three types of motoneurons (only two are shown). One type is the somatic motoneuron that extends an axon along the spinal cord to innervate muscular processes that form synapses abutting the cord. The second type is the visceral motoneuron that projects through the dorsal root to innervate the pterygial muscle. Lampreys have in their spinal cord only somatic motoneurons whose axons project out through ventral roots, and in their hindbrain only dorsal exiting motoneurons, referred to as branchiomotoneurons because they innervate muscle derived from branchial arches. Lampreys have three populations of ocular motoneurons in the midbrain hindbrain region, some of which have the appearance of somatic motoneurons. Modified from Bullock and Horridge (1965), Fritzsch and Northcutt (1993), and Fritzsch (1998).

Motoneurons are found throughout the spinal cord and as far rostral as the primary motor center between the second and third dorsal nerves. An additional set of motoneurons encompasses the so-called visceral motoneurons. These are the most ventral neurons in the nervous system, situated virtually at the floor plate (Bone, 1960). The axons of these cells exit through the dorsal roots and supply the pterygeal muscle with cholinergic endings (Bone, 1960; Bone *et al.*, 1996).

In cyclostomes, somatic motoneurons are present only in the spinal cord and in the oculomotor nuclei near the isthmus, whereas branchial motoneurons are only present in the brainstem (Fritzsch and Northcutt, 1993; Fritzsch, 1998). Absence of extraocular motoneurons in hagfish may represent a primitive condition and, at least for oculomotor and trochlear motoneurons, may be related to a different organization of the isthmus and the apparent absence of a cerebellum. We will discuss this issue below when we consider the evolution of the midbrain/hindbrain boundary (MHB). It is important to note that cyclostomes do not have autonomic ganglia and lack visceral motoneurons that innervate such ganglia. It appears that autonomic ganglia arose with gnathostomes and in association with the formation of preganglionic parasympathetic motoneurons in the head and the caudal part of spinal cord (the craniosacral parasympathetic preganglionic motoneurons) and preganglionic sympathetic motoneurons in the thoracic and lumbar spinal cord (Fritzsch, 1998).

In summary, the motoneurons of deuterostomes show certain basic similarities across taxa but also exhibit unique taxon-specific features that do not appear to follow a progressive evolutionary transformation but rather indicate independently derived transformations that may have a common root in the basiepithelial nerve plexus of other deuterostome taxa (Figures 7 and 8).

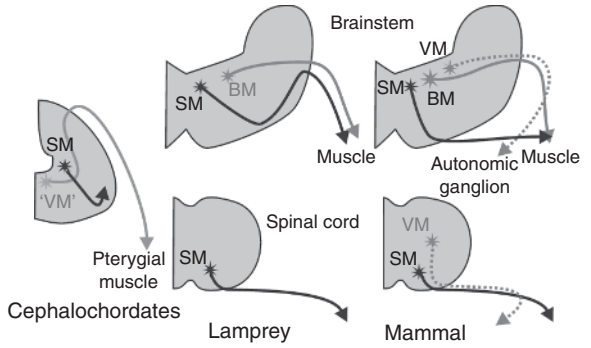

Figure 8 Motoneurons of craniates and cephalochordates are compared. Two motoneurons have been characterized in cephalochordates, the visceral motoneurons (VM) that innervate the pterygial muscle and exit through dorsal roots and the somatic motoneurons that do not exit the neural tube but rather form synapses with muscle processes at the lateral wall of the spinal cord. Lampreys have only somatic motoneurons that exit through the ventral root in the spinal cord. Mammals have evolved visceral motoneurons in the spinal cord that migrate into a distinct position and project to autonomic ganglia. The brainstem of lamprey has mainly branchiomotoneurons (BM) that project through the dorsal root. Whether the abducens motoneurons are somatic motoneurons (SM) in lampreys is unclear as they also project through the dorsal root. In mammals there are additionally visceral motoneurons in several cranial nerves that project to the parasympathetic ganglia. These visceral motoneurons can be regarded as special branchiomotoneurons. Modified from Fritzsch and Northcutt (1993) and Fritzsch (1998).

9.2.3.3 Sensory afferents We next turn to the distribution of afferents, a feature that is highly indicative of specific sensory modalities in craniates (Fritzsch and Northcutt, 1993). As outlined above, sensory input through cranial nerves in craniates can be simply summarized: all sensory systems, except for olfaction and the visual system, reach the brain through nerves that terminate in the rhombencephalon (Nieuwenhuys *et al.*, 1998). More precisely, all sensory input is into the alar plate of the rhombencephalon or the alar plate of the midbrain (vision) or the forebrain (olfaction). The only noncraniate deuterostomes for which a reasonably detailed knowledge of central sensory projections exists are the cephalochordates. In these animals, the central projections of rostral nerves and the first pair of dorsal nerves have been described using tract tracing (Fritzsch, 1996) and serial electron microscopic reconstruction (Lacalli, 1996, 2004). Both approaches show that the first pair of dorsal nerves passes ventrally along the lamellar body and derives from the organs of de Quatrefages as well as some of the numerous sensory cells of the rostrum. However, interpretations of the likely homology of this region differ substantially. The serial reconstruction data have been interpreted to indicate that cephalochordate larvae have a midbrain that receives not only the fibers from the rostral sensory cells and organs, but also from the frontal eye that is a likely homologue of the lateral eyes of craniates (Lacalli, 1996, 2004). In contrast, the tracing studies have been interpreted to show that the termination area near the lamellar body is homologous to the alar plate of the hindbrain (Fritzsch, 1996). Under the second interpretation, cephalochordates lack a midbrain and hindbrain features start as far rostral as the first and second dorsal nerve. This interpretation was recently supported

by an analysis of gene expression (Takahashi and Holland, 2004) and will be discussed below (see Section 9.3). Further studies as well as the clarification of the relationships of the various sensory organs of chordates across taxa is needed (Fritzsch and Beisel, 2004; Lacalli, 2004; Mackie and Singla, 2004; Mazet *et al.*, 2004; Holland, 2005). More molecular markers, in particular various transcription factors, need to be investigated to provide more plausibility to the various scenarios proposed.

Urochordates also have sensory input to their ganglia that is derived from cells that share certain developmental steps with the neural crest (Meinertzhagen *et al.*, 2004), but much more analysis is required in these species before arguments about homology can be made.

9.2.3.4 Immunocytochemically identified neuron populations We turn next to certain populations of immunocytochemically identified neurons in chordates. We will here focus only on three neuron types, those expressing the oligopeptide neurotransmitter FMRFamide, those expressing tyrosin hydroxylase (TH), the rate-limiting enzyme for synthesis of catecholamine neurotransmitters, and those expressing GABA, the principal inhibitory neurotransmitter. These examples are selected because the data are most revealing for the overall question of similarities and uniqueness of features across deuterostomes. We should emphasize, however, that immunohistochemical identification of proteins (such as TH) in lower deuterostomes using antibodies raised against the craniate proteins should be regarded as tentative without independent confirmation using other molecular techniques.

9.2.3.4.(i) FMRFamide FMRFamide is widely distributed in the nervous system of invertebrates, including coelenterates (Katsukura *et al.*, 2003) and may be among the oldest neurotransmitters (Cazzamali and Grimmelikhuijzen, 2002; Seipel *et al.*, 2004). In deuterostomes, this small peptide has been demonstrated in echinoderms (Garcia-Arraras *et al.*, 1991) and immunoreactivity is abundantly present in the central and peripheral nervous system of cephalochordates (Uemura *et al.*, 1994; Bone *et al.*, 1996). In contrast, craniates seem to have only a few RFamide peptides (Hinuma *et al.*, 2000; Yano *et al.*, 2004), and the almost complete absence of FMRFamide in the peripheral nervous system and the gastrointestinal system of craniates stands in stark contrast to the abundant presence of this peptide in protostomes, coelenterates, echinoderms, and cephalochordates. This difference supports the notion that the enteric nervous system of the cephalochordate atrium is not related to the enteric system of craniates as has been previously suggested (Bone, 1961; Fritzsch and Northcutt, 1993). This may be related to a more recent evolution of the vertebrate enteric nervous system from the neural crest (Fritzsch and Northcutt, 1993).

In cephalochordates, the visceral motoneurons and possibly some of the somatic motoneurons are FMRFamide-immunoreactive (Pestarino and Lucaroni, 1996). No FMRFamide immunoreactivity has been reported for craniate motoneurons, indicating that the visceral motoneurons and some somatic motoneurons of cephalochordates may resemble a more primitive condition characteristic of coelenterates and protostomes. In this regard, it will be important to assess the expression of FMRFamide in urochordates and hemichordates.

9.2.3.4.(ii) Catecholaminergic neurons The TH gene has been sequenced in several deuterostomes and shown to be a single orthologue with high sequence similarity between cephalochordates and vertebrates and lower sequence similarity between either of these and the urochordates. Immunocytochemistry and *in situ* hybridization in cephalochordates shows a rostral and dorsal distribution near the anterior end of the neural tube and the first two dorsal nerves. This has been interpreted as indicating similarities with the di-, mes-, and rhombencephalic catecholaminergic neuron groups known in craniates. In this context, it is important to note that a genetic basis of the development of catecholaminergic and serotonergic neurons in mammals has been studied extensively and upstream regulators have been identified (Qian *et al.*, 2001; Brunet and Pattyn, 2002; Pattyn *et al.*, 2003a). Serotonergic neurons have been found to form through a positionally and temporally regulated fate switch of visceral motoneuron progenitors (Pattyn *et al.*, 2003b). If such developmental linkage is conserved, one would expect serotonergic neurons to form only near motoneurons, which closely fits the currently known distribution of serotonergic neurons near the first motor center in cephalochordates (Lacalli, 1996; Moret *et al.*, 2005). Most interesting is the case of catecholaminergic neurons related to the solitary tract in vertebrates (Qian *et al.*, 2001). These neurons form a longitudinal column in the hindbrain and depend on several transcription factors that are longitudinally expressed (Brunet and Pattyn, 2002). Interestingly, in cephalochordates a longitudinal column of putative catecholaminergic neurons is located adjacent to the fibers of sensory cells on the rostrum that enter through the first nerves

(Fritzsch, 1996; Lacalli, 1996). This molecular and topographic relationship could indicate that at least some of these fibers are chemosensory and that they terminate in the equivalent of the solitary tract. Given the paucity of data, other interpretations are possible.

In urochordates, a small population of dopamine + and TH+ cells is found in the ventral region of the rostral ganglion in both *Ciona* (Moret *et al.*, 2005) and *Oikopleura* (Søviknes and Glover, unpublished data). Moret *et al.* (2005) surmise on the basis of this location that the ventral part of the rostral ganglion is homologous to the vertebrate hypothalamus, which also contains catecholaminergic neurons. As far as we know, catecholaminergic neuron populations have not yet been described in hemichordates.

9.2.3.4.(iii) GABA GABA is the principal inhibitory transmitter in vertebrates and is also a major inhibitory transmitter in invertebrates. It therefore appears to be a common currency for inhibition throughout the animal kingdom. In invertebrates, GABA-immunopositive neurons typically occupy specific locations within ganglia either as distinct single neurons or clusters of neurons (reviewed in Søviknes *et al.*, 2005). In vertebrates, GABA-immunopositive neurons have widespread distributions and nearly all regions of the brain are replete with GABA-immunopositive terminals (Anadon *et al.*, 1998a; Melendez-Ferro *et al.*, 2000, 2002, 2003). However, the developmental origins of mammalian GABA-immunopositive neurons are much more discrete, with subsequent migration giving rise to their far-flung positions (Stuhmer *et al.*, 2002a, 2002b).

In the lamprey, the earliest GABA-immunopositive neurons appear in late embryos in the basal plate of the isthmus, in the caudal rhombencephalon, and in the rostral spinal cord (Melendez-Ferro *et al.*, 2002, 2003). Somewhat later, GABA appears in the prosencephalon, first in the diencephalon and later in the cortex. GABA neurons then appear elsewhere, but with distinct regional differences in distribution.

In the urochordate *Oikopleura*, GABA-immunoreactive neurons also originate at discrete sites within the CNS, both in the rostral ganglion and in the caudal ganglion (Søviknes *et al.*, 2005). GABA-immunoreactive neurons are not found, however, in the nerve cord, in contrast to the extensive population of GABA neurons in the spinal cord of vertebrates. Thus, GABA neurons appear to be regionally patterned in both urochordates and vertebrates, but there seems to be an increasingly broader distribution of GABA neurons in higher taxa, perhaps in conjunction with an increasing demand for local inhibitory inputs to provide finer regulation of sensory and motor information traffic.

In summary, comparative analysis of common neuronal cell types indicates a variety of patterns of topology and homology. Enough similarities exist to suggest that most of the apparent differences can probably be interpreted as variations on a theme that may already have been set up in the last common ancestor of all deuterostomes.

9.3 Making and Placing Neurons: The Evolution of Cell Fate and Regional Patterning

The expression of multiple genes in a nested anteroposterior pattern in the basiepithelial nerve plexus of hemichordates (Holland, 2003; Lowe *et al.*, 2003), combined with the presence of neuron-specific patterning genes and transcription factors in coelenterates (Seipel *et al.*, 2004) make it likely that ancestral deuterostomes had a skin brain that was regionally patterned by virtue of specific gene expression. Obviously, this basiepithelial nervous system had no dorsoventral patterning, since this would first arise in chordates in conjunction with the process of invagination of the neural plate into the neural tube. It has been noticed in both protostomes and deuterostomes that many genes governing the processes of invagination and of dorsoventral patterning are different (Arendt and Nubler-Jung, 1999). It seems therefore plausible that both processes were derived independently from an organism that already had evolved anteroposterior patterning. This would explain the utilization of two different sets of similar genes to regulate the two different processes (Meinhardt, 2004). It is fair to say that the relationship of most of the patterning genes to cell fate-determining genes and, ultimately, to neuronal organization is largely unknown. Thus, understanding how these genes relate within the context of the ancestral basiepithelial nerve net may provide insight into their functional relationships in the context of the brain of higher taxa. Examples such as otx, which helps specify the forebrain as an element of anteroposterior patterning in gnathostomes but is also expressed regionally in the brainless coelenterates (Ghysen, 2003), illustrate the possibility of an ancestral role that presumably was co-opted along with the neurogenic genes into the process of forming and patterning the brain *per se*.

In recent years, a number of cell fate determining genes have been identified in the nervous system and their functions experimentally determined (Lee, 1997; Anderson, 1999; Bermingham *et al.*, 1999; Brunet and Pattyn, 2002). Many of these patterning genes code for basic helix-loop-helix (bHLH) transcription factors. Much recent work has shown that the bHLH genes are ancient and are found not only in bilaterians, but also in coelenterates (Muller *et al.*, 2003; Seipel *et al.*, 2004). These findings begin to shed light on a major question in neurobiology: what is the origin of the neuron, the basic building block of any brain or nerve net? We propose here that the evolution of cellular diversification is closely associated with the evolutionary divergence of the bHLH genes and their restricted expression in the peripheral and central nervous system (Figure 9). The evolutionary expansion of this gene family as well as the currently known expression patterns of its members is consistent with a previously proposed hypothesis of cellular diversification as the basis for brain evolution (Mackie, 1990).

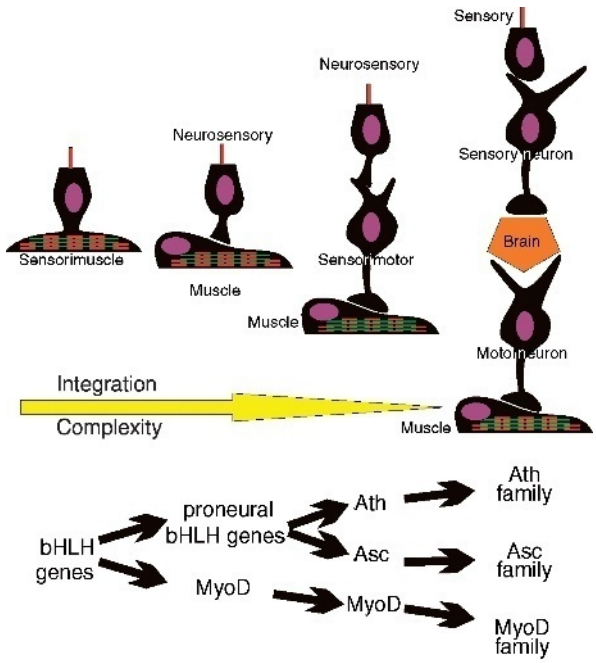

Figure 9 The proposed co-evolution of cellular complexity and expansion of the bHLH gene family that guides development of cellular diversity. It is hypothesized that the original neuronal cell type was a sensorimuscle cell that had both sensory capacity and contractile capacity. As the *bHLH* gene family expanded by generation of additional paralogous family members, the repertoire of neuronal cell types increased. Eventually, specialized sensorimotor cells that interconnected pure neurosensory cells and pure muscle cells evolved, thus providing the basis for integration of input from multiple sensory cells to govern the activity of multiple muscle fibers. These sensorimotor neurons ultimately diverged to give rise to the entire interneuronal compartment of the brain, an evolution that occurred in parallel with the increased specialization and diversification of sensory organs to govern more complex motor output. Modified from Mackie (1990) and Seipel *et al.* (2004).

Essential for the formation of any CNS is the specification of a neural cell fate in the developing ectoderm: cells have to be diverted from an epidermal fate to a neural fate, which is to say into neurons instead of skin cells. This fate switch is likely to be triggered by activating the bHLH genes that specify neuronal fate. We therefore turn our attention now to the question of how neuronal induction might have evolved.

Over the last 10 years, the predominant view has been that Spemann's organizer generates signals that can change cell fate from epidermal to neuronal through the generation of bone morphogenic proteins (BMP) antagonists (De Robertis *et al.*, 2000; Munoz-Sanjuan *et al.*, 2002). Based on several transplantation and *in vitro* experiments, it has been proposed that neuronal induction is a direct consequence of BMP inhibition in the ectoderm and that the neural fate can be considered to be a ground state that is revealed in the absence of the instructive (negative) BMP signals and the ectopic expression of proneural bHLH genes (Ma *et al.*, 1996; Lee, 1997). One possibility is that in the ancestral deuterostome, BMP expression was suppressed, or at least downregulated, in a scattered pattern, thus triggering neurogenesis at the scattered locations characteristic of the basiepithelial nerve net. bHLH genes evolved already in coelenterates, where they are involved in specifying neuronal precursors in the ectoderm and endoderm (Seipel *et al.*, 2004).

In the chicken, Spemann's organizer, but not previously defined BMP inhibitors, leads to neurogenesis, and it has been claimed that it is FGF signaling that is elicited by the organizer and that antagonizes BMP signaling (Munoz-Sanjuan *et al.*, 2002). However, recent data from ascidian embryos suggest that FGF signaling alone, without acting through BMP inhibition, is a direct inducer of early neurogenic genes (Bertrand *et al.*, 2003). More recent work seems to generate a unified position and suggests that FGF is not only inhibiting BMP signaling, but also has an independent and direct neural inductive capacity possibly conserved among chordates (Delaune *et al.*, 2004; Kuroda *et al.*, 2005). Specifically, it now appears that FGFs can directly activate neuralization through the MAPK pathway rather than by overriding the BMP-mediated inhibition (Kuroda *et al.*, 2005). Unfortunately, the function of FGFs in the context of neural induction in other deuterostomes has not been fully analyzed

(Davidson *et al.*, 2002). It is important to note that a cooperative FGF/BMP signaling system also exists in insects, but not in connection with CNS development. The FGF pathway is used for branching morphogenesis of trachea (Sutherland *et al.*, 1996) and null mutants of the single known *Drosophila* FGF ligand (branchless) do not show overt brain development deficits (Hirth *et al.*, 2003). This difference is significant, as in the past it appeared that both insect and vertebrate neural induction relied on BMP/dpp suppression (Urbach and Technau, 2004). Now it appears that either chordates have evolved the new feature of FGF involvement, or, conversely, that FGF involvement in neural induction was lost in insects. Clearly, the emerging issues regarding phylogenetic differences in the signaling that underlies neural induction support the model that neurulation in protostomes and deuterostomes may have evolved independently (Lowe *et al.*, 2003; Meinhardt, 2004). These divergent views need to be reconciled by more detailed analysis of nonchordate deuterostomes. Such data could help to resolve the basic question of conservation of neural induction across phyla, which as of this writing is controversial.

Upon invagination, the vertebrate neural tube becomes patterned in the transverse plane according to an intricate process involving multiple transcription factors (Figure 10). In a simplified version, the dorsoventral expression domains of these transcription factors are established by a bipolar gradient of diffusible signaling molecules that act in concert (Sander *et al.*, 2000; Vallstedt *et al.*, 2001; Maklad and Fritzsch, 2003; Pattyn *et al.*, 2003b), a fact that can be unmasked if one pole of the gradient is experimentally abolished (Litingtung and Chiang, 2000). Several of these patterning genes have been identified across deuterostome phyla (Wada and Satoh, 2001). However, it is also clear that factors such as the Wnts are expressed differently in cephalochordates and craniates (Holland *et al.*, 2000; Schubert *et al.*, 2001), suggesting that certain aspects of dorsoventral patterning may not be fully conserved across deuterostomes. Clearly, more work on other relevant dorsoventral patterning genes is needed in more deuterostomes before any firm conclusion can be drawn.

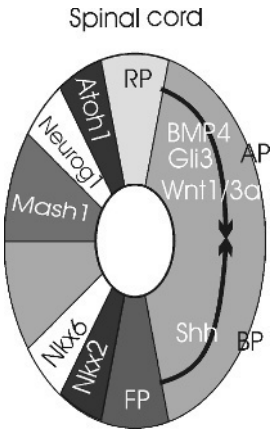

Figure 10 Genes involved in the dorsoventral patterning of the neural tube. The floor plate produces sonic hedgehog (*Shh*), which diffuses through the basal plate (BP) to interact with GLI-Kruppel family member 3 (*Gli3*). Opposing diffusion gradients are set up by bone morphogenic factor 4 (*BMP*4) and wingless-type MMTV integration site family member 1/3a (*Wnt1/3a*). The interaction of these gradients sets up domains of transcription factor expression that govern more directly the cell fate of neurons developing in their expression area. For example, the expression of NK6 transcription factor related, locus 1 (*Nkx6.1*) is essential for the formation of somatic motoneurons. Modified from Sander *et al.* (2000), Vallstedt *et al.* (2001), Maklad and Fritzsch (2003), and Pattyn *et al.* (2003b).

One specific brain region that has attracted intensive studies and has generated a large set of comparative gene expression data is the MHB (Figure 11). The formation of the MHB has been shown to be critically dependent on several transcription factors that interact with each other to stabilize the boundary (Wang and Zoghbi, 2001). If any of these genes is mutated, the boundary does not form and nearby neuron populations, including certain extraocular motoneurons, fail to differentiate (Fritzsch *et al.*, 1995). Certain genes expressed in this region, such as Pax2/5/8, can be used as markers to delineate the MHB (Wada *et al.*, 1998). It is interesting that no midbrain seems to exist in noncraniate deuterostomes in the sense of the specific overlapping gene expression patterns that are found in gnathostomes. Likewise, it is unclear whether a true isthmus region with the organizer capacity of gnathostomes exists in any nonvertebrate deuterostome (Figure 11). Given that the MHB is so important for cerebellar, oculomotor motoneuron, and trochlear motoneuron development, it is conceivable that the absence of a cerebellum as well as of all extraocular motoneurons in hagfish may reflect a primitive absence of a true MHB. Likewise, the absence of a mesencephalic root of the trigeminus nerve in cyclostomes may be related to a different pattern of gene expression in this region.

Another long-standing comparative issue is the number and significance of neuromeres and how they relate to gene expression domains and neuronal differentiation. Based on gnathostome data, it appeared that most cranial motoneuron nuclei showed a simple relationship to specific rhombomeres in a pattern that was reasonably well conserved across gnathostome phyla (Fritzsch,

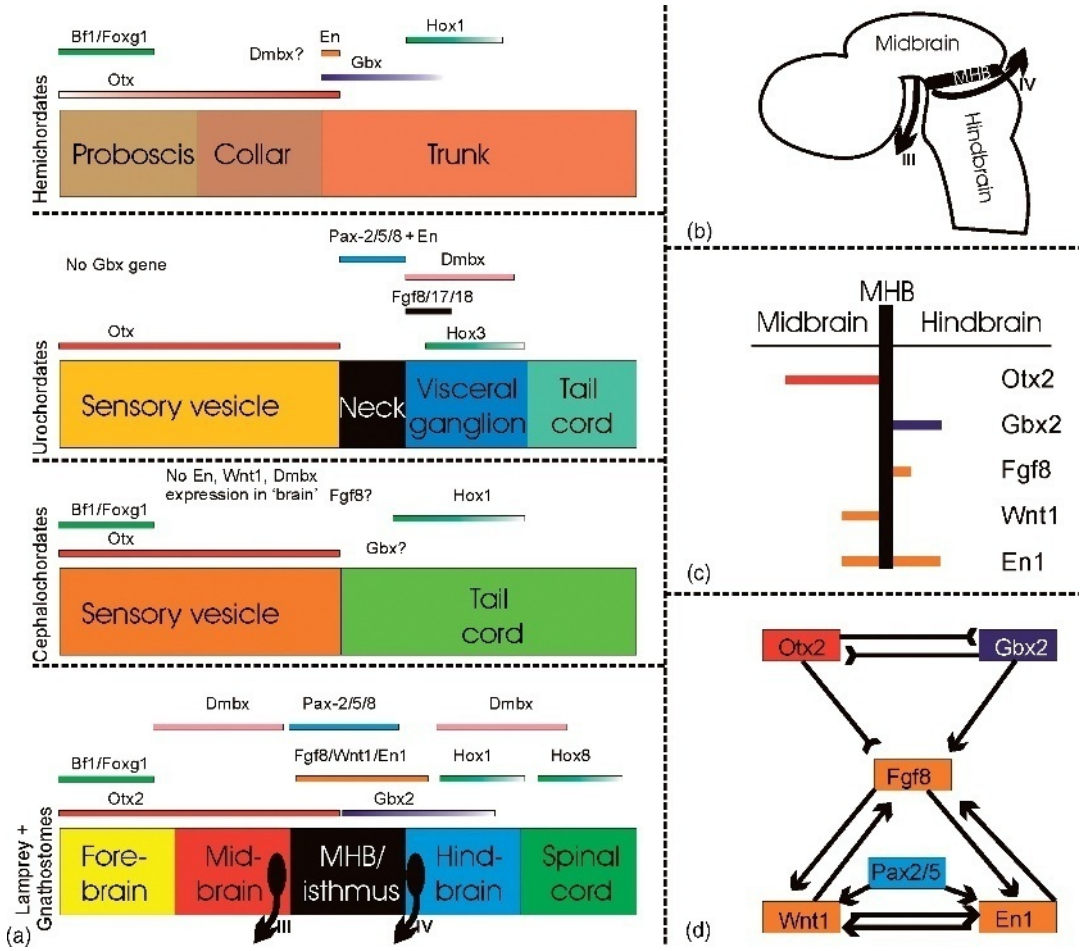

Figure 11 The evolution of gene expression at the MHB is shown for deuterostomes. The MHB forms in gnathostomes where *Otx2* and *Gbx2* expression is abutting. This stabilizes the expression of *Fgf8*, which in turn stabilizes the expression of *Wnt1* and *En1*. Mutation of *Otx2*, *Gbx2*, *Fgf8*, or *Wnt1* eliminates the MHB. *Pax2/5/8* are also expressed at the MHB, whereas the expression of *Dmbx* occurs immediately rostral to the MHB and later in the hindbrain and spinal cord. Cephalochordates have *Dmbx*, *Wnt1*, and *Engrailed* genes, but these are not expressed in any close relation to the *Otx* expression domain. The expression of *Fgf*s and *Gbx* has not yet been characterized in cephalochordates. Urochordates lack a *Gbx* gene and have nonoverlapping *Pax* and *Fgf* expression domains meeting between the visceral ganglion and the neck, with *Dmbx* expression caudal to *Pax* expression as opposed to rostral to *Pax* expression as is seen in gnathostomes. Hemichordates have overlapping expression of *Gbx*, *Otx*, and *En* in the rostral trunk. Outgroup data suggest that coelenterates have a *Dbmx* orthologue, thus raising the possibility that hemichordates also have a *Dmbx* gene, but neither this gene nor the *Fgf* and *Pax* genes have been characterized in terms of expression pattern in hemichordates. Together these data show that certain gene expression domains are topographically conserved (*Hox*, *Otx*), whereas others show varying degrees of overlap. It is conceivable that the evolution of nested expression domains of transcription factors is causally related to the evolution of specific neuronal features such as the evolution of oculomotor and trochlear motoneurons around the MHB. Modified from Fritzsch (1996), Wang and Zoghbi (2001), Lowe *et al.* (2003), and Takahashi and Holland (2004).

1998; Glover, 2001; Murakami *et al.*, 2004; Kiecker and Lumsden, 2005). However, a number of exceptions have been noted among cyclostomes in the organization of hindbrain motor nuclei compared to mammals (Figure 12). These data suggest that while the formation of rhombomeres might be a constant feature of craniate hindbrains (with the possible exception of hagfish) the content of rhombomeres is not stably conserved. The distribution of motoneurons in the adult hindbrain is complicated by longitudinal and radial migrations that make comparison among craniates somewhat tentative. For example, facial motoneurons migrate in hagfish and mammals, whereas they remain near their ventricular origin in lampreys. Rhombomeric differences in the distribution of craniate motoneurons have recently been analyzed during development in cyclostomes and correlated with gene expression patterns (Murakami *et al.*, 2004). Beyond the branchiomotor neurons analyzed in this study, the abducens nucleus has also been found not to have boundaries coinciding with rhombomere

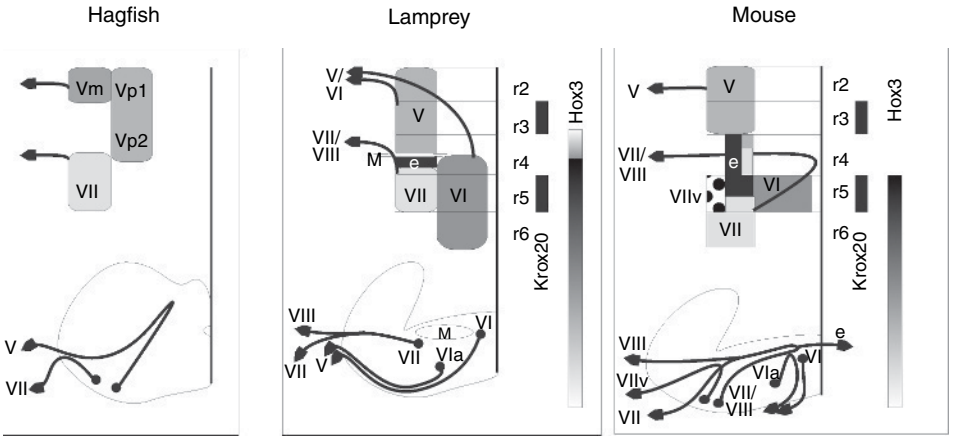

Figure 12 The distribution of motoneurons relative to rhombomeres is shown for three adult craniates in combination with gene expression in a dorsal view (top) and a coronal section (bottom) of the left half of a hindbrain. Hagfish and lampreys have a much larger contingent of trigeminal (V) than facial motoneurons (VII). Hagfish are unusual in the trajectories taken by their motoneuron axons, a feature that is related to the overall unusual organization of the hindbrain. In lampreys, the abducens (VI) and facial motoneurons (VII) originate from rhombomeres 2.5 and 1.5, respectively, and trigeminal and facial motoneurons approximate each other in the middle of rhombomere 4 near the Mauthner cell (M) and the inner ear efferents (e). Lampreys have two adjacent populations of motoneurons that each innervates distinct ocular muscles. Fibers of the abducens exit through the trigeminal nerve (V). In mice, the facial branchial (VII) and visceral (VIIv) motoneurons originate in rhombomeres 4 and 6, respectively. The facial branchiomotor neurons migrate during development from rhombomere 4 to 6, trailing their axons behind them. Each of the three motoneuron types generated in rhombomere 4/5 has a distinct exit either bilaterally through the octaval nerve (VIII) for efferents (e) or through the intermediate nerve for facial visceral motoneurons (VIIv) or through the facial nerve for facial branchiomotoer neurons (VII). Note that abducens fibers exit in mammals as a ventral root (VI). Gene expression studies show that rhombomeres 3 and 5 are characterized across phyla by the expression of *Krox20,* whereas *Hox* expression changes from a midrhombomere 4 expression in lampreys to a rhombomere 4/5 boundary expression in mice. No expression data exist on hagfish. Modified from Fritzsch (1998), Glover (2001), and Murakami *et al.* (2004).

boundaries (Fritzsch, 1998). This detailed analysis has confirmed earlier assessments of motoneuron distribution and shows a correlation of motoneuron populations not with rhombomeric boundaries *per se* but with Hox gene expression domains, as had previously been hypothesized for craniates in general (Glover, 2001). Indeed, the details of distribution of motoneurons generated in a given rhombomere are not stable across craniate evolution, as illustrated by the formation of a visceral motor component in rhombomere 5 of the mammalian hindbrain, a motoneuron population that is entirely missing in cyclostomes. Experimental manipulation of gene expression is now needed in lampreys to show that the boundaries of motoneuron domains are specified by *Hox* gene expression.

Overall, these data show that rhombomeres are not fully invariant with respect to motoneuron composition, and similar conclusions have been reached with respect to reticulospinal and vestibulospinal neurons (Auclair *et al.*, 1999; Diaz and Glover, 2002; Diaz *et al.*, 2003; Maklad and Fritzsch, 2003). This instability in detail relates to the unresolved problem of the origin of neuromeres. While past research assumed that this might have happened early in deuterostome evolution and may have been related to segmentation from a hypothetical common urbilaterian ancestor (Arendt and Nubler-Jung, 1999), more recent data based on the study of the *Krox20* gene suggests that neuromeres evolved first among craniates. This conclusion is warranted, as in null mutants of that gene there is selective disappearance of rhombomere 3 and 5 (Voiculescu *et al.*, 2001). Genetic analysis has shown that in the absence of *Krox20* the remaining cells of rhombomeres 3 and 5 acquire rhombomere 2 or 4 identities, demonstrating that *Krox20* is essential for odd- and even-numbered territories in the hindbrain. *Krox20* is not expressed in the brain of cephalochordates, thus indicating that rhombomere formation probably evolved after *Krox20* was co-opted into hindbrain patterning (Knight *et al.*, 2000).

In summary, these data indicate a promising beginning but also show, despite their current paucity, that gene expression domains do not constitute a magic bullet that reveals the basic homology of neuromeres across phyla. Evolutionary alterations of gene expression and/or neuronal population differentiation patterns within any given neuromere will make the road ahead as difficult as the road was until here. Still, experimental manipulation of the emerging framework of nested gene expression through gain and

loss of function studies, combined with more sophisticated tracer studies, should reveal the origins of region-specific neuronal phenotypes and their relationships to specific gene expression domains.

9.4 Multiplying and Diversifying Sensory Systems for More Detailed Sensory Analysis and More Appropriate Motor Responses: The Conundrum of the Co-Evolution of Ever More Sophisticated Input, Processing, and Output Processes as a Basis for a Runaway Selection

The chicken and egg question to be addressed next is the co-evolution of sophisticated sensory systems and the brains necessary to process the information they provide. As with the evolution of brain regionalization outlined above, it is likely that the evolution of genes predated the evolution of sensory systems, which, in turn, predated the evolution of sophisticated brains. We base this assertion on the simple fact that even animals with a basiepithelial nerve plexus, like some jellyfish, may have both eyes and ears (Kozmik *et al.*, 2003). Conversely, to date, no animal is known that has a sophisticated brain but lacks sensory inputs entirely. Indeed, in animals in which a specific sensory system becomes the leading sensory input, the brain areas dedicated to this input tend to increase in absolute size and complexity (Nieuwenhuys *et al.*, 1998). Clearly, among deuterostomes the degree of development of sensory systems and brain go rather well with each other. For example, no specialized sensory system is known for hemichordates or echinoderms (Bullock and Horridge, 1965). Urochordates have some specialized systems associated with water intake (Mackie and Singla, 2004) and have two simple receptors in the rostral ganglion (Meinertzhagen *et al.*, 2004). The most sophisticated sensory system of cephalochordates encompasses the organs of de Quatrefages and their function is still unknown (Lacalli, 2004). This raises the problem of ancestral craniate senses that are causally linked to the progressive evolution of the brain. Judging from the central representation size and abundance of receptors, it appears that chemical sense and tactile sense are the dominant sensory inputs to the hagfish brain (Braun, 1996, 1998), but it remains unclear whether this is a primitive or a derived condition. Overall, craniate evolution is clearly correlated with greater sophistication of eyes and ears. We therefore provide below a brief comparison of these two organs in craniates and gnathostomes.

9.4.1 Eyes

Vertebrate eyes have long been regarded as uniquely derived features that are homoplastic to arthropod eyes. This is related to the unusual development as an evagination of the forebrain not found in any other phylum. However, recent years have highlighted a number of transcription factors and, more recently, opsin proteins that are related across phyla (Kozmik *et al.*, 2003; Arendt *et al.*, 2004). Indeed, a molecular link between the photoreceptors and opsins of invertebrates and vertebrates was recently proposed (Arendt *et al.*, 2004) and may be extendable to deeper evolutionary connections between eyes and mechanoreceptors (O'Brien and Degnan, 2003; Fritzsch and Beisel, 2004; Niwa *et al.*, 2004; Piatigorsky and Kozmik, 2004). Minimally, this raises the possibility that at least the molecular building blocks of eyes are homologous across phyla and arose already in coelenterates, thus indicating a deep homology of all eyes. While it cannot be excluded that hemichordates and echinoderm ancestors had more sophisticated eyes, it seems more plausible to assume that lack of eyes in these two taxa is primitive. If so, eye evolution would begin in deuterostomes with the chordates. It has been proposed that the frontal organ of cephalochordates is homologous to the lateral eyes of craniates and this suggestion is backed by some connectional data as well as by the expression of Pax6 (Lacalli, 2004). Furthermore, some data suggest that all retinal neurons may be directly related to the sensory receptors (Arendt, 2003) and past differences perceived in opsins among metazoans have recently been reconciled (Arendt *et al.*, 2004).

This may be so, but we want to explore here the basic organization of cyclostome retinas that appears to be primitively different from gnathostome retinas in several respects (Figure 13). First, like the submeningeal layer of the brain, the vitreal part of the lamprey and hagfish retina is devoid of nerve fibers, which run instead at the level of the inner nuclear layer. Second, most of the ganglion cells are located within the inner nuclear layer and do not form a distinct ganglion cell layer. Last, but not least, cyclostome eyes receive a proportionally large GABAergic retinofugal input from apparently homologous efferent nuclear centers in the midbrain (Fritzsch and Collin, 1990; Fritzsch, 1991; Nieuwenhuys *et al.*, 1998) and this input develops before receptor cells mature (Fritzsch, 1991; Anadon *et al.*, 1998b). These data suggest that cyclostome retinas are only partially transformed from their original neuroectoderm-like cell and fiber layering. The organization of the gnathostome

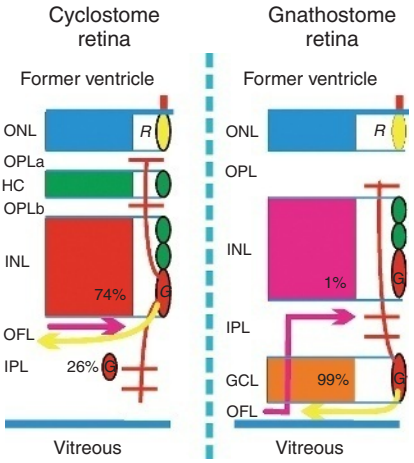

Figure 13 Differences in the fiber and cell layers of the cyclostome and gnathostome retinas. Note that the receptor processes of the outer nuclear layer (ONL) protrude into the former ventricle. In lampreys, horizontal cells are separated from the ONL and inner nuclear layer (INL) by two sublayers of the outer plexiform layer. Lampreys have about 74% of their ganglion cells (G) located in the INL, whereas only 1% of ganglion cells of jawed vertebrates are found in this layer, with 99% being in the ganglion cell layer (GCL). Afferent (yellow) and efferent (lilac) fibers project in lamprey along the INL. In jawed vertebrates, such fibers run in the outer fiber layer (OFL) at the vitreous. It is conceivable that the formation of myelin necessitated a different distribution of myelinated fibers near the vitreous and farther away from the receptors in jawed vertebrates. Reorganization of ganglion cells into the IPL to eventually form the ganglion cell layer may have been a consequence of this fiber reorganization. HC, horizontal cell; IPL, inner plexiform layer; OPLa, outer plexiform layer a; OPLb, outer plexiform layer b. Modified from Fritzsch (1991) and Anadon *et al.* (1998b).

retina seems to be a transformation of this ancestral pattern (Figure 13). It is important here to realize that the eye of cyclostomes is moved by a different organization of eye muscles and that the pattern of innervation of these eye muscles is also different from gnathostomes (Fritzsch, 1998). In addition, lampreys lack a ciliary muscle and accommodation must happen by other means, potentially related to the triangular shape of the lens. Overall, these data on the eye support the monophyly of cyclostomes and show a radical reorganization of retina organization and eye muscles in gnathostome vertebrates.

9.4.2 Ears

Like the eye, the ear has been proposed as an example of deep homology with several transcription factors shared between deuterostomes and coelenterates (Kozmik *et al.*, 2003; Fritzsch and Beisel, 2004; Seipel *et al.*, 2004; Fritzsch and Piatigorsky, 2005). Comparable to the retina, the ear of cyclostomes also shows an apparently primitive organization different from gnathostomes: cyclostome ears have only two canal cristae, lacking the horizontal canal and crista, and they have only a single otoconia-bearing epithelium (Fritzsch and Beisel, 2004). It has been suggested that a gene otherwise related to the forebrain is causally linked to the evolution of the horizontal canal (Cantos *et al.*, 2000; Fritzsch *et al.*, 2001). *Otx1* appears in the gnathostome lineage through duplication and has acquired a novel expression and function in the ear that is not shared by *Otx2* (Morsli *et al.*, 1999). Moreover, several other genes discussed above in the MHB context (see Section 9.3) all appear in the ear, again emphasizing that evolutionary change in expression, multiplication of an ancestral gene, and acquisition of a novel function need to be considered when gene expression is to be related to evolutionary alterations. In this context, the evolutionary relation with structures in other deuterostomes that show expression of *Pax2* also have a phenotype in *Pax2*-null mutants (Burton *et al.*, 2004). *Pax 2* has been discussed as a major factor relevant for ear evolution (Wada *et al.*, 1998; Mazet *et al.*, 2003) and has been used to suggest homologies across phyla. However, this idea should be regarded at the moment as tentative and requires support through the expression of other genes (Fritzsch and Beisel, 2004; Holland, 2005). It is fair to say that the ear can be viewed as a miniature problem of craniate head evolution and unraveling the molecule basis of its evolution might pave the way for furthering our understanding of head evolution in general.

Overall, the sensory systems reviewed here show a remarkable progression between cyclostomes and gnathostomes and indicate that these sensory systems present unique components of cyclostomes that set them apart in a likely primitive way from gnathostomes. More detailed understandings of the molecular alterations underlying jaw formation (Shigetani *et al.*, 2002) need to be revealed and related to the sensory and motor reorganizations of the cyclostomes. At the moment, it is safe to say that we do not understand the molecular basis of these changes in enough detail. More experimental work is needed to support the current notion of nested gene expression patterns and their potential evolutionary significance.

9.5 Summary and Conclusion

Attempts at linking the evolution of organisms and organs to the evolution of transcription factors that direct developmental processes are greatly complicated by the multitude of poorly understood transcriptional regulatory networks. Emerging issues are the apparent pleiotropic effects of many

transcription factors, the modularity of development, and the evolution of *cis*-acting regulation of transcription factors (Carroll *et al.*, 2005). Conflicts between morphology and genetics, such as the many examples of morphological divergence arising from apparently identical transcription factor expression across taxa, are bound to leave conclusions on molecular and developmental homologies controversial for the near future. For example, recent progress in the long-standing issue of the molecular basis of organ formation has shown that certain *Pax* genes are relevant for the formation of eyes (*Pax6*) and ears (*Pax2*) across many phyla and have evidently arisen from an ancestral *Pax2/6* gene, *PaxB*, that is found in cnidarians (Sun *et al.*, 1997). Moreover, *PaxB* is expressed in the eye and statocyst of cubomedusan jellyfish, cnidarians that already contain these sophisticated organs for vision and mechanoreception (Piatigorsky and Kozmik, 2004). This suggests that evolution may have used paralogues of a single ancestral *Pax* gene to organize the development of both of these peripheral organs, no matter what shape, cell types, and transducer molecules are involved (Kozmik *et al.*, 2003; Piatigorsky and Kozmik, 2004). Since *PaxB* is also expressed in sponges (Hoshiyama *et al.*, 1998), its involvement in sensory development may even have predated the formation of a central nervous system (Lowe *et al.*, 2003; O'Brien and Degnan, 2003; Piatigorsky and Kozmik, 2004; Fritzsch and Piatigorsky, 2005). Such ideas imply that *Pax2/6* expression in the brain and the use of *Pax2/5/8* in the MHB is a secondary co-option of these genes from their original involvement in sensory organ development. As an extension of the principles governing the determination of organs, the fates of individual cells within organs have to be similarly directed. Much as with *Pax* genes, a number of common cell fate-determining transcription factors are co-utilized in the eyes and ears of different species. Examples include the *bHLH* gene *atonal/Atoh1/Atoh5* (Ben-Arie *et al.*, 2000; Niwa *et al.*, 2004), and the Pou domain factor *Pou4f3* (Liu *et al.*, 2001; Wang *et al.*, 2002). It is noteworthy that the selective co-expression pattern of these genes, which appear to be situated downstream of the *Pax2* gene regulating development of the optic nerve and ear (Torres *et al.*, 1996), suggests their specialization for the specification of cells within these two sensory organs. Another example is the *Eya1/Six1* signaling system, which is essential for development of the vertebrate and insect eye and the vertebrate ear (Zou *et al.*, 2004) as well as being used in other aspects of development (Piatigorsky and Kozmik, 2004). Extending this line of inquiry into the CNS, several examples exist of transcription factors controlling the same neuronal phenotype across taxa, such as the *Islet/LIM* genes, which are involved in specifying the motoneuron phenotype in urochordates as well as vertebrates (Price and Briscoe, 2004; Katsuyama *et al.*, 2005), and the *Unc30/Pitx2* gene, which has been shown to control the determination of the GABAergic phenotype in vertebrates as well as the nematode *C. elegans* (Westmoreland *et al.*, 2001). Clearly, a number of the transcription factors and developmental cascades that determine cell fates are shared across phyla and across sensory organs and the CNS. Assuming that these similarities are more than coincidental and in fact indicative of a co-evolutionary history of eyes, ears, and brains, the present challenge is to trace the common origins of these structures to conserved developmental programs. In keeping with the recently emerging concept of a second code for gene regulation (Pennisi, 2004), namely the *cis*-acting regulatory elements, it is possible that enhancers orchestrating the development of specific structures in different species share motifs and properties that have led to the modular use of common transcription factors during the evolution of those structures. One of the challenges ahead is therefore the careful mapping of regulatory elements associated with the transcription factor genes that play pivotal roles in such modules. Obtaining an overarching conceptual framework of the molecular characterization of brain development is likely to provide more than unifying insight into the evolution of the brain and senses. Evolutionary molecular neurobiology is likely also to provide novel insights into human diseases common to such apparently dissimilar organs as the eye and ear, such as Usher syndrome (Fritzsch and Beisel, 2004), myosin IIIa-associated nonsyndromic hearing loss, and retinal degeneration (Walsh *et al.*, 2002), choroideremia (Starr *et al.*, 2004), and Norrie disease and exudative vitreoretinopathy (Xu *et al.*, 2004). Understanding the regulation of common transcription factor modules, and linking this to their expression in topographically distinct contexts, may ultimately lead to a better understanding of the causes and differential penetrance of such inherited diseases.

Acknowledgments

This work was supported by grants from NIH (RO1 DC005590; BF), NASA (NAG 2-1611; BF), and the European Union (QLG2-CT-2001-01467; JG). This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant Number 1 C06 RR17417-01 from the

National Center for Research Resources, National Institutes of Health. We wish to dedicate this chapter to Ted Bullock, a great mentor and friend.

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10 Evolution of the Nervous System in Fishes

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Glossary

actinopterygians	Sistergroup of sarcopterygians, include all ray finned fishes, that is, bichirs (*Polypterus*) and the reedfish (*Calamoichthys*), together forming the cladistians, the stur geons (chondrosteans), the gars (*Lepisosteus*; ginglymodes), and the bowfin (*Amia*; halecomorphs), as well as the manifold modern ray finned fishes, the teleosts.
agnathan(s)	Descriptor for all jawless fishes; the two extant groups, lampreys (petromyzontids) and hagfishes (myxinoids) are not considered monophyletic here, since petromy zontids are more closely related to gnathostomes.
Bauplan	Set of ancestral characters shared by all organisms (or one of their organs; e.g., the brain) forming a given taxon.
chondrichthyans	Outgroup of remaining gnathos tomes, including all cartilaginous fishes, that is, elasmobranchs (sharks, skates, and rays) and holocephalans (chimaeras).
cladogram	Branching diagram of taxa exclu sively based on shared derived characters (synapomorphies).
gnathostomes	Vertebrates with true jaws (chon drichthyans, actinopterygians, sarcopterygians).
monophyletic group	Taxon that includes all descendants of a last common ancestor.
neuromeres	Transverse units (segments) of developing neural tube (rhombo meres, prosomeres).
neuromeric model	Assumes transverse (neuromeres) as well as longitudinal units (roof, alar, basal, floor plates) along the entire anteroposterior neural tube axis, and that their arrangement is guided by selective regulatory gene expression that allows for regiona lized developmental processes.
organizing centers and patterning	Restricted regions of the embryo that secrete specific signalling mole cules, responsible for specifying distinct domains (molecularly, ana tomically, functionally distinct) in competent neighbouring tissues. This process is called patterning.
phyletic method	Uses cladistic methodology (clado grams, outgroup comparison) for establishing evolutionary polarity (i.e., ancestrality vs. derivedness) of characters.
sarcopterygians	Sistergroup of actinopterygians, including lobe finned fishes, that is, *Latimeria* (actinistians) and the lungfishes (dipnoi), and all land vertebrates (tetrapods).

10.1 Introduction: *Scala Naturae* Concept is Hard to Kill

The designation of fishes and amphibians as 'lower' vertebrates and of amniotes – or even mammals only – as 'higher' vertebrates expresses the pervasive

ladder concept of linear progress along the vertebrate phylogenetic tree (*Scala naturae*). Instead of considering the mammalian condition as the peak of vertebrate Bauplan perfection – a very idealistic viewpoint of the ladder of progress concept – Charles Darwin and his followers have argued that one should rather view the evolution of species in general, and the evolution of vertebrates in particular, as having occurred in a bush-like fashion (see Butler and Hodos, 2005 for recent review). Viewing evolution as a bush and not as a ladder implicates that each living species is neither 'higher' nor 'lower' than the others. Species simply diverged from each other at different time points during phylogenesis. This separation always included changes in early development, resulting in different animal forms and functions. Thus, many modern, derived features evolved independently in various vertebrate lineages, at the same time as many ancestral traits were shared by all vertebrates, representing the inheritance of their common ancestor. Most animal forms became extinct at some point or another, the extant species representing merely a relatively small selection of those forms that survived for reasons of adaptation or other factors, such as geographic isolation or escaping mass extinctions.

To reconstruct the evolution of the vertebrate brain, modern neurobiologists use the comparative (cladistic) method for establishing the evolutionary polarity of brain traits. This method analyzes single-organism characters instead of entire organisms or their brains, and allows to determine whether a neural character is ancestral (plesiomorphic) or derived (apomorphic) using well-supported cladograms (Figure 1). Cladograms are exclusively based on shared derived characters (synapomorphies). Sistergroups (e.g., sarcopterygians–actinopterygians in Figure 1a) are characterized by synapomorphies inherited from their last common ancestor, separating them from the outgroup taxa (e.g., cartilaginous fishes in Figure 1a). The outgroup comparison determines the evolutionary polarity of particular neural characters. If two conditions occur in sistergroups (evagination of telencephalic hemispheres in sarcopterygians; eversion in actinopterygians), the condition in the outgroups is investigated (evagination in cartilaginous fishes) which, for reasons of parsimony (i.e., principle of choosing the simplest explanation), is considered to represent the ancestral condition.

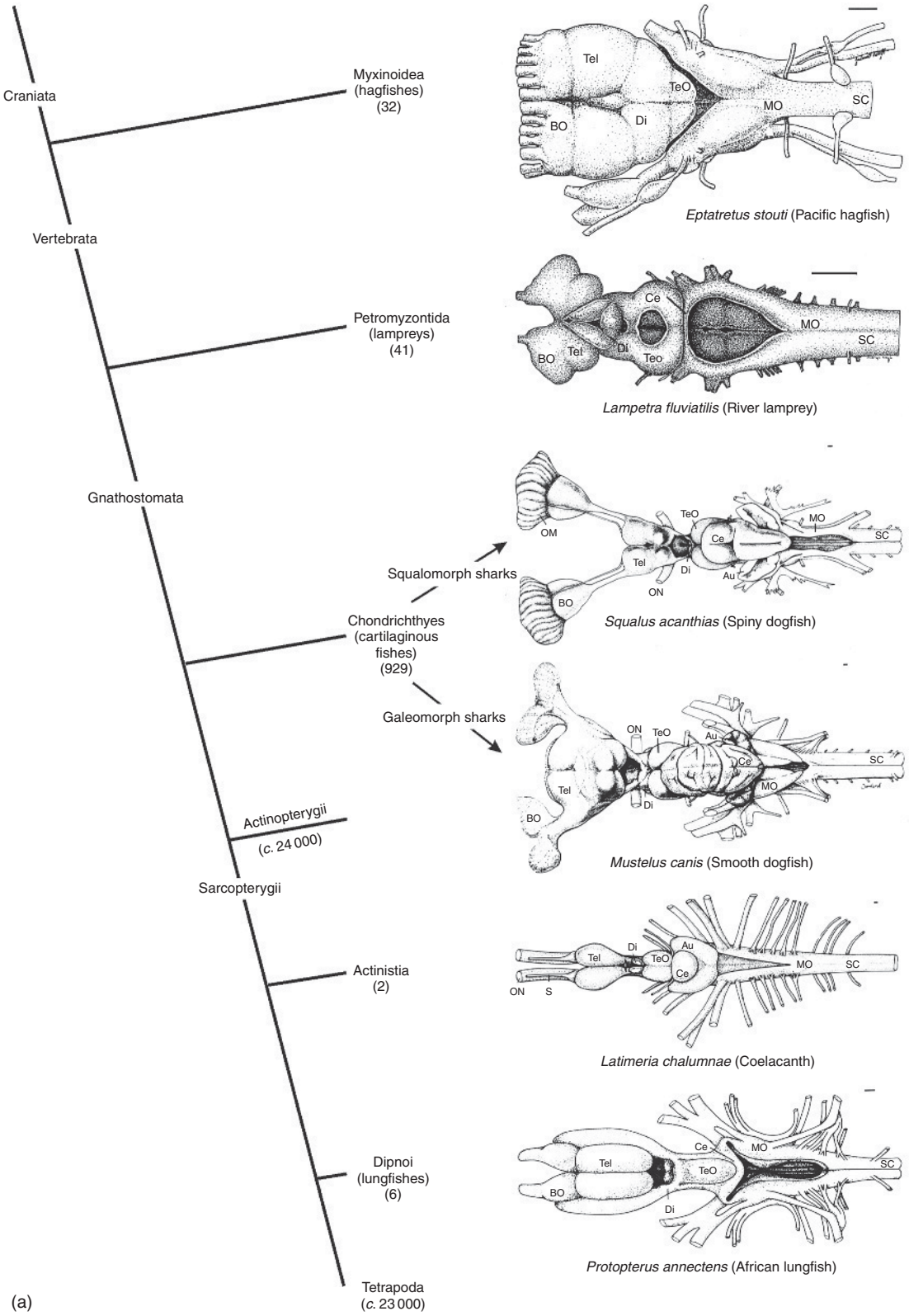
Despite this meanwhile well-accepted change of perspective brought about especially by the cladistic school in evolutionary theory, the metaphoric value of the *Scala naturae* concept is hard to kill. Especially the vertebrate brain evolution is still often viewed as proceeding from an assumed simple brain in various fish groups or amphibians to increasingly larger and more complex brains with greater capacity in turtles, lepidosaurs (lizards and snakes) and birds, with mammals being at the summit of the ladder, and this phenomenon is believed to be notable in the brain diversity of extant vertebrates. However, this view is highly biased by anthropocentric, teleological thinking. In the following, we discuss the notion of progress during vertebrate brain evolution, with special emphasis on fishes, and ask in particular whether *Scala naturae* thinking is supported by brain weight/body weight data or degree of histological and morphological complexity (see Section 10.2), by functional neuroanatomy (see Section 10.3), and by neurochemical brain organization (see Section 10.4).

10.2 Diversity and Bauplan of Fish Brains from Agnathans to Lungfishes

What are fishes? Fishes represent a way of life rather than a monophyletic craniate group (Figures 1a–1c). Extant fish groups include species such as the Comoran and Indonesian coelacanths (Actinistia; two species) and the lungfishes (Dipnoi; six species), both of which are sarcopterygians (lobe-finned fishes), the taxon that includes tetrapods (Figure 1a). Within the tetrapod radiation of approximately 23 000 species, amphibians amount to almost 4000 (18%), reptiles to more than 6000 (26%), birds to almost 9000 (38%), and mammals to slightly more than 4000 (19%) species.

The sistergroup of the sarcopterygians is comprised of the actinopterygians (ray-finned fishes, often regarded as the 'true fishes'). Actinopterygians are the most successful vertebrate radiation, since they represent half of all living vertebrate species (almost 24 000), forming a symmetrical dichotomy in vertebrate evolution. Thus, it is hard to argue objectively that there is a linear increase in evolutionary success going from fishes to mammals. The outgroup to sarco- and actinopterygians are the chondrichthyans or cartilaginous fishes (holocephalans, sharks, and batoids, i.e., rays and skates), a rather successful ancient gnathostome radation (around 1000 extant species). Two more outgroups to gnathostomes exist, namely the extant agnathan petromyzontids (lampreys; around 41 species), which constitute the vertebrates together with the gnathostomes and myxinoids (hagfishes; 32 species), which complement the vertebrates in the formation of the craniates (Figure 1a).

The craniate taxon indeed entails a large increase in anatomical and physiological complexity, as



(a)

Figure 1 (*Continued*)

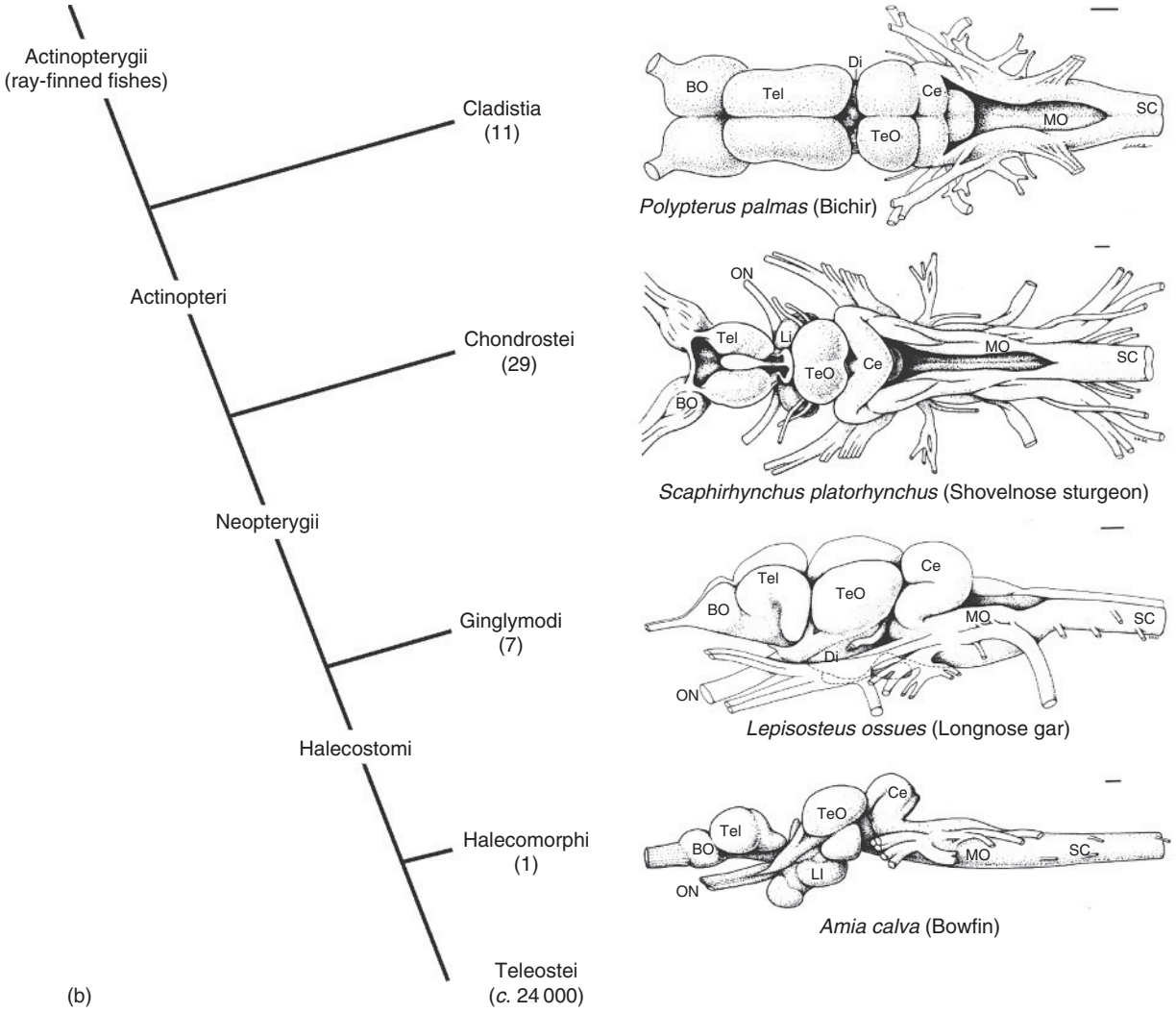


Figure 1 (*Continued*)

compared to its protochordate outgroups, that is, cephalochordates (amphioxus) and urochordates (ascidians). The recent advances of genome sequencing have rejuvenated the idea put forward originally by S. Ohno that the generation of craniate morpho-functional complexity relies upon the generation of a genetic complexity (Ohno, 1970). Indeed, craniates have on average 2 times more genes than protochordates, mainly resulting from the duplication of already existing genes (Furlong and Holland, 2002). Although no agreement has been reached on the mechanisms at the origin of this increased genetic complexity (Hughes and Friedman, 2003), a double duplication of the whole genome of a chordate ancestor (the 2R hypothesis) may have been instrumental in the emergence of craniates, more than 500 Mya (Lynch and Conery, 2000; Levine and Tjian, 2003).

Considering actinopterygians (Figure 1b), modern teleosts are as remote from their Paleozoic ray-finned fish ancestors as modern mammals differ from their Early Mesozoic sauropsid ancestors. Moreover, ray-finned fishes (actinopterygians) flourished several times in evolution (Carroll, 1988), first as chondrosteans (especially the palaeoniscoids) in the Paleozoic, and continuing with the (historically called) holosteans in the Mesozoic. These two actinopterygian radations independently generated many forms apparently adapted to all sorts of enviroments, probably from deep sea and free water to coral reefs. In contrast, the living descendants (i.e., cladistians, chondrosteans, gynglimodes, halecomorphs; Figure 1b) of nonteleost actinopterygians are small in species number.

Interestingly enough, the analysis of the whole genome of several teleost fishes, and gene data from

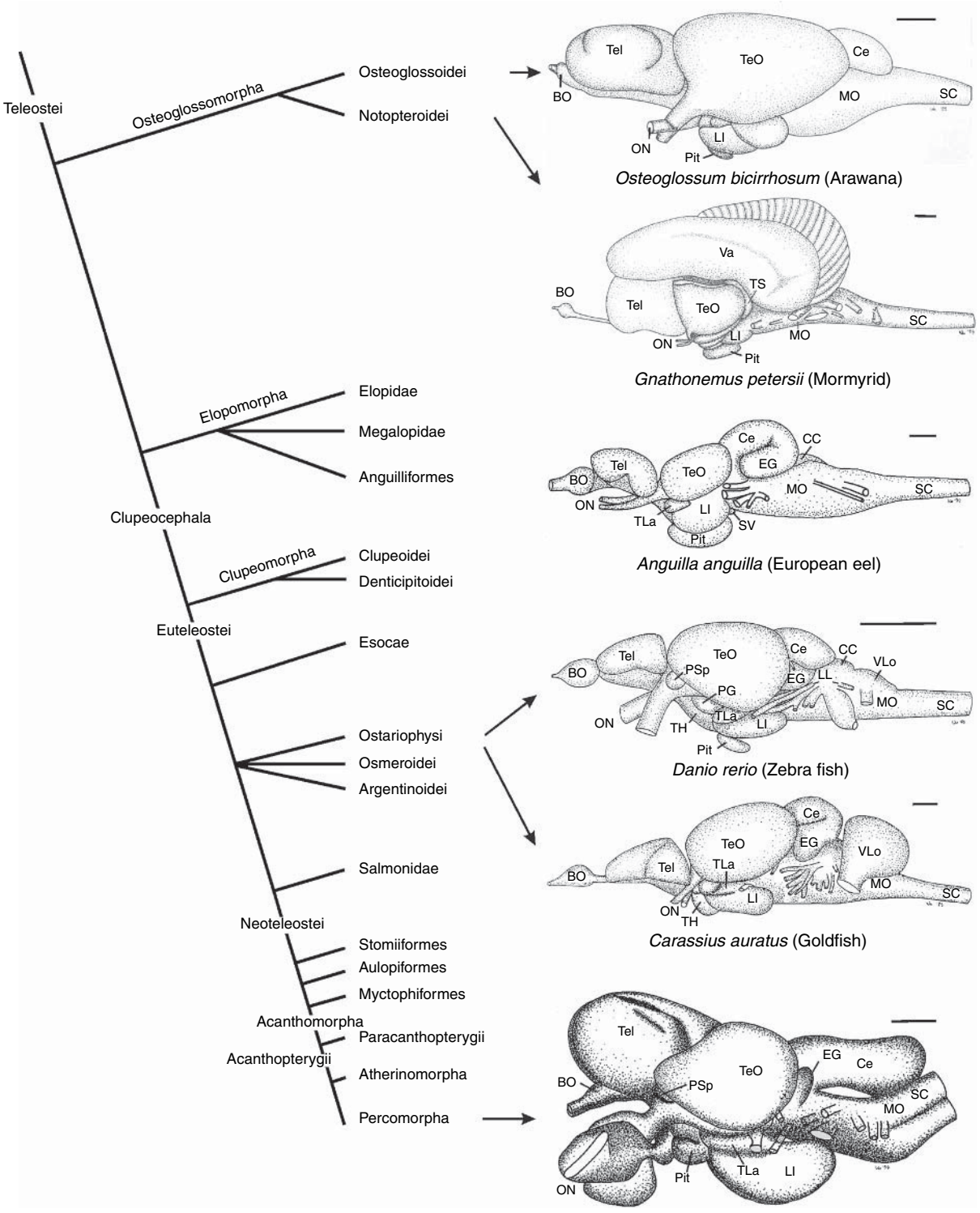


Figure 1 Cladograms depict systematics of extant a, craniate; b, actinopterygian; c, teleostean taxa. Lateral or dorsal views of representative brains are shown on the right side. Au, auricle; BO, olfactory bulb; CC, crista cerebellaris; Ce, cerebellum; Di, diencephalon; EG, eminentia granularis; LI, hypothalamic inferior lobe; LL, lateral line nerves; MO, medulla oblongata; OM, olfactory mucosa; ON, optic nerve; PSp, parvocellular superficial pretectal nucleus; Pit, pituitary; PG, preglomerular area; S, secondary olfactory peduncle; SC, spinal cord; SV, saccus vasculosus; Tel, telencephalon; TeO, optic tectum; TH, tuberal hypothalamus; TLa, torus lateralis; TS, torus semicircularis; Va, valvula cerebelli; VLo, vagal lobe. Scale bars: 1 mm. Adapted from Lauder, G. V. and Liem, K. F. 1983. The evolution and interrelationships of the actinopterygian fishes. *Bull. Mus. Comp. Zool.* 150, 95 197. Some drawings courtesy of Helmut Wicht (*Eptatretus*), Christoph Weigle (*Lampetra*) and R Glenn Northcutt (cartilaginous fishes, sarcopterygians, nonteleost actinopterygians). Species numbers according to Berra (1981), except cartilaginous fishes (Hamlett, 1999).

other groups of actinopterygians, has consistently shown that an additional round of genome duplication occurred between nonteleost actinopterygians and teleosts around 335–404 Mya (Hoegg *et al.*, 2004). Accordingly, teleost fishes have about 2 times more genes than the other craniate/vertebrate groups including mammals, a fact that also breaks the ladder of an assumed increasing complexity from fish to mammals. It is plausible that this genetic complexity of teleosts may have been critical for their tremendous species diversification, leading to the invasion of all aquatic environments covering 80% of the Earth's surface (Meyer and Van de Peer, 2005). The fossil origin of teleosts (Figure 1c) lies in the Early Mesozoic (Late Trias, as does that of mammals; Carroll, 1988), when teleosts started to form free water fish swarms (pholidophorids, leptolepids). But teleost speciation, in particular that of the acanthomorphs (which include the percomorphs), increased tremendously toward and after the cretaceous–tertiary boundary. Thus, acanthomorphs and placental mammals originate in the fossil record at around the same geological time.

10.2.1 Bauplan: The Shared Ancestral Brain Morphotype

Despite the apparent considerable diversity of general morphology (Figure 1) and internal organization of fish brains (see Sections 10.2 and 10.3), one can nevertheless establish a brain Bauplan or morphotype, that is, use the comparative method to demonstrate shared primitive characters that define the ancestral craniate or vertebrate brain (Northcutt, 1985; Wicht and Northcutt, 1992). Such a comparison makes clear that no stepwise addition of brain parts at the anterior pole of the neuraxis occurred during craniate evolution, as had been envisaged historically by E. Haeckel and followers (terminal addition and recapitulation; see Butler and Hodos, 2005, for review). Rather, it shows that most basic brain parts were initially present in craniate/vertebrate ancestors, with the possibility to develop novelties and peculiarities from a common morphotype, as originally proposed (in a different form) by von Baer (1928).

All gnathostome fishes exhibit the five conventionally recognized amniote brain parts (shown in Figure 2): from rostral to the caudal telencephalon, diencephalon (both together: forebrain), mesencephalon (midbrain), metencephalon (including the cerebellum), and myelencephalon (both together: hindbrain). The latter two, without the cerebellum, are often referred to as medulla oblongata. Classical embryology describes that the vertebrate brain develops from a three-vesicle stage (rhombencephalic vesicle including metencephalon and myelencephalon, mesencephalic vesicle, prosencephalic vesicle including diencephalon and telencephalon) into a five-vesicle stage (representing the primordia of the five adult brain parts mentioned). The rejuvenation of paradigms of neuromeric organization further suggests that the craniate rhombencephalon develops from seven to eight transitory neuromeres (rhombomeres), and that the prosencephalon does so from at least three more neuromeres and a less clearly segmented secondary prosencephalon (prosomeres; Puelles and Rubenstein, 1993, 2003; cf. the discussion of molecular data below). Neuromeres, originally described by von Baer (1928), are both morphologically defined entities, as well as territories of gene expression representing a useful framework to compare and interpret morphological observations from one species to another.

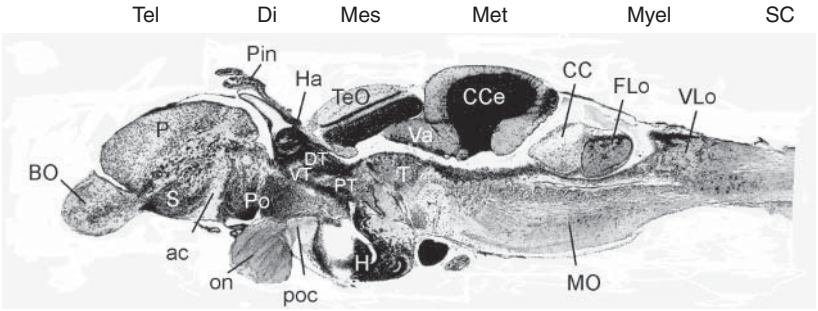


Figure 2 Sagittal section of adult zebra fish brain shows histology of major brain parts. ac, anterior commissure; BO, olfactory bulb; CC, crista cerebellaris; CCe, corpus cerebelli; Di, diencephalon; DT, dorsal thalamus; FLo, facial lobe; H, hypothalamus; Ha, habenula; Mes, mesencephalon; Met, metencephalon; MO, medulla oblongata; Myel, myelencephalon; on, optic nerve; P, pallium; Pin, pineal organ; Po, preoptic region; poc, postoptic commissure; PT, posterior tuberculum; S, subpallium; SC, spinal cord; T, tegmentum; Tel, telencephalon; TeO, optic tectum; Va, valvula cerebelli; VLo, vagal lobe; VT, ventral thalamus (prethalamus). Modified from Wullimann, M. F., Rupp, B., and Reichert, H. 1996. Neuroanatomy of the Zebrafish Brain. A Topological Atlas. Birkhäuser Verlag.

The vertebrate rhombencephalon is ancestrally characterized by its association with the majority of cranial nerves and their primary motor and sensory centers, that is, the trochlear (IV), trigeminal (V), abducens (VI), facial (VII), otic (VIII), glossopharyngeal (IX), and vagal (X) nerves, as well as the lateral line nerves (including a mechano- and an electroreceptive component).

The mesencephalon of vertebrate fishes includes dorsally an optic tectum (visual-multisensory; corresponding to mammalian superior colliculus) and a torus semicircularis (auditory-lateral line; corresponding to mammalian inferior colliculus) which may become somewhat ventrally displaced during ontogeny in certain taxa, as well as a ventral tegmentum which is dominated by motor structures, for example, oculomotor nerve (III) and nucleus.

Classically, the vertebrate diencephalon has been described in dorsoventral order to consist of epithalamus, dorsal thalamus, ventral thalamus, posterior tuberculum, and hypothalamus, with the pretectum intricately intermingled with diencephalic cell groups. The neuromeric model instead (Puelles and Rubenstein, 1993, 2003) proposes that pretectum, dorsal thalamus, and ventral thalamus (prethalamus) represent three transverse neural tube units or prosomeres along the longitudinal brain axis. The posterior tuberculum of fishes develops from the ventral portions of prosomeres 2 and 3, and the region of the nucleus of the medial longitudinal fascicle represents the ventral portion of prosomere 1. The optic nerve (II) enters the diencephalon at the ventral boundary region of preoptic region and hypothalamus.

The telencephalon of all fishes includes a pallium and a subpallium representing, together with the hypothalamus (as well as eminentia thalami and preoptic region), the most anterior, prechordal part of the neural tube (proposed to represent three prosomeres of the secondary prosencephalon; see the discussion above). Thus, the diencephalon gains a new meaning in the neuromeric model, since the classical dorsoventral order of diencephalic divisions transforms into a caudorostral sequence of the anterior neural tube. The olfactory nerve (I) enters the olfactory bulb at the anterior pallial pole of the telencephalon. The terminal nerve (0) is also associated with the telencephalon.

Most of the above-discussed vertebrate neural characters also apply to myxinoids (craniates). However, in contrast to gnathostomes, hagfishes have no recognizable cerebellum, and lampreys also only have a rudiment of it, lacking Purkinje cells or other major rhombic lip-derived elements. Interestingly, the lamprey rhombic lip region also lacks *Pax6* expression which is mandatory for the development of cerebellar structures in gnathostomes (Murakami *et al.*, 2005). Another novelty of gnathostomes is the presence of three semicircular canals in the vestibular inner ear, whereas hagfishes and lampreys have a simpler labyrinth. This is directly related to the absence of expression of one *Otx* gene in the otic placode of agnathans in contrast to gnathostomes (Germot *et al.*, 2001). Therefore, both agnathan groups offer no reasonable distinction between met- and myelencephalon.

Furthermore, myxinoids, but not lampreys, lack external eye muscles, as well as the associated cranial nerves and nuclei (e.g., III, IV, VI) and a terminal nerve, as well as the electroreceptive – but not the mechanoreceptive – component of the lateral line nerves (Braun, 1996; Wicht, 1996; Wicht and Northcutt, 1998). The absence of these and additional neural characters may be part of the ancestral condition for craniates, corroborating that myxinoids form the outgroup to vertebrates. Unfortunately, the lack of an appropriate outgroup to craniates prevents a test of an alternative explanation, namely that these myxinoid characteristics evolved as secondary reductions.

In any case, considerable evidence from modern developmental studies is in support of a basic craniate/vertebrate Bauplan just outlined.

10.2.2 Extension of Bauplan: Phylotypic Stage in Brain Development

Neuromeres are useful paradigms for comparing brain morphologies. They are also morphogenetic units resulting from the specification of the neural phenotype, which is an ongoing succession of cell-fate determination in spatially defined regions of the neuroepithelium. The conservation of neuromeres throughout the craniate taxon reflects major constraints on the development of the neural tube. During neurulation, the neural tube closes and becomes patterned along the anteroposterior axis (neuromeres) and along the dorsoventral axis (roof, alar, basal, floor plates). This patterning corresponds to the restriction of cell movements and to polyclonal cell divisions that become confined to a neuromeric unit.

A key feature of this cell patterning in the neural tube is that it also corresponds to gene patterning. In other words, morphogenetic units are also territories of defined gene expression. A probable proximal cause of this gene patterning is the existence of local sources of inductive signals located in the so-called organizing centers such as in the roof plate (members of secreted bone morphogenetic

proteins (BMPs) and wingless-related factors – WNTs) in the floor plate and zona limitans intrathalamica (ZLI) (secreted Hedgehog factors, Shh; induced after expression in notochord and prechordal plate), in the anterior neural ridge (ANR) or in the midbrain–hindbrain boundary (MHB), both secreting FGF8. The signals emitted by these centers coordinate the action of proliferation-related neurogenic or proneural genes at specific locations to maintain or inhibit proliferation of neural progenitors, leading to the formation of segments or neuromeres (Wurst and Bally-Cuif, 2001; Bertrand *et al*., 2002; Lekven *et al*., 2003; Buckles *et al*., 2004; Wilson and Houart, 2004; Figure 3). In addition to neurogenesis, local signals control the expression of other classes of genes, providing an identity, that is, a restricted fate of differentiation, to the precursor born in one of these neuromeres, and linking neurogenesis to neural differentiation.

Thus, there is a stage during neural development where the segmental or neuromeric organization of the neural tube is easy to recognize. During this stage, the acquisition of positional identity of neuroblasts under the control of signaling coordinates and specific genetic networks takes place. The important aspect of this paradigm is that neural determination and subsequent differentiation is acquired in a strict spatially defined manner, similar to the so-called phylotypic stage in the hourglass model of development by Duboule (1994) and Raff (1996). Before this phylotypic step, gastrulation and neurulation can significantly vary from one vertebrate species to another, provided they lead to a neural tube that is spatially organized into morphogenetic units, which may be neuromeres or finer units. The direct consequence of the phylotypic gene regulations is generation and differentiation of neural precursors in a time-and-space-defined manner within morphogenetic units. Neural precursors produced in a given morphogenetic unit will then proliferate, migrate, and establish connections with other brain parts, often losing their original spatial distribution. These events may differ from one species to another and render the neuromeric organization difficult to recognize at later stages of development or in adults.

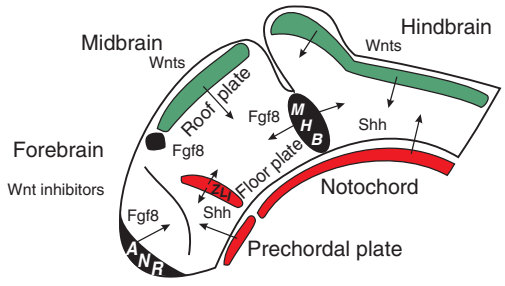


Figure 3 Schematic lateral view of early zebra fish brain with some major signaling centers indicated. Their activity underlies dorsoventral and anteroposterior regionalization leading to the formation of a Bauplan during the phylotypic brain development stage. ANR, anterior neural ridge; MHB, midbrain hindbrain boundary; ZLI, zona limitans intrathalamica. Adapted from Buckles, G. R. Thorpe, C. J., Ramel, M.-C., and Lekven, A. C. 2004. Combinatorial Wnt control of zebrafish midbrain hindbrain boundary formation. *Mech. Dev.* 121, 437 447 and Wilson, S. W. and Houart, C. 2004. Early steps in the development of the forebrain. *Dev. Cell* 6, 167 181.

Thus, this central nervous phylotypic stage results from tight constraints of neural differentiation, and certainly accounts for the striking conservation of the neuromeric organization of the neural tube in vertebrates, providing an easily recognizable framework for brain comparisons between species. From an evolutionary point of view, the phylotypic stage is also the developmental master theme on which many species-specific brain variations emerge depending on functional adaptation.

The best example of phylotypic structures is found in the neuromeric organization of the vertebrate rhombencephalon. Here, the combined action of proneural genes and positional cues, which depends on the spatially defined expression of *Hox* and *Nkx*-related genes (the so-called *Hox* code for acquiring positional identity), will, for example, specify the identity of motor neurons in the cranial nerve nuclei and of serotoninergic neurons in the reticular raphe nuclei (Cordes, 2001). In most vertebrates, the pattern of cranial nerves is highly similar, highlighting the power of segmental specification. Still, differences exist, such as the position of the trigeminal and facial nerve nuclei which are not in register with rhombomeres 2 through 4 in lampreys as they are in jawed vertebrates (Murakami *et al*., 2005). This is due to a rostral shift of *Hox3* expression in lampreys, revealing that positional information is retained, but in this case, independent of rhombomere segmentation.

Also in the forebrain, an astonishing degree of similarity of brain patterning has been described between zebra fish, *Xenopus*, and mouse at critical time point (zebra fish: 2–3 days postfertilization, *Xenopus*: stage 48, mouse: embryonic day 12.5/13.5). This morphogenetic pattern nicely corresponds to the differential presence of neurogenic and proneural gene expression, which later affects the differentiation of neurotransmitter phenotypes. For example, in the mammalian brain, γ-aminobutyric acid (GABA)-ergic cells are born and determined in the embryonic ventral subpallium (i.e., the medial ganglionic eminence), the determination of which depends on a genetic pathway that includes the regionalized expression of *Dlx1/2*, *Nkx21* and *Lhx6* and

of the proneural *Mash1* gene. A very similar situation is observed in *Xenopus* and zebra fish (Wullimann *et al.*, 2005; Mueller *et al.*, 2006). In the mouse, a large fraction of these ventrally born GABA neurons later migrate tangentially into the pallium, that is, the future cortex (reviewed in Wullimann and Mueller, 2004) where they become interneurons. In the zebra fish, migration of GABA neurons into the pallium likely also occurs (Mueller *et al.*, 2006), but the adult arrangement of interneurons in the zebra fish pallium remains to be described precisely. Interestingly, the absence of *Nkx2.1* expression in the lamprey subpallium correlates with the absence of GABA cells in the pallium (Murakami *et al.*, 2005). In contrast, the determination and differentiation of glutamatergic cells of the pallium (cortex) depends on the concerted activity of *Neurogenin1* and *NeuroD* in areas where *Pax6*, *Emx1/2*, *Tbr1*, and *Lhx9* are expressed in a regionalized manner (Wullimann and Mueller, 2004, and references therein).

These observations suggest that there are indeed strictly defined temporal and spatial requirements for a given neuronal phenotype to be differentiated (e.g., for GABAergic neurons), reflected in the strict spatiotemporal patterning of gene expression at the phylotypic stage. It is also the stage when proliferation of neural precursors is the most tightly regulated, affecting thereby relative final size of brain areas and total brain size as a consequence. Assuming comparable changes in cell cycle lengths during development of different species, the later the cell divisions stop the larger the brain will be. This requires that sufficient energy is produced by the organism to support brain metabolism, linking brain size to body size.

10.2.3 Brain Weight–Body Weight Data

How do vertebrate brain weights compare with a *Scala naturae* concept? All organs increase in size/weight with increase in body size. The brain does so at a coefficient of 0.66 of the body weight on average. In other words, the steepness of the regression line reveals a negative allometric growth of brain weight compared with body weight (van Dongen, 1998; Jerison, 2001). A common measure for relative brain size (degree of encephalization) is real brain weight over the expected brain weight. This value is 1.0 if a brain weight lies on the regression line. If the brain is twice the expected size, the value would be 2.0. In such a comparison, humans amount to 6.45, whereas the trout is at a value of 1.2. This may appear supportive of a linear increase in relative brain weight from fish to human and of *Scala naturae*. However, the goldfish amounts to 2.2 and the African electric fish *Gnathonemus petersii* reaches 5.5 (calculated based on brain weights/body weights given in Nilsson, 1996) Furthermore, within mammals, independent brain enlargement is seen in primates, carnivores, whales, and elephants (van Dongen, 1998). This clearly shows that there is independent increase in relative brain weight between and also within major vertebrate taxa and is in accord with a bush-like evolution of relative brain weight as discussed above.

Another way of looking at relative brain weight is to construct minimum convex polygons (Figure 4). It is true that, in such a comparison, the mammalian and bird polygons lie above agnathans, amphibians, and reptiles, that is, mammalian and avian brains are always much larger for a given body weight compared to these other three groups (Jerison, 2001) Interestingly, fossil information (endocasts) shows an increase in relative brain weight during geological times (i.e., tertiary) in mammals, but not in diapsid reptiles (van Dongen, 1998). A similar tendency of brain enlargement is seen in fossil versus extant birds. Thus, based on their evolutionary history of brain enlargement, mammals and birds might be considered 'higher' vertebrates. However, a large fraction of cartilaginous fish and certain teleost (i.e., mormyrid) brain weights overlap with the avian and mammalian polygons. The fact that the mormyrid data have only recently been added (Jerison, 2001) furthermore indicates that the values for ray-finned fishes may not be representative in the face of their large species number (see the discussion above). The fact that many extant cartilaginous fishes as well as at least the mormyrid fishes among ray-finned fishes lie in a range as to overlap with the mammalian or bird polygons also shows that there is no rule that keeps early diverging vertebrate groups intrinsically constrained by way of their systematic alliance to have small relative brain weights. Moreover, in contrast to the general belief that all brain parts increase to the same degree, relative brain enlargement in cartilaginous fishes and teleosts may largely be accounted for by disproportional growth of the cerebellum.

Consequently, these big-brained sharks, rays, and mormyrids would have to be considered as 'higher' vertebrates, together with mammals and birds, which is not useful. Clearly, brain weight–body weight data do not support the common ladder notion of *Scala naturae*, but rather show that independent cases of relative brain enlargement did occur in mammals, birds, cartilaginous fishes, and ray-finned fishes – several times in each –and, thus, represent cases of homoplastic (convergent) evolution of brain enlargement.

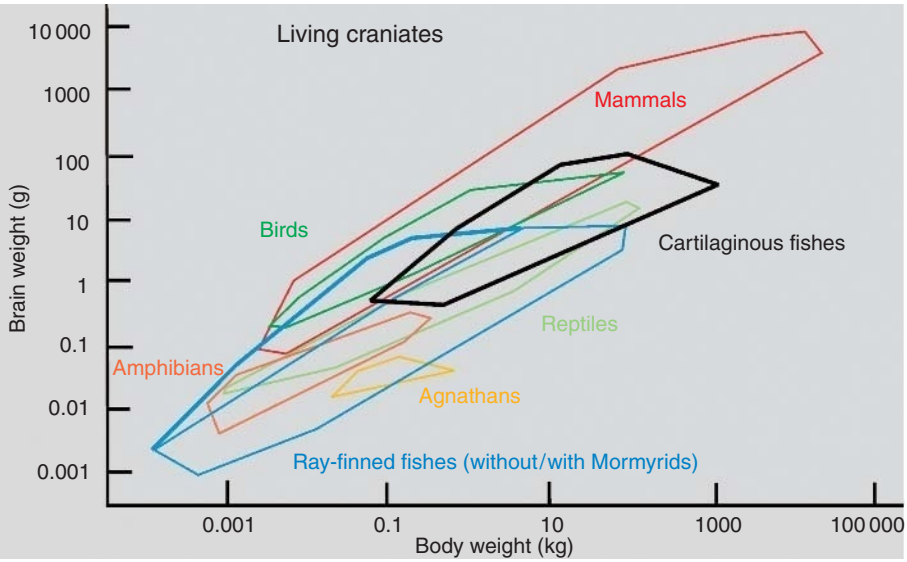


Figure 4 Brain weight/body weight relationships of living craniates. Note that both cartilaginous and ray-finned fish polygons overlap greatly with those of mammals and birds in comparable body size ranges. Adapted from Jerison, H. 2001. The evolution of neural and behavioral complexity. In: Brain Evolution and Cognition (*eds.* G. Roth and M. F. Wullimann), p. 523. Spektrum Akad Verlag/Wiley.

The analysis of environmental factors that may have guided the evolution of relative brain enlargement fits into this picture (van Dongen, 1998). The need for improved sensory and neural processing for finding and discriminating food has been suggested in primates and bats where fruit eaters have larger brains than herbivores/insectivores. Also, socially demanding environments for any given species generally seem to correlate with larger brain size (Striedter, 2005). Alternatively, the high-energy content of certain foods may allow for brain enlargement. Similarly, food-storing birds have a considerably larger hippocampus than closely related birds that do not store food. The degree of precociality at birth in birds – but interestingly not in mammals – is negatively correlated with brain size (van Dongen, 1998). The fact that apparently no general evolutionary factor does account for brain enlargement in mammals and birds, let alone in cartilaginous or ray-finned fishes, is in further support of the convergent nature of brain enlargement in various vertebrate taxa.

10.3 Functional Neuroanatomy of Fish Brains

10.3.1 How the World and Brain Interconnect: The Peripheral Nervous System

The peripheral nervous system of the head is represented by the cranial nerves. They connect the brain with the sensory and motor periphery and, thus, represent a natural starting point for understanding functional neuroanatomy. As already noted, myxinoids lack terminal, oculomotor, trochlear, and abducens nerves, as well as electrosensory (but not mechanosensory) lateral line nerves, while the situation in lampreys for sensory systems and cranial nerves is similar to gnathostomes in this respect. Therefore, here, we will describe only briefly the ancestral set of cranial nerves and sense organs that defines vertebrates (reviews: agnathans: Braun, 1996; teleosts: Wullimann, 1998; cartilaginous fish: Hofmann, 1999).

The relationship of cranial nerves and brain can only be understood in the larger context of how the vertebrate head is developmentally constructed (Northcutt and Gans, 1983). The interactions of the three embryonic germ layers during neurulation and their anatomical consequences are considerably more complex in the head than in the vertebrate body trunk (see Wilson and Houart, 2004; Butler and Hodos, 2005). For our purpose, it is important to keep in mind that in addition to neural tube (all somato- and visceromotor nerve components) and neural crest (sensory nerve components) – which are involved in spinal nerve development as well – a third set of neuroectodermal structures, namely the placodes, are involved in cranial nerve development. Placodes are embryonic epidermal thickenings representing neurogenic tissues that give rise to most special head sensory organs and – together with the head neural crest – to their innervating sensory ganglia and nerves.

Of the classical twelve cranial nerves recognized in human neuroanatomy, the hypoglossal (XII,

motor innervation of tongue) and spinal accessory nerve (XI, motor innervation of some neck and larynx muscles) are unique to tetrapods. The remaining ten nerves characterize all vertebrates and, thus, are present in lampreys and gnathostome fishes. The olfactory nerve (I) consists of primary sensory cells in the olfactory epithelium with an axon that projects to the olfactory bulb. In contrast, the terminal nerve (0) is formed by ganglion cells that often lie close to the ventral olfactory bulb and send a peripheral dendrite toward the olfactory epithelium and a central axon into the telencephalon beyond the olfactory bulb. The optic nerve originates from ganglion cells of the retina and is, thus, part of the central, not the peripheral, nervous system. The oculomotor (III), trochlear (IV), and abducens (VI) motor nerves innervate the extraocular eye muscles, with the oculomotor nerve including a parasympathetic component controlling pupillary light reflex. Branchiomeric nerves (V, VII, IX, X) are related to the innervation of one or more (only X) branchial arches or their derivatives (Butler and Hodos, 2005). The trigeminal (V) nerve is concerned with somatosensation of face and oral cavity. The facial (VII), glossopharyngeal (IX), and vagal (X) nerves all include a gustatory component innervating taste buds which may also lie outside the oral cavity on the body surface in fishes where they are always innervated by the facial nerve. Some teleosts have separate primary sensory facial and vagal lobes in the medulla oblongata (cf. Figure 2). All branchiomeric nerves in fishes have a motor contribution for innervating the jaw musculature (V), the hyoid arch (VII) or gill arch musculature (pharynx; IX, X), as well as a viscerosensory and parasympathetic component related to the innervation of head glands or viscera. Clearly, the ancestral vertebrate condition involves more than those ten cranial nerves just discussed. The lateral line nerves of vertebrate fishes – as many as six may be ancestral for gnathostomes (Northcutt, 1989) – innervate mechanosensory neuromasts (hair cell receptors) and electroreceptors on the body surface (Bullock *et al.*, 1983). Closely associated developmentally and functionally is the otic nerve (VIII) which innervates the mechanoreceptive hair cells of the labyrinth. Next to the vestibular sense, an auditory component is meanwhile assumed to be ancestral for vertebrates. Northcutt and various co-workers (summary in Northcutt and Bemis, 1993) furthered the comparative and embryological study of placodes, their developmental fate, and adult configuration of cranial nerves which resulted in a new understanding of the vertebrate head and its evolutionary history. This modern view gives a clear definition of sensory cranial nerves including a distinct placodal origin, the resulting peripheral ganglion and sensory receptor structures, and, most importantly, separate primary central nervous projection nuclei. This led to the falsification of the so-called octavolateralis hypothesis, which assumed that lateral line mechanoreceptors on the fish body surface were internalized in evolution into the labyrinth to serve tetrapod auditory function. The related concept of a primary sensory octavolateralis region in fishes where lateral line and otic nerve input forms an overlapping input is also factually false. The ancestral condition for vertebrate fishes is that they have, from dorsal to ventral, three separate sensory medullary columns dedicated to receive segregated lateral line electrosensory, mechanosensory, and otic nerve information (McCormick, 1992).

10.3.2 Sensory Systems from Primary Sensory to Higher Order Integrative Centers

There is a great general similarity in the synaptic relay from the primary sensory centers throughout the ascending neuraxis into the subpallium and/or pallium between amniotes and fishes.

10.3.2.1 Actinopterygians In teleosts, almost all sensory system pathways have been neuronally traced from primary sensory centers into the telencephalon (for detailed review of original literature, see Wullimann, 1998), which definitely receives largely nonoverlapping information from all sensory systems. Although the homology of sensory pathways between teleosts and tetrapods is not certain in each single case, the degree of similarity is nevertheless of great functional interest. Secondary olfactory input reaches a limited pallial territory in teleosts (in particular the posterior zone of the dorsal telencephalon, Dp, which is considered the homologue of the lateral pallium or olfactory cortex; Figure 5d) as well as most subpallial areas. The teleostean visual system has been described to display a direct retino-thalamofugal and an indirect retino-tecto-thalamofugal system with synaptic relays in the dorsal thalamus. In contrast to amniotes, both teleost visual pathways may be terminating in the subpallium and not in the pallium. However, tectofugal visual information reaches the pallium in certain teleosts via the preglomerular region, a complex of migrated nuclei lateral to the posterior tuberculum. The sensory systems which ascend multisynaptically in the lateral longitudinal fascicle via mesencephalic torus semicircularis and diencephalon to the pallium, that is, audition, lateral line mechanoreception, and electroreception,

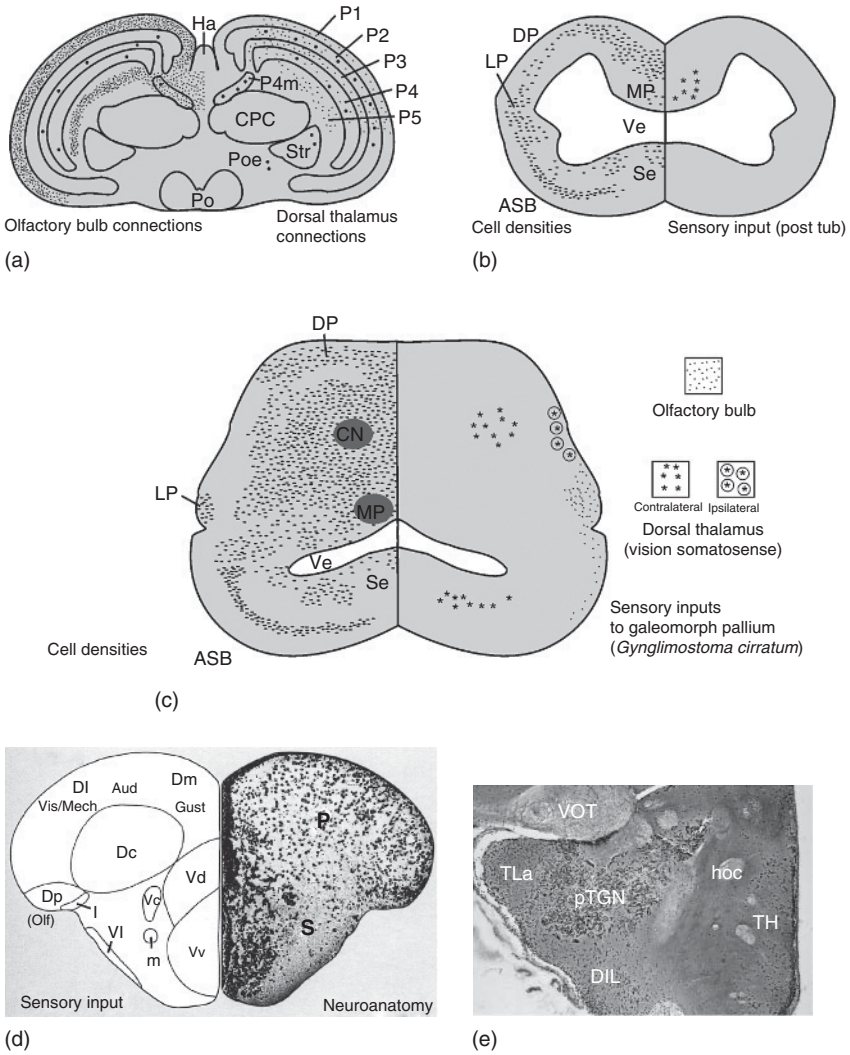


Figure 5 Telencephalic sensory input and output relationships in various fish taxa. a, *Eptatretus stouti* (Pacific hagfish). Left side: Olfactory bulb input to two pallial layers throughout most of their mediolateral extent. Right side: Dorsal thalamic input to all pallial layers. Note also reciprocity of connections with both sources of sensory input. b, *Squalus acanthias* (spiny dogfish, a squalomorph shark). Left side: Medial, dorsal, and lateral pallial divisions dorsal to the subpallium. Right side: Multimodal sensory input from posterior tuberculum to medial pallium. c, *Mustelus canis* (smooth dogfish, a galeomorph shark). Left side: A large pallial central nucleus is recognized in addition to three conventional pallial divisions. Right side: Telencephalic sensory input (established in another galeomorph, the nurse shark *Gynglimostoma cirratum*). d, *Danio rerio* (zebra fish). Right side: Histology of the teleostean pallial dorsal telencephalic area (P) and subpallial ventral telencephalic area (S). Left side: Pallial locations of olfactory bulb (Olf), visual (Vis), lateral line mechanosensory (Mech), auditory (Aud), and gustatory (Gust) inputs (originating in preglomerular nuclei lateral to the posterior tuberculum) as established in various other species (for details, see Wullimann and Mueller, 2004; Northcutt, 2006). e, Photomicrograph of the preglomerular gustatory projection nucleus to the telencephalon in percomorph teleosts (*Hemichromis lifalili*). ASB, area superficialis basalis; CN, central nucleus; CPC, central prosencephalic complex; Dc, Dl, Dm, and Dp, central, lateral, medial, posterior zones of area dorsalis telencephali (pallium); DIL, diffuse nucleus of inferior lobe; DP, dorsal pallium; Ha, habenula; hoc, horizontal commissure; l, lateral olfactory tract; LP, lateral pallium; m, medial olfactory tract; MP, medial pallium; P1 5, pallial layers; P4m, medial part of pallial layer; Po, preoptic region; Poe, external preoptic region; pTGN, preglomerular tertiary gustatory nucleus; Se, septum; Str, striatum; TH, tuberal hypothalamus; TLa, torus lateralis; Vc, Vd, Vl, and Vv, central, dorsal, lateral, ventral nuclei of area ventralis telencephali (subpallium); Ve, ventricle; VOT, ventrolateral optic tract. a, Adapted from Wicht, H. and Northcutt, R. G. 1998. Telencephalic connections in the Pacific hagfish (*Eptatretus stouti*), with special reference to the thalamopallial system. *J. Comp. Neurol.* 395, 245 260. b, Adapted from Northcutt, R. G. 1981. Evolution of the telencephalon in nonmammals. *Ann. Rev. Neurosci.* 4, 301 350; Smeets, W. J. A. J. and Northcutt, R. G. 1987. At least one thalamotelencephalic pathway in cartilaginous fishes projects to the medial pallium. *Neurosci. Lett.* 78, 277 282; Bodznick, D. A. 1991. Elasmobranch vision: Multimodal integration in the brain. *J. Exp. Zool.* Suppl., 108 116. c, Adapted from Ebbesson, S. O. E. 1980. On the organization of the telencephalon in elasmobranchs. In: Comparative Neurology of the Telencephalon (*ed.* S. O. E. Ebbesson), pp. 1 16. Plenum; Luiten, P. G. M. 1981. Two visual pathways to the telencephalon in the nurse shark (*Gynglimostoma cirratum*). II: Ascending thalamo-telencephalic connections. *J. Comp. Neurol.* 196, 539 548; and Bodznick, D. A. 1991. Elasmobranch vision: Multimodal integration in the brain. *J. Exp. Zool.* Suppl., 108 116. e, Adapted from Ahrens, K. and Wullimann, M. F. 2002. Hypothalamic inferior lobe and lateral torus connections in a percomorph teleost, the red cichlid (*Hemichromis lifalili*). *J. Comp. Neurol.* 449, 43 64.

are very comparable to the lateral lemniscal system of tetrapods. Gustation in teleosts reaches the diencephalon and telencephalon via a medullary secondary gustatory nucleus, which is comparable to the parabrachial nuclear region of mammals. Finally, teleosts possess a direct spinal ascending somatosensory system similar to the mammalian anterolateral (protopathic) system in addition to indirect spinal ascending projections which are relayed at the obex level, comparable to the mammalian medial lemniscal (epicritic) system.

A notable difference between teleost and amniote ascending sensory circuitry is that the predominant diencephalic targets of teleostean ascending sensory projections are not in the dorsal thalamus, but in the preglomerular nuclei located in the lateral periphery of the posterior tuberculum (Wullimann, 1998; Northcutt, 2006). Specific sensory preglomerular nuclei exist for the auditory, the lateral line mechanosensory, the electrosensory, and the gustatory systems (Figure 5e). There is also a preglomerular nucleus relaying visual information from tectum to telencephalon, at least in some teleosts. Finally, somatosensory information is relayed in the preglomerular region (Finger, 2000). Furthermore, these preglomerular nuclei – and not the dorsal thalamic ones – provide the major diencephalic input to the pallial zones of the area dorsalis telencephali (Figure 5d, left side). Also, the preglomerular nuclei in teleosts clearly display a higher degree of cytoarchitectonic differentiation and interspecific variation compared to the dorsal thalamus. Thus, the functional similarities between the teleostean preglomerular region and the amniote dorsal thalamus are striking: both make up a large proportion of the diencephalon, are subdivided into many nuclei associated with specific sensory systems, and most of them have reciprocal connections with the pallium.

The teleostean telencephalon is divided into a subpallial ventral telencephalic and a pallial dorsal telencephalic area (shown, for the zebra fish, in Figure 5d, right side). Teleostean pallial masses are topologically different from the usual vertebrate location of medial, dorsal, and lateral pallium resulting from evagination of bilateral telencephalic hemispheres (illustrated, for sharks, in Figure 5b). In teleosts, pallial masses are everted (Nieuwenhuys and Meek, 1990) and a recently proposed theory of partial eversion attempts to explain this topology by a developmental mechanism (Wullimann and Mueller, 2004). For our purpose, it is important to note that the posterior zone of the dorsal telencephalic area (Dp) is the major recipient of secondary olfactory input and is considered as the homologue of the lateral pallium (or olfactory cortex). The pallial lateral zone has been described as a visual area, the lateral, central, and medial zones as lateral line mechanosensory, the lateral and medial zones as auditory, the medial and central zones as somatosensory, and the medial zone as gustatory recipient zones in various teleosts species (for review of original literature, see Wullimann, 1998 and Northcutt, 2006).

10.3.2.2 Chondrichthyans Historically, the smell-brain theory suggested that the telencephalon of fishes is largely dominated by secondary olfactory input. Thus, ascending sensory systems reaching the telencephalon were believed to exclusively characterize amniotes, or even mammals only. However, the situation summarized above suggests that there is a pattern of ascending sensory pathways to the telencephalon common to tetrapods and actinopterygians. Since sarcopterygians include tetrapods and are the sistergroup of actinopterygians (Figure 1a), it is pivotal to analyze the information on cartilaginous fishes as an outgroup to reveal the ancestral condition of ascending sensory pathways and centers in gnathostomes.

We will first consider the location of diencephalic sensory targets in cartilaginous fishes (for original literature, see Smeets *et al.*, 1983; Wullimann, 1998; Hofmann, 1999). Visual information reaches the dorsal thalamus both directly from the retina and via the optic tectum. It is unclear which diencephalic region is involved in chondrichthyan audition, although autoradiographic deoxyglucose data suggest that it is the dorsal thalamus. Cartilaginous fishes also have a lateral lemniscal system. The ascending lateral line mechanosensory information reaches the dorsal thalamus, as well as the region lateral to the posterior tuberculum. Electroreception, on the other hand, does not reach the dorsal thalamus, but is represented in a ventral nucleus, lateral to the posterior tuberculum and in a hypothalamic nucleus, and both nuclei project to the telencephalon (Fiebig and Bleckmann, 1989). Further, directly ascending spinal somatosensory pathways exist up to the dorsal thalamus in chondrichthyans, though the presence of an indirect somatosensory system relayed at the obex level is unclear in these fishes. Ascending gustatory pathways have not been investigated in cartilaginous fishes.

These data in cartilaginous fishes suggest that a dual innervation of the diencephalon (dorsal thalamus/posterior tubercular region) by at least some ascending sensory systems is the ancestral pattern

for gnathostomes. Furthermore, it may be a gnathostome plesiomorphy that hair cell sensory organs in the labyrinth (audition, vestibular sense) are represented in the dorsal thalamus and the remaining hair cell sensory organs (mechanoreception, electroreception) are present in the posterior tubercular region. If so, the evolutionary loss of the latter sensory systems in amniotes may directly explain the dominance of the dorsal thalamus as the diencephalic sensory region in amniotes.

We turn now to the question where sensory systems are represented in the chondrichthyan telencephalon. Cartilaginous fish display evaginated telencephalic hemispheres with medial, dorsal, and lateral pallial divisions located dorsal to the subpallium (illustrated, in the spiny dogfish, by Northcutt, 1981; Figure 5b, left side). As noted above, many cartilaginous fish species have relatively large brains (Figures 1a and 4). In fact, galeomorph sharks and myliobatiforms (stingrays) among batoids show independent brain enlargement, while holocephalans, squatinomorphs, and squalomorph sharks, as well as most skates and rays other than stingrays, remain modest in brain size (Northcutt, 1978; cf. Figures 1a, 5b, and 5c). Apart from the cerebellum (compare *Squalus* and *Mustelus* in Figure 1a), particularly the telencephalon is also enlarged in these groups. Galeomorph sharks (e.g., *Mustelus canis*, the smooth dogfish; Figure 1a) display for example a conspicuous large central nucleus in the dorsal pallium (Figure 5c). Pioneer discoveries by Ebbesson and co-workers (summarized in Ebbesson, 1980) revealed that the telencephalon of the (galeomorph) nurse shark (*Gynglimostoma cirratum*) receives only very restricted secondary olfactory projections from the olfactory bulb to pallial (lateral pallium) and subpallial territories (Figure 5c) and, furthermore, that the nurse shark central pallial nucleus receives substantial contralateral and the lateral dorsal pallium receives ispilateral dorsal thalamic input (unspecified modality). Later, the central pallial nucleus of the nurse shark has been demonstrated to be recipient of a retino-thalamofugal and a retino-tecto-thalamofugal system (Luiten, 1981). Electrophysiological evidence also indicates that visual, somatosensory, and lateral line information is processed in the nurse shark central pallial nucleus (Bodznick, 1991). Furthermore, the medial pallium of the squalomorph spiny dogfish (*Squalus acanthias*) also receives dorsal thalamic as well as posterior tubercular inputs and it has been identified electrophysiologically as a multisensory region (vision, electrosense; Figure 5b right side, Smeets and Northcutt, 1987; Bodznick, 1991). These findings falsify the smell-brain theory because they show that the ancestral situation for gnathostome vertebrates is already characterized by ascending pathways of most, if not all, sensory systems reaching the telencephalon.

10.3.2.3 Agnathans Finally, turning to petromyzontids and myxinoids, we shall focus on the forebrain (original literature cited in Braun, 1996; Wicht, 1996). Both subpallial and pallial divisions may be recognized in the myxinoid telencephalon (*Eptatretus stouti*; Figures 1a and 5a). However, the myxinoid pallium apparently does not show three pallial divisions typical of gnathostomes (see the discussion above), but is rather homogeneously organized as a cortex, exhibiting five distinct neuronal layers throughout (Wicht and Northcutt, 1998). Olfactory bulb input covers most of the mediolateral extent of the hagfish pallium, but this input remains restricted to two pallial layers (P1, P5; Figure 5a). Secondary olfactory projections are also extensive – although to a lesser degree – in the lamprey pallium (Polenova and Vesselkin, 1993). However, all hagfish pallial layers receive additional dorsal thalamic input (Wicht and Northcutt, 1998). Furthermore, there are reciprocal connections both with the olfactory bulb and, importantly, with the dorsal thalamus. Also, the lamprey pallium receives dorsal thalamic input (Polenova and Vesselkin, 1993). An outgroup comparison of these findings may indicate that early vertebrates and craniates possessed a more olfactory dominated telencephalon or pallium than early gnathostome vertebrates. However, this is not to revive the smell-brain theory for two reasons. First, extant agnathans are very different from their ancestors (Carroll, 1988) and their life habits are seemingly very specialized for olfactory orientation, possibly representing later adaptive specializations. Second, and more importantly, the fact that diencephalic sensory input reaches the pallium in both lampreys and myxinoids demonstrates that, also in these highly olfactory guided animals, the telencephalon (and pallium) is not exclusively of olfactory nature as the smell-brain theory would predict.

10.3.3 Integrative and Motor Systems

Regarding the functional organization of two additional major integrative centers next to the telencephalon, namely optic tectum and cerebellum, surprising similarity is seen between gnathostome fishes and tetrapods. Also, both extant agnathan groups have an optic tectum that shares various inputs and outputs with those of gnathostomes. It

is beyond the scope of this contribution to review this information (for details on all groups, see Nieuwenhuys *et al*., 1998; for cartilaginous fishes, see Northcutt, 1978; Smeets *et al*., 1983; Hofmann, 1999; for teleosts, see Wullimann, 1998). The cytoarchitectonic and modular organization of the craniate optic tectum, its segregated multimodal input, and the topographical representation of this input and output to the reticular formation provide very likely an ancestral neuronal machinery apparently exquisitely designed for integrative orientation tasks, such as object identification and location, and coordinated motor control.

As noted above, a very rudimentary cerebellum may be identified in lampreys, but not in myxinoids. However, gnathostomes clearly have ancestrally a large cerebellum that exhibits the typical three-layered cortex with comparable cell types and internal circuits. Also the afferent and efferent connections of the chondrichtyan and actinopterygian cerebellum are similar to tetrapods, and this suggests that the cerebellum may have ancestral functions in motor learning and coordination in all gnathostomes (see also The Evolution of the Cerebellum).

What remains to be discussed is how the fish brain manages to access the efferent structures, that is, the primary motor nuclei of brain and spinal cord, for displaying a particular behavior. Except for the long palliospinal and palliopontine tracts, which represent independently evolved derived characters of (some) mammals and birds, also the motor (spinal and cranial nerve motor nuclei; see the above discussion) and premotor systems of gnathostome fishes resemble those of tetrapods. As in mammals, descending spinal projections in chondrichthyans (Smeets *et al*., 1983; Cruce *et al*., 1999) and actinopterygians (reviewed in Wullimann, 1998) originate in all divisions of the reticular formation, in the caudal (inferior) raphe region (but not in the superior raphe), in vestibular and sensory trigeminal nuclei, and even in a nucleus ruber. Furthermore, the nucleus of the medial longitudinal fascicle is the locus of an ancestral craniate premotor system descending to medullary and spinal levels. Also, in both agnathan groups, all parts of the reticular formation, as well as vestibular and sensory trigeminal nuclei, give rise to descending spinal projections (Ronan, 1989). However, they both lack a nucleus ruber, likely related to the absence of extremities.

As noted above, both optic tectum and cerebellum act on various premotor centers, in particular onto the reticular formation. However, the forebrain control centers of fish spinal descending systems are less well understood compared to tetrapods. Even more than in the case of the ascending sensory systems, studies in cartilaginous fishes and agnathans are urgently needed in order to understand the ancestral gnathostome and craniate condition of multisynaptically descending (extrapyramidal) systems.

10.4 Neurochemical Organization

The adult functional fish brain anatomy of excitatory neurotransmitters, such as glutamate and aspartate, as well as of inhibitory neurotransmitters GABA and glycine, remains to be described in detail, although these are certainly involved in sensory, motor, and higher-order circuitry just discussed. Recent descriptions of GABA systems in lampreys or teleosts basically show a similar degree of conservation as seen in the sensorimotor networks they contribute to form. A more complete picture may be drawn here on modulatory neuroactive substances of the fish brain, such as dopamine, noradrenaline, serotonin, histamine, and acetylcholine, with a particular focus on the involvement of these transmitters in the ascending modulatory systems.

10.4.1 Dopaminergic, Noradrenergic, Serotoninergic, Histaminergic, and Cholinergic Systems

The neurons that synthesize monoamines and acetylcholine in the craniate brain include the neuromodulatory systems, which regulate all basic functions of the central nervous system (i.e., many aspects of motor programming and sensory processing; Nieuwenhuys, 1985). They are also the main substrate of more specific behavioral processes such as reward and motivation, awareness, aggression or escape, sleep, or thirst and hunger. Accordingly, modulatory systems send most of their projections anteriorly in a very divergent manner, likely accompanying the evolutionary invention of the telencephalon of craniates. They act in target cells through activation of membrane receptors, which belong to different classes (see Kapsimali *et al*., 2003), and mediate different types of responses in the neural networks they control.

10.4.1.1 Actinopterygians Neurons synthesizing catecholamines (mostly dopamine and noradrenaline in craniates) were primarily studied by immunohistochemistry of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis, and sometimes by direct analysis of dopamine or noradrenaline distribution in various

actinopterygian brains (for reviews, see Kaslin and Panula, 2001; Rink and Wullimann, 2001). Here, we will focus on the model animal zebra fish (*Danio rerio*), as there is complementary immunohistochemical information on the serotoninergic, histaminergic, and cholinergic systems, as well as on critical ascending modulatory forebrain and spinal connections.

The zebra fish noradrenergic system (Kaslin and Panula, 2001; Figure 6a) includes medullary cells close to the viscerosensory column/area postrema (comparable to mammalian groups A1/A2; Smeets and Reiner, 1994) and a locus coeruleus (mammalian A6). The other noradrenergic neurons corresponding to the A3–A5 and A7 groups of mammals are not distinguishable separately in the teleost brain. Neurons of mammalian A1/2 exert local control on the respiratory pacemaker and related functions (e.g., swallowing, response to pH changes). These mixed dopaminergic/noradrenergic neurons are highly conserved in vertebrates and probably induce very similar cell responses in a broad range of species. The axons of the zebra fish locus coeruleus (Figure 7b) mainly project anteriorly, with a smaller contingent going to the hindbrain and spinal cord (Ma, 1997). Anterior projections reach virtually all midbrain and forebrain structures as they do in other gnathostomes. In mammals, the locus coeruleus is crucial for two basic components of behaviors, namely arousal (as opposed to sleep or resting states) and awareness, the latter being necessary for focusing on specific aspects of sensory perceptions. Three receptor classes mediate the effect of noradrenaline, α_1, α_2, and β, each of which comprising typically 3–4 subtypes, highlighting the large variety of cellular actions promoted by this neurotransmitter. No precise distribution of all receptors are available yet in teleosts, although α_{2A} and β_2 receptors seem to be more concentrated in anterior pallial areas and β_1/β_2 receptors in pretectal and cerebellar target areas (Zikopoulos and Dermon, 2005). The remaining tyrosine hydroxylase positive cells rostral to the locus coeruleus in the zebra fish brain are dopaminergic (Ma, 1997; Kaslin and Panula, 2001).

Some dopaminergic cell clusters in the zebra fish posterior tuberculum with a ventral telencephalic (likely striatal) projection were recently proposed to be homologous to the most anterior part (now interpreted as basal diencephalic instead of mesencephalic) of the amniote substantia nigra/ventral tegmental area (mammalian A9/A10; groups 1, 2, 4 of Rink and Wullimann, 2001; Figures 6a and 7a). Posterior tubercular zebra fish dopamine neurons project to telencephalic ventral (septum) and dorsal (basal ganglia) divisions, but some projections from the posterior tuberculum may also reach dorsal (pallial) areas, as is the case in mammals and other amniotes. The action of dopamine on these structures is mediated by classes of receptors (D1 and D2), comprising also 2–4 subtypes. In subpallial structures, two receptor subtypes are mainly found, the D_{1A} and D_2 subtypes, which are located on different populations of neurons (Kapsimali *et al.*, 2003). They likely mediate integration of sensorimotor cues in automatic programs of movements (dorsal striatum), as in other gnathostomes. In contrast, the D_{1B} receptors are clearly present in an area located at the Dm–Dl junction, which has been proposed to be homologous to the mammalian hippocampus (Kapsimali *et al.*, 2000; Salas *et al.*, 2003). In addition, some dopaminergic cells in the zebra fish posterior tubercular area project to the spinal cord (McLean and Fetcho, 2004) and, thus, may correspond to A11. Other diencephalic zebra fish dopamine cells include a ventral thalamic group corresponding to mammalian zona incerta (A13; group 0 of Rink and Wullimann, 2001; Figure 6a). Preoptic zebra fish dopamine cells may partially correspond to group A14, as there are strong preoptic projections to the ventral telencephalon (likely to septum; Rink and Wullimann, 2004). Other preoptic dopamine cells in teleosts project on the GnRH producing cells of the ventral hypothalamus, where they exert a highly variable, mostly inhibitory effect on gametogenesis and ovulation via D_2 receptors (Dufour *et al.*, 2005). A homologue of A15 as seen in some mammals additionally in the preoptic region is doubtful in the zebra fish. As all vertebrates, teleosts possess olfactory bulb (A16) and retinal (A17) dopamine cells, where dopamine acts mostly on D2-like receptors likely to increase discrimination for the two sensory pathways as in amniotes.

Clearly, teleostean posterior tubercular and hypothalamic dopamine populations are more numerous (groups 3, 5–7, 9–11; Figure 6a) than those of amniotes and they include three distinct cell types. Liquor-contacting cells (zebra fish groups 3, 5, 7, 10; Figures 6a and 7a) are absent in mammals (but present in all other vertebrates). Zebra fish large pear-shaped dopamine cells are long-distance projections neurons (see above and Figure 7a). Thus, only small round dopamine cells remain as possible candidates for an A12 (mammalian hypothalamic dopamine cells) homologue. Accordingly, numerous nuclei in the ventral and dorsal hypothalamic regions are targets of dopamine neurons. In some teleosts, the D_{1A} and D_{1B}

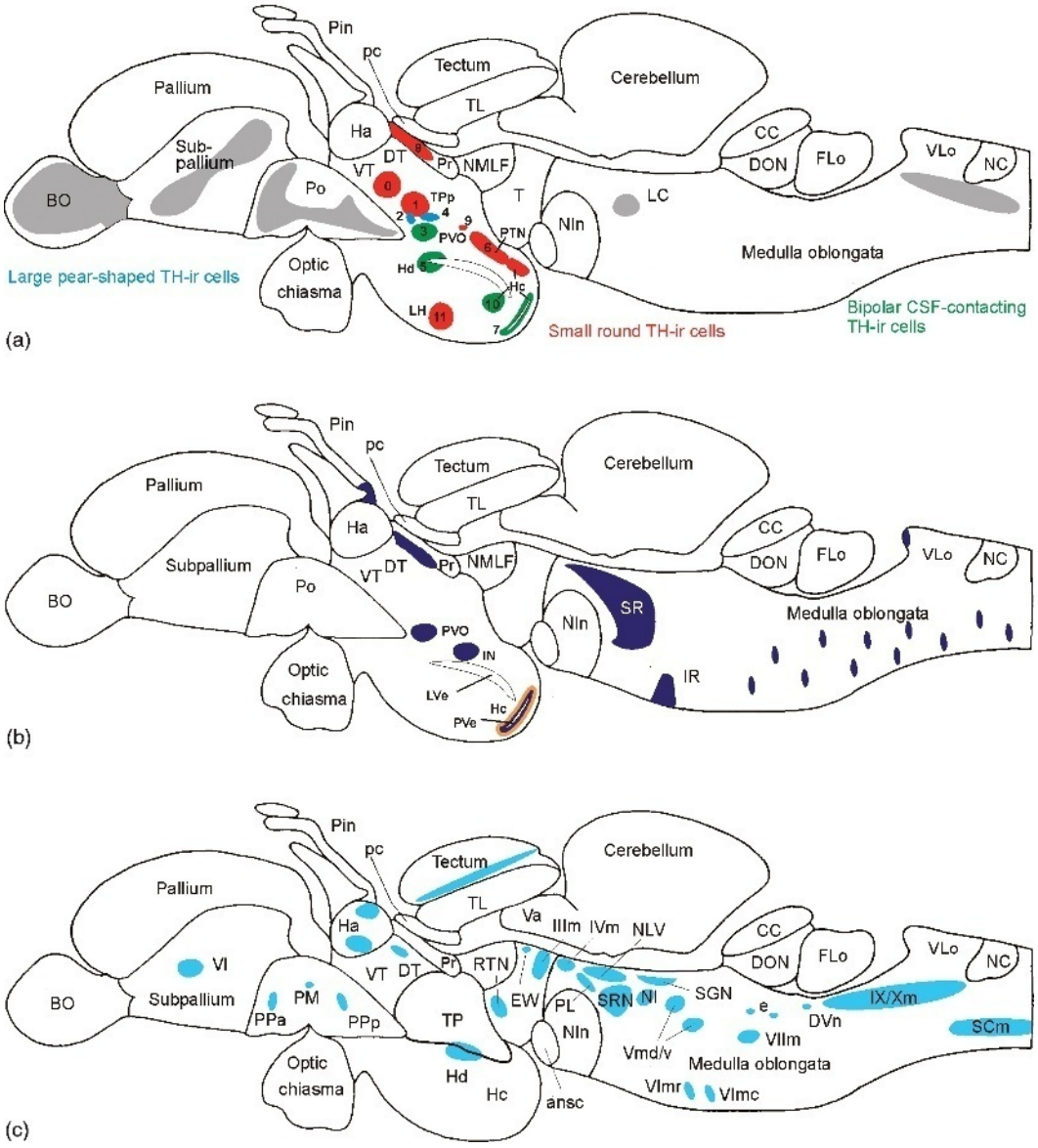


Figure 6 Adult neurochemical organization of the teleost brain (zebra fish). a, Dopaminergic and noradrenergic systems revealed by tyrosine hydroxylase distribution. Noradrenergic cells are only present in locus coeruleus and medulla oblongata. b, Serotoninergic and histaminergic (orange) systems. c, Cholinergic system revealed by choline acetyltransferase distribution. ansc, ansulate commissure; BO, olfactory bulb; CC, cerebellar crest; DON, descending octavolateralis nucleus; DT, dorsal thalamus; DVn, cholinergic neurons associated with DV; e, two medullary populations of efferent octavolateralis cells; EW, Edinger-Westphal nucleus; FLo, facial lobe; Ha, habenula; Hc and Hd, caudal, dorsal periventricular hypothalamic zones; IN, intermediate nucleus (of Rink and Wullimann, 2001); IR, inferior raphe; LC, locus coeruleus; LH, lateral hypothalamus; LVe, lateral recess ventricle; NC, commissural nucleus of Cajal; NI, nucleus isthmi; NIn, interpeduncular nucleus; NMLF, nucleus of medial longitudinal fascicle; NLV, nucleus lateralis valvulae; pc, posterior commissure; Pin, pineal organ; PL, perilemniscal nucleus; PM, magnocellular preoptic nucleus; Po, preoptic region; PPa, anterior part of parvocellular preoptic nucleus; PPp, posterior part of parvocellular preoptic nucleus; Pr, periventricular pretectum; PTN, posterior tubercular nucleus; PVe, posterior recess ventricle; PVO, paraventricular organ; RTN, rostral tegmental nucleus; SCm, spinal cord motoneurons; SGN, secondary gustatory nucleus; SR, superior raphe; SRN, superior reticular nucleus; T, tegmentum; TL, torus longitudinalis; TP, posterior tuberculum; TPp, periventricular nucleus of posterior tuberculum; Va, valvula cerebelli; VI, lateral nucleus of area ventralis telencephali; VLo, vagal lobe; VT, ventral thalamus (prethalamus); IIIm, oculomotor nerve nucleus; IVm, trochlear nerve motor nucleus; Vmd/v, dorsal/ventral trigeminal nerve motor nucleus; VImr, rostral abducens nerve motor nucleus; VImc, caudal abducens nerve motor nucleus; VIIm, facial nerve motor nucleus; IX/Xm, glossopharyngeal/vagal nerve motor nucleus. a, Adapted from Ma, P. M. 1997. Catecholaminergic systems in the zebrafish. III: Organization and projection pattern of medullary dopaminergic and noradrenergic neurons. *J. Comp. Neurol.* 381, 411 427; Rink, E. and Wullimann, M. F. 2001. The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). *Brain Res.* 889, 316 330. b, Adapted from Kaslin, J. and Panula, P. 2001. Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). *J. Comp. Neurol.* 440, 342 377. c, Adapted from Mueller, T., Vernier, P., and Wullimann, M. F. 2004. The adult central nervous cholinergic system of a neurogenetic model animal, the zebrafish *Danio rerio. Brain Res.* 1011, 156 169.

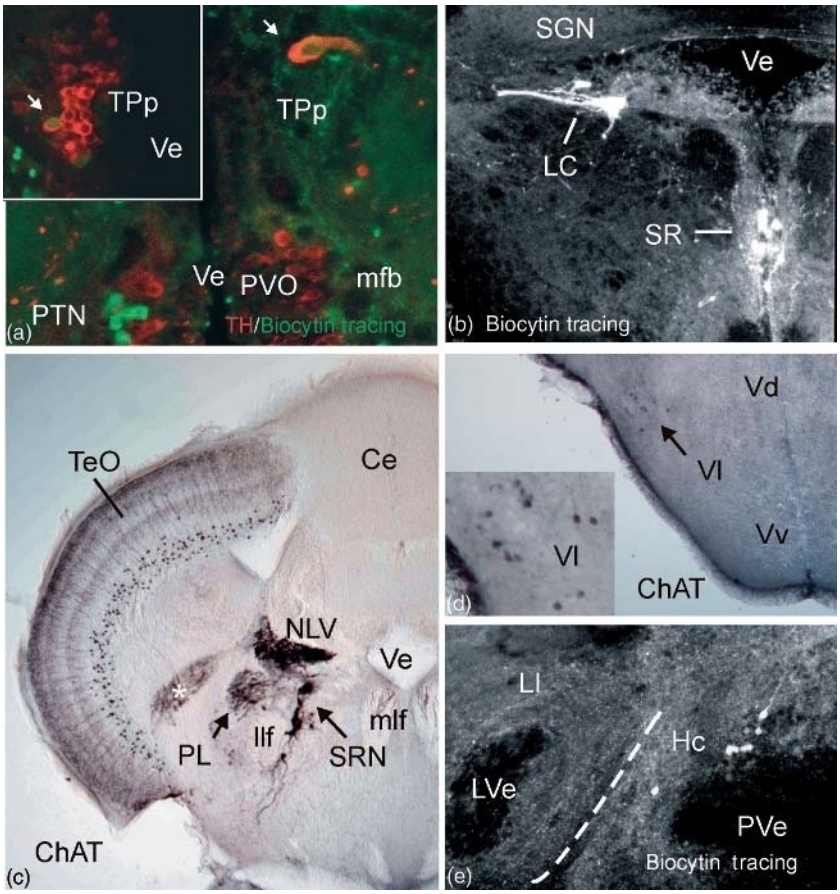


Figure 7 Ascending modulatory systems in the zebra fish brain shown in transverse sections. a, Photomontage shows tyrosine hydroxylase (TH)-containing (i.e., dopaminergic) small (left arrow) and large (right arrow) neurons in periventricular posterior tuberculum which were traced at the same time for projections to ventral telencephalon. b, Telencephalic projection neurons in noradrenergic locus coeruleus and serotoninergic superior raphe. c, Cholinergic neurons in superior reticular nucleus. d, Cholinergic neurons in lateral nucleus of ventral telencephalic area. e, Telencephalic projection neurons in caudal hypothalamus (likely histaminergic). These neurons, as well as neurons in periventricular posterior tuberculum, locus coeruleus, superior raphe, and superior reticular nucleus are all telencephalic projection nuclei (Rink and Wullimann, 2004), but double-label experiments for showing both neurochemical nature and projections of a given cell are only available for TH-containing neurons in the zebra fish. Ce, cerebellum; ChAT, choline acetyl transferase; Hc, caudal periventricular hypothalamus; LC, locus coeruleus; Ll, inferior lobe; llf, lateral longitudinal fascicle; LVe, lateral recess ventricle; mfb, medial forebrain bundle; mlf, medial longitudinal fascicle; NLV, nucleus lateralis valvulae; PL, perilemniscal nucleus; PTN, posterior tubercular nucleus; PVe, posterior recess ventricle; PVO, paraventricular organ; SGN, secondary gustatory nucleus; SR, superior raphe; SRN, superior reticular nucleus; TeO, optic tectum; TPp, periventricular posterior tuberculum; Vd, Vl, and Vv, dorsal, lateral, ventral nuclei of area ventralis telencephali (subpallium); Ve, ventricle. a, Adapted from Rink, E. and Wullimann, M. F. 2001. The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). *Brain Res.* 889, 316 330. c and d, After Mueller, T., Vernier, P., and Wullimann, M. F. 2004. The adult central nervous cholinergic system of a neurogenetic model animal, the zebrafish *Danio rerio. Brain Res.* 1011, 156 169.

receptor transcripts have been detected in preoptic nuclei and in the dorsal and ventral periventricular hypothalamic areas. In addition, the D_{1C} receptor, a dopamine receptor subtype which has been lost in mammals, is found in a few restricted areas of the dorsal hypothalamus, including the liquor-contacting cells. Pretectal dopamine cells (group 8; Figure 1a), which project to tectal layers where D_{1A} receptors are found, likely are ancestral for sarco- and actinopterygians, as they are absent in chondrichthyans and agnathans (and, again, absent in mammals). Telencephalic (subpallial) dopamine cells occur ancestrally in agnathans, chondrichthyans, and actinopterygians and are lost in tetrapods, while mammals appear to evolve convergently subpallial dopamine cells.

Turning now to serotoninergic zebra fish brain populations, there are also some striking correspondences to amniotes (Kaslin and Panula, 2001; Figure 6b). However, although the five classes of serotoninergic receptors, which have been isolated in mammals, also exist in teleosts, very few studies have addressed the relationship of serotonin projections and receptor localization. The large

serotoninergic population in the superior raphe, which has a telencephalic projection (Rink and Wullimann, 2004; Figure 7b), is almost certainly homologous to mammalian dorsal and central superior raphe nuclei (B6–8; Nieuwenhuys, 1985). The main target areas of these raphe neurons are probably hypothalamic nuclei where a high amount of receptor binding sites have been evidenced, as well as striatal and pallial areas. Serotonin cells in the zebra fish inferior raphe and in the more caudolaterally located reticular formation (both with spinal projections; Wullimann, 1998) may correspond to mammalian nucleus raphes magnus (B3) and nuclei raphes pallidus/obscurus (B1/2), respectively. There is a distinct population of serotonin cells in the posterior tuberculum, and two more in the hypothalamus of the zebra fish (Figure 1b). The situation in the amphibian posterior tuberculum and hypothalamus is similar (Dicke *et al*., 1997), and sauropsids – but not mammals – also have serotonin cells in the posterior tuberculum (Smeets and Steinbusch, 1988; Challet *et al*., 1996). The zebra fish hypothalamic intermediate nucleus exclusively exhibits serotonin cells, while the paraventricular organ as well as the caudal hypothalamus contain both dopamine and serotonin cells in the zebra fish (Figure 6b). Although absent in amniotes, serotonin cells seen in the teleost pretectum may be ancestral for vertebrates, as they occur in amphibians (Dicke *et al*., 1997), chondrichthyans, and lampreys (see the discussion below), but those in the pineal stalk may be unique to actinopterygians. As in tetrapods, histaminergic cell populations in the zebra fish brain are present exclusively in the most caudal hypothalamus (Kaslin and Panula, 2001; Figures 6b and 7e).

Also the cholinergic system of the zebra fish easily reveals great similarity to the amniote pattern (Figure 6c; for discussion see Mueller *et al*., 2004). First, all motor cranial nerve nuclei (as discussed above) expectedly are cholinergic. Second, there are cholinergic subpallial as well as brainstem neurons possibly corresponding to amniote cholinergic basal forebrain (Figure 7d) and ascending reticular (i.e., pedunculopontine-laterodorsal tegmental; Figure 7c) systems. Also, the zebra fish secondary gustatory nucleus is at least partially cholinergic and projects to the hypothalamus. Furthermore, a cholinergic isthmic nucleus (comparable to the mammalian parabigeminal nucleus) projects to the optic tectum.

10.4.1.2 Chondrichthyans The noradrenergic system of cartilaginous fishes also exhibits rhombencephalic groups including cells close to the viscerosensory column and a locus coeruleus (summarized by Smeets and Reiner, 1994). Chondrichthyan dopamine cells are present in olfactory bulb, subpallium, preoptic region, zona incerta, posterior tuberculum, and hypothalamus, where the situation is very comparable to actinopterygians (Smeets and Reiner, 1994). However, elasmobranchs (sharks and skates/rays) have large dopamine cell groups in the mesencephalic tegmentum resembling the amniote substantia nigra/ventral tegmental area. In contrast to all elasmobranchs investigated, *Hydrolagus collei* (a holocephalian, the sistergroup of elasmobranchs) lacks basal mesencephalic dopamine cells (Stuesse and Cruce, 1991); such cells are restriced to the directly adjacent posterior tuberculum (similar to actinopterygians) and this possibly represents the ancestral vertebrate condition (see discussion below). Unfortunately, there is practically no functional information on this system in cartilaginous fishes. Interestingly, chondrichthyans exhibit dopamine cells in the pallium and habenula, features they share (apparently convergently) only with mammals (pallial cells also with some reptiles).

Chondrichthyan serotoninergic cells are abundant in the extensive raphe region and reticular formation (Stuesse *et al*., 1990, 1991; Stuesse and Cruce, 1991). Also, similar to actinopterygians, posterior tuberculum and hypothalamus contain many serotonin cells. The pretectum contains serotonin in some, but not all, chondrichthyan species. There is no report on histamine in chondrichthyans.

The cholinergic system in chondrichthyans (summarized by Rodríguez-Moldes *et al*., 2002) shares many ancestral features with that in actinopterygians, that is, the motor nuclei, potential cholinergic basal forebrain (subpallial) cells, brainstem reticular ascending cholinergic system, an isthmic nucleus, and a possible secondary viscerosensory nucleus. Interestingly, there are pallial cholinergic cells in chondrichthyans, otherwise only seen in mammals.

10.4.1.3 Agnathans Noradrenergic cells in lampreys are definitely present in a brainstem group (corresponding to a locus coeruleus) that projects to the telencephalon (nucleus reticularis medius; Pombal *et al*., 1997). The dopamine system of lampreys includes retinal, olfactory bulb, preoptic, and possibly some subpallial cells (Pombal *et al*., 1997). The pattern in the posterior tuberculum and hypothalamus is similar to that in chondrichthyans and actinopterygians. In particular, a projection of dopaminergic posterior tubercular cells to the striatum has been shown, supporting the ancestral presence in vertebrates of a diencephalic homologue of the substantia nigra/ventral tegmental area, as

also seen in some chondrichthyans and all actinopterygians investigated.

In hagfishes, tyrosine hydroxylase containing cells are present in a brainstem group, likely representing a noradrenergic locus coeruleus, as well as in hypothalamic and posterior tubercular positions, presumably representing dopaminergic cells (Wicht and Northcutt, 1994).

The serotoninergic system in lampreys (Pierre *et al*., 1992) and hagfishes (Kadota, 1991) includes neuronal groups apparently corresponding to raphe nuclei, posterior tubercular, and hypothalamic populations; lampreys also contain a pretectal one, as seen in other vertebrates.

Histaminergic neurons in lampreys have been reported in the hypothalamus and, unlike all other vertebrates, in the midbrain–hindbrain boundary region (Brodin *et al*., 1990).

Regarding cholinergic systems in the lamprey brain, they also exhibit many ancestral characteristics, that is, motor nuclei of cranial nerves, nucleus isthmi, possibly a secondary viscerosensory population, and even a small cholinergic basal forebrain group (Pombal *et al*., 2001).

10.5 Conclusions

Modern comparative research in developmental biology, functional neuroanatomy, and neurochemical central nervous system organization has fundamentally changed our view of vertebrate brain evolution. The metaphor of the vertebrate brain climbing slowly up the ladder of progress from fish to human has been replaced by the common theme of a largely conservative Bauplan of vertebrate brain organization, upon which uncounted variations are independently generated along various major phylogenetic lines.

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11 Evolution of the Amphibian Nervous System

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Glossary

basal optic neuropil (BON) Situated in the ventral tegmentum. It obtains afferents from all quadrants of the retina. Neurons of the BON are sensitive to horizontal and vertical direction of stimulus movement and, together with the thalamus and pretectum, constitute the circuitry for optokinetic responses.

basolateral amygdala It is disputed whether amphibians possess an amygdalar complex homologous to the mammalian basolateral amygdala of pallial origin.

bed nucleus of the stria terminalis (BNST) Part of the extended central amygdala.

central amygdala Occupies the caudal ventral telencephalon around the ventricle medial to the caudal pole of the striatopallidum in frogs. In salamanders, it is located more rostrally extending ventral to the striatopallidum. It is characterized by reciprocal connections with visceral autonomic brain centers.

cerebellum Composed of the corpus cerebelli, the auricular lobes, and the cerebellar nucleus. A mossy fiber and a climbing fiber system are present. The cerebellar nucleus is considered homologous to the deep cerebellar nuclei of mammals. Like that of other vertebrates, the cerebellum is involved in sensorimotor integration and motor coordination.

dorsal column nucleus (DCN) Situated in the transition zone between the medulla oblongata and medulla spinalis. It receives somatotopically organized input from the skeletal system. The ascending tracts reach ipsi and/or contralateral mesencephalic and diencephalic structures.

dorsal pallium Forms the dorsal part of the telencephalon and consists of a dorsomedial and a dorsolateral portion. Neurons of both portions display only intratelencephalic projections. It has associative limbic functions.

dorsal striatopallidum Occupies the ventrolateral wall of the telencephalic hemisphere; its neurons resemble the medium spiny neurons of the mammalian caudate putamen. The rostral portion of this complex is now regarded dorsal striatum proper and the caudal portion dorsal pallidum.

hypothalamus Part of the diencephalon consisting of a preoptic and an infundibular region, which

	have wide connections with nuclei of the limbic system and brainstem nuclei. It consists of the preoptic region, the partly cholinergic magnocellular pre optic nucleus, the suprachiasmatic nucleus, the posterior entopeduncular nucleus, the periventricular dorsal, ventral and lateral nucleus, the posterior tubercle, and the periventricular organ.
isthmic nucleus	Situated in the caudal tegmen tum and essential for object localization and selection. It is homologous to the parabigem inal nucleus of mammals. Retinotectal transmission is facilitated by a cholinergic isth motectal projection, which is topographically organized and in register with the retinal map.
lateral line system	Present in fully aquatic species and in species with biphasic lifestyle during larval stages, but absent in direct developing or life bearing taxa. It is involved in directional current detection and current related postural adjustments.
lateral pallium	Occupies the dorsolateral por tion of the telencephalon. It is divided into a rostral intermedi ate, precommissural, and a caudal postcommissural part. Neurons of the former portion project to the medial, dorsal, and ventral pallium and to the main olfactory bulb, while those of the latter portion send their dendrites and axons along the olfactohabenular tract to the dorsal and medial pallium and to the septum.
main olfactory amygdala	Region in the ventrolateral part of the caudal pallium dorsolat eral to the vomeronasal amygdala. It is connected to olfactory structures and to the hypothalamus.
medial pallium	Occupies the dorsomedial por tion of the telencephalon. The dorsal portion of the medial pallium is considered homolo gous to the mammalian Ammon's horn and the ventral portion to the subiculum; a dentate gyrus seems to be absent. It is believed to be involved in learning and mem ory formation.
motor nuclei	Classically divided into viscero , branchio , and somatomotor nuclei. Motor pools display a somatotopic organization and form a medial and a lateral col umn in the spinal cord of most amphibian species.
nucleus accumbens/ ventral striatopallidum	Found in the rostral ventro medial telencephalon. It extends caudally to what is now considered the ventral pallidum.
nucleus of the diagonal band of broca	Situated ventral to the medial septal nucleus and now believed to be part of the medial septal complex.
pallidum	The caudal part of the dorsal striatum is now considered the dorsal pallidum. The ventral pallidum is situated in the ven tromedial telencephalon. It is a shell like caudal continuation of the nucleus accumbens/ven tral striatum.
parabrachial nuclei	The nucleus visceralis secundar ius of amphibians is considered homologous to the parabrachial nuclei of amniotes.
pedomorphosis	A form of heterochrony, in which traits that characterize larvae or juveniles of ancestral taxa are maintained in the adult stage of descendant taxa. It involves different degrees of retardation, reduction, or absence of traits in otherwise fully developed organisms.
posterior tubercle	Situated in the caudal ventral diencephalon. It contains dopa minergic cells and is homologous to the mammalian substantia nigra pars compacta.
preoptic area/region	See hypothalamus.
pretectum	Transition zone between dience phalon and mesencephalon (also called synencephalon). Deep and laterally migrated neurons are distinguished with reciprocal connections with other visual centers. Neurons are direction ally selective and involved in optokinetic nystagmus.
raphe nuclei	Situated along the ventral mid line of the entire brainstem. They have extensive ascending projections to all parts of the brain. The exact contribution of

	the different raphe nuclei for targets in the forebrain is unknown.
reticular formation	Situated in the brainstem and composed of a median, medial, and lateral zone. These zones differ in the distribution of neurotransmitters. Numerous descending pathways converge onto the zones. Nuclei of the amphibian reticular formation are assumed to correspond to that of mammals.
rostral pallium	Occupies the rostral pole of the pallium and projects to all other pallial regions and, like the ventral pallium, to the dorsal edge of the striato pallidum.
secondary simplification	Arises from pedomorphosis. A mosaic of fully adult, weakly expressed and missing traits appears at terminal ontogenetic stages. Accordingly, brains have fewer cells, a lower degree of morphological differentiation and reduced cellular migration, but retain the plesiomorphic organization found in other vertebrates.
septum	Located between medial pallium and nucleus accumbens. A medial complex including a ventrally situated nucleus of the diagonal band of Broca, a lateral, and a central complex are now distinguished.
solitary tract	Runs inside the dorsolateral medulla oblongata and receives general and gustatory viscerosensory fibers from the IXth and Xth cranial nerves. It is accompanied by the nucleus of the solitary tract.
striatopallial transition area (SPTA)	Located dorsal to the striatopallidum. It is considered as part of the ventral pallium. Projects to the (lateral) vomeronasal amygdala and hypothalamus.
tectum mesencephali	Laminated structure forming the dorsal midbrain. It is the main center for visual perception and visuomotor functions. It possesses neuronal types with specific connections to the forebrain and/or brainstem. Here, like in amniotes, object recognition is based on population coding and occurs in a parallel distributed fashion.
tegmentum mesencephali	Forms the ventral mesencephalon and consists of a dorsal and ventral part with a classical distinction of tegmental nuclei comparable to mammals.
thalamus	The dorsal thalamus contains an anterior, central, and posterior periventricular and an anterior and posterior lateral nucleus. Sensory afferents terminate in the ventral thalamus consisting of a periventricular nucleus and a number of migrated nuclei. In contrast to mammals, the dorsal thalamus does not process unimodal sensory (lemnothalamic) information. The anterior dorsal nucleus combines traits of the mammalian anterior, dorsomedial, midline, and intralaminar nuclei. The central dorsal nucleus of amphibians is regarded homologous to the nucleus rotundus of reptiles and birds.
torus semicircularis	Consists of a principal, laminar, and magnocellular nucleus. It is the major audiomotor interface and the center of convergence of ascending auditory, vestibular, somatosensory, and lateral line pathways as well as descending pathways from the forebrain.
ventral pallium	Situated between lateral pallium and striatopallidum and includes the SPTA. It projects to the accessory olfactory bulb, the vomeronasal amygdala and preoptic region, and hypothalamus.
vestibular nuclei	Situated in the medulla oblongata and divided into four nuclei, which receive projections from sensory epithelia of the canal ampullae, utriculus, sacculus, and lagena of the inner ear.
vomeronasal amygdala	Situated in the caudal ventrolateral telencephalon. It is continuous with the SPTA covering the area formerly called 'lateral amygdala' and is characterized by its massive input from the accessory olfactory bulb and its projections to the preoptic area and hypothalamus via the stria terminalis.

11.1 Introduction

In this article, we give an overview of the central nervous system (CNS) (see Basic Nervous System "Types": One or Many?, Origin and Evolution of the First Nervous System), i.e., spinal cord and brain, of amphibians in a comparative and evolutionary context. A comprehensive description of sense organs and the CNS of amphibians is beyond the scope of this article, and we restrict a more detailed description to those parts of the CNS that are best studied and of greatest interest for a comparative and evolutionary approach, namely (1) the visual system including retina, optic tectum, pretectum, and thalamus; (2) thalamotelencephalic pathways; (3) telencephalic pallial regions; and (4) telencephalic limbic centers including the basal ganglia. For a more extended overview of the amphibian nervous system, the reader is referred to volume 2 of Nieuwenhuys *et al.* (1998). However, in our article, we include substantial data from more recent studies.

11.2 Phylogeny of Amphibians

Modern amphibians, the *Lissamphibia*, form the three orders Anura (anurans: frogs and toads; presently 29 families, about 5086 species), Urodela (urodeles: newts and salamanders; 10 families, about 545 species), and Gymnophiona (caecilians; six families, 170 species) (Frost, 1985; Duellman and Trueb, 1986; Amphibiaweb, 2006). Members of the order Anura are distributed worldwide; those of the order Caudata are found in the northern hemisphere of Eurasia as well as in North America, Central America, and the northern part of South America; and the order Gymnophiona is restricted to the tropics and subtropics of the Old and New World.

Most authors now assume that lissamphibians form a monophyletic group, but a minority assumes a polyphyletic origin for the three orders (cf. Pough *et al.*, 2001). It is assumed that the lungfishes (Dipnoi, six species) are the living sister group to tetrapods and the closest nontetrapod relatives of modern amphibians (Zardoya *et al.*, 1998; Tohyama *et al.*, 2000; Brinkmann *et al.*, 2004).

The earliest limbed vertebrates, the labyrinthodontids (stegocephalians), appeared in the Upper Devonian. Living amphibians have ancient roots and each may have originated in the Paleozoic (San Mauro *et al.*, 2005). Different authors consider them to be either a sister group or descendants of the temnospondyls (living about 340 Mya) (Ruta *et al.*, 2003) or the even more ancient microsaurs (Laurin, 1998a, 1998b). Most temnospondyls were clumsy-looking animals, up to several meters long with a thick, scaly skin, but some, the dissorophoids, were small and gracile and thought by some to be ancestors of the lissamphibia. Modern amphibians are mostly small to very small animals with thin, mostly smooth skin allowing cutaneous respiration: hence the name Lissamphibia (i.e., amphibians with smooth skin). They exhibit many additional traits that distinguish them from their paleozoic ancestors; this suggests that they have undergone substantial evolutionary transformation in the process of pedomorphosis, as will be discussed below.

The relationship between the three amphibian orders is still controversial, but most authors adhere to the hypothesis that salamanders and caecilians are more closely related to one another than to frogs (cf. Pough *et al.*, 2001). Despite their presumed monophyly, the three amphibian orders differ greatly in skeletal structure and way of life. While urodeles retained much of the bodily appearance of ancestral amphibians, anurans have a greatly reduced vertebral column and strongly developed hind limbs. Caecilians, finally, evolved a highly ossified skull and lost their limbs. The ancestors of amphibians most probably underwent metamorphosis including an aquatic larval stage, but many taxa from all three orders developed direct development (i.e., loss of a larval stage), and some of them developed viviparity (Duellman and Trueb, 1986).

11.3 Structure and Function of the Amphibian CNS

The CNS of amphibians consists of the spinal cord and the brain, which is divided into five parts, i.e., medulla oblongata, cerebellum, mesencephalon, diencephalon, and telencephalon, as indicated in Figure 1.

11.3.1 Spinal Cord

11.3.1.1 Gross morphology The amphibian spinal cord possesses cervical and lumbar enlargements characteristic of tetrapods. In transverse sections, the gray matter of the spinal cord typically has an H shape in frogs and a compact, oval appearance in salamanders (cf. Figure 2). The frog spinal cord is divided into a dorsal, lateral, central, ventromedial, and ventrolateral field; the spinal cord of salamanders consists of dorsal, intermediate, and ventral zones. In frogs, the dorsal horns are separated by dorsal funiculi; the substantia gelatinosa is difficult to delimit within the dorsal horn. In salamanders, the dorsal horn mainly consists of the

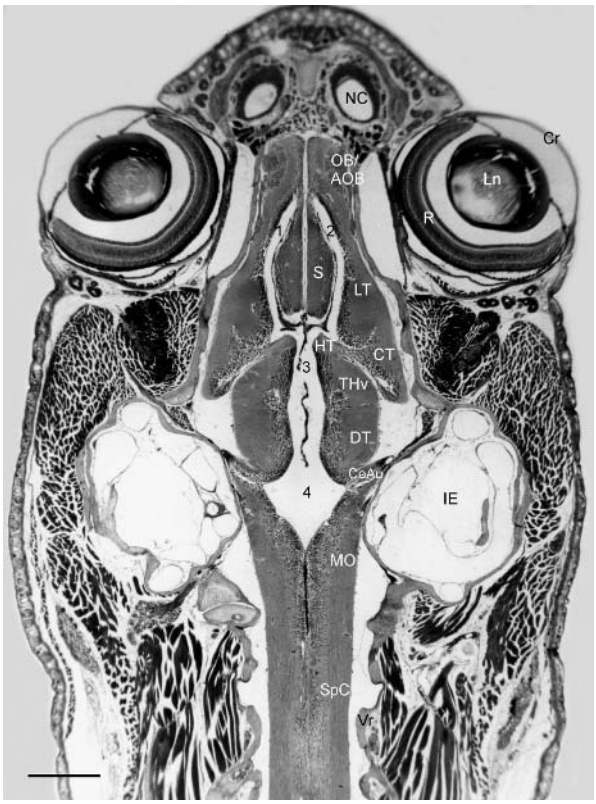

Figure 1 Horizontal section through the head of the salamander *Plethodon dunni* showing the gross anatomy of the brain and spinal cord, eye, nose, and inner ear. 1, 2, 3, 4, ventricles; NC, nasal cavity; OB, olfactory bulb; AOB, accessory olfactory bulb; Cr, cornea; Ln, lens; IE, inner ear; R, retina; S, septum; LT, lateral telencephalon; CT, caudal telencephalon; HT, habenular tract; THv, ventral thalamus; DT, dorsal tegmentum; CeAu, cerebellar auricle; MO, medulla oblongata; SpC, spinal cord; Vr, vertebra. Scale bar: 500 μm.

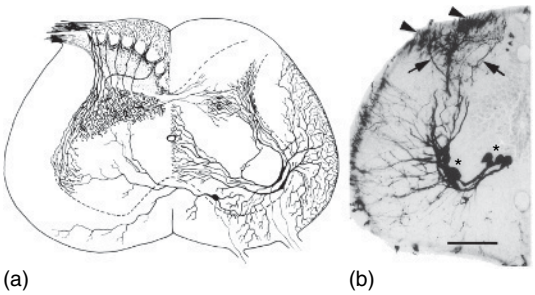

Figure 2 a, Schematic representation of a cross section through the lumbosacral region of the spinal cord of the frog. Motor neurons (right side) belong to the dorsolateral and ventromedial group of spinal motor neurons; their dendrites constitute spatially separate dendritic arrays. On the left side, the projection of dorsal root fibers is illustrated. A lateral bundle of fibers descends into the ventral horn, and establishes contact with motor neurons. The dotted area of the dorsal horn represents the substantia gelatinosa. b, Microphotograph of a transverse section through the spinal cord of *Plethodon jordani* at the level between the third and fourth spinal nerves. After HRP labeling of the superficial ramus of the brachial nerve, motor neurons (asterisks) and sensory fibers are stained. Some primary dendrites (arrows) extend to the dorsal or dorsolateral sensory fiber bundles (arrowheads). Scale bar: 100 μm. a, Reproduced from Frog Neurobiology, 1976, pp. 765 792, Organization of locomotion, Sźekely, G. and Czeh, G. With kind permission of Springer Science and Business Media. b, From Dicke, U. and Muhlenbrock-Lenter, S. 1998. Primary and secondary somatosensory projections in direct-developing plethodontid salamanders. *J. Morphol.* 238, 307 326.

substantia gelatinosa. The ventral zone comprises the motor neurons, which are arranged in motor columns along the rostrocaudal axis. Ependymal cells line the central canal, and radial glial cells send processes toward the pia. These types of cells are present throughout the CNS; inside the brain, they constitute the layer lining the ventricles.

11.3.1.2 Primary afferents Afferent fibers originate from end organs in the skin, joints, and muscles, from free nerve endings of the skeletal system and the inner organs. Dorsal root fibers entering the spinal cord bifurcate into descending and ascending fiber bundles; a lateral division of sensory fibers runs in a bundle comparable to the Lissauer tract of mammals. In the cervical spinal cord, afferent fibers cross the midline and terminate in the corresponding contralateral gray matter. In frogs and salamandrid salamanders, primary afferents ascend to the hindbrain and enter the cerebellum, whereas in plethodontid salamanders they reach the rostral medulla oblongata (Antal *et al.*, 1980; Muñoz *et al.*, 1997; Dicke and Muhlenbrock-Lenter, 1998). A somatotopic arrangement has been described for primary afferents that terminate in the dorsal column nucleus (DCN) situated in the rostral spinal cord (Muñoz *et al.*, 1994a, 1995, 1998). In frogs, primary afferents of the somatosensory system constitute a tract with thick myelinated fibers running in the medial dorsal horn, while thin myelinated fibers run ventrally and laterally to the entrance of the dorsal root. Cutaneous afferent fibers run within the dorsal tract and form a dorsal neuropil, while muscle afferent fibers form a ventral neuropil of the ventral tract, which is contacted by dendrites of motor neurons (Figure 2a). In plethodontid salamanders, both types of afferents form a dorsal and dorsolateral tract and corresponding neuropils, which are both contacted by motor neuron dendrites (Figure 2b).

A variety of neuropeptides involved in transmission of somatosensory and/or nociceptive stimuli (opioids, tachykinins, and FMRFamides) as well as serotonergic, histaminergic, and catecholaminergic fibers have been demonstrated in primary afferent fibers and/or in the dorsal spinal cord of amphibians (Lorez and Kemali, 1981; Danger *et al.*, 1985; Adli

et al., 1988; Salio *et al.*, 2001; Sanchez-Camacho *et al.*, 2001b; Partata *et al.*, 2002; Chartrel *et al.*, 2002; Guedes *et al.*, 2004).

11.3.1.3 Autonomic neurons Sympathetic preganglionic somata are situated dorsal to the central canal and form a continuous column between the level of the third and the seventh/eighth spinal nerves; parasympathetic preganglionic neurons are situated in the most caudal part of the spinal cord.

11.3.1.4 Motor neurons In most amphibians, motor neurons are arranged in a medial and a lateral column (Matesz and Székely, 1978; Wake *et al.*, 1988; Kim and Hetherington, 1993) (Figures 2, 3d, and 3e). Among salamanders, interspecific differences exist; in bolitoglossines, for example, a clear distinction of the two motor columns is absent (Figures 3e and 3h, right). The medial motor column consists of pear-shaped cells and the lateral one of spindle-shaped cells. Primary dendrites of the latter motor neurons extend to the dorsal horn and overlap with primary afferent fibers and their neuropils. Within the motor columns, motor pools innervating different muscles considerably overlap in the rostrocaudal axis and in the transverse plane. Nevertheless, motor pools show a somatotopic organization in the sense that more caudally located motor neurons innervate more distally located limb muscles. Spinal circuits form building blocks for movement construction and have been described as isometric force fields. During limb behavior, motor elements are combined in chains and in combination contingent on the interaction of feedback and central motor programs (Giszter *et al.*, 1993; Kargo and Giszter, 2000).

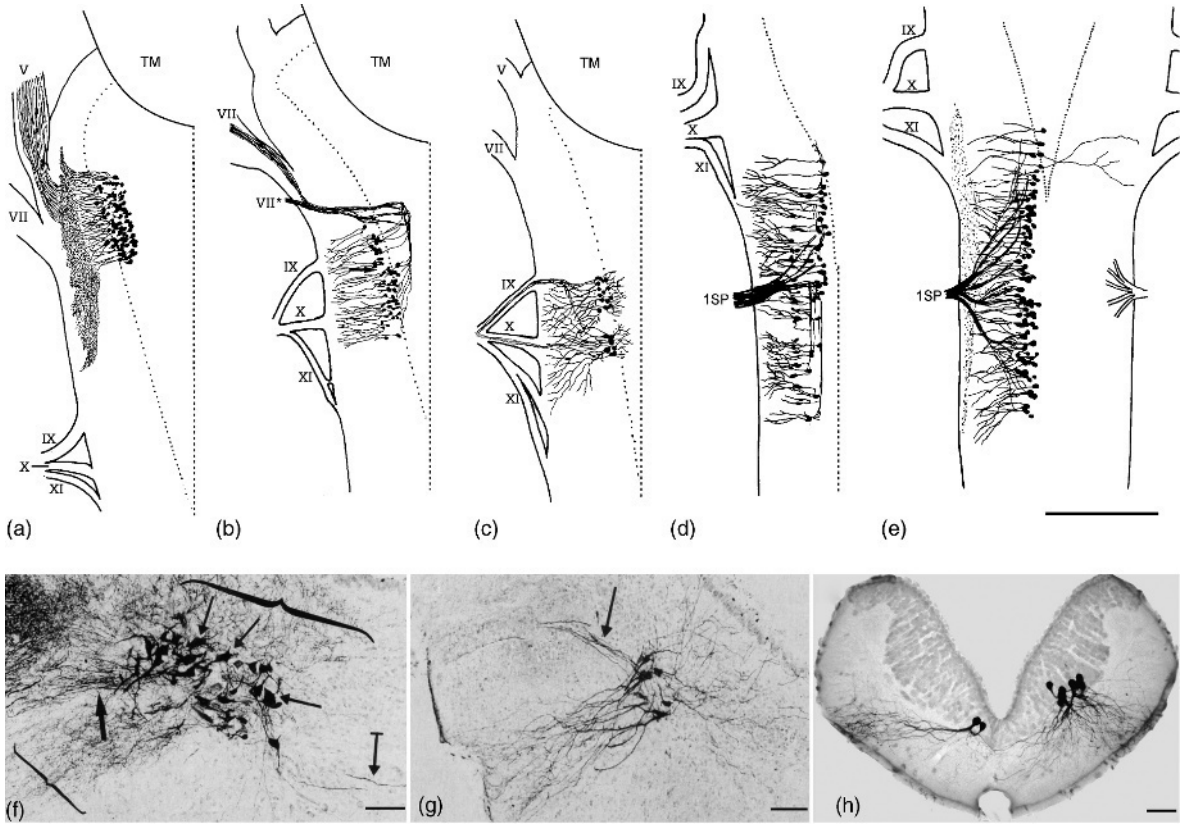

Figure 3 a e, Camera lucida reconstruction of motor nuclei of salamanders after application of horseradish peroxidase (HRP) to the nerve stumps of the trigeminal (a) facial; (b) glossopharyngeal; (c) cranial nerves, the hypoglossal/first spinal; (d, e) in the species *Plethodon jordani* (a, c), *Salamandra salamandra* (b, d), and *Hydromantes italicus* (e). Note the presence of a medial and lateral motor column in (d) and the absence of a lateral motor column in (e). f h, Microphotographs of transverse sections through motor nuclei. Motor neurons of the trigeminal (f) and facial (g) cranial nerve of *Rana esculenta*, and motor neurons of the Xth cranial nerve (h, left side) and the first spinal nerve (h, right side) of *Plethodon jordani*. Scale bar: 500 μm in a e and 100 μm in f h. V, VII, X, XI, XII, cranial nerves; VII*, lateral line nerve; TM, mesencephalic tectum; 1SP, first spinal nerve. a e, From Roth, G., Rottluff, B., and Linke, R. 1988a. Miniaturization, genome size and the origin of functional constraints in the visual system of salamanders. *Naturwissenschaften* 75, 297 304. f and g, Reproduced from *Adv. Anat. Embryol.*, Vol. 128, 1993, pp. 1 92, The efferent system of cranial nerve nuclei: A comparative neuromorphological study, Sźekely, G. and Matesz, C. With kind permission of Springer Science and Business Media.

11.3.1.5 Secondary projections

11.3.1.5.(i) Descending projections from brain centers In all amphibian taxa, extensive descending pathways arise from the reticular formation, the octavolateral area, the locus coeruleus, the laterodorsal tegmental nucleus, the raphe nucleus, and sensory nuclei of the medulla oblongata. Descending projections from the cerebellum, mesencephalon (tectum, torus, and tegmentum), pretectum, posterior tubercle, ventral thalamus, hypothalamus, and the amygdaloid complex were likewise found (ten Donkelaar *et al.*, 1981; Luksch *et al.*, 1998; Dicke, 1999; Roth and Grunwald, 2000; Sanchez-Camacho *et al.*, 2001a, 2001b).

11.3.1.5.(ii) Ascending projections of the spinal cord Projections from the spinal cord ascend via the lateral and/or the ventral funiculus to the reticular formation, mesencephalon, and thalamus. Neurons of the DCN and the lateral cervical nucleus (LCN) form a contralaterally ascending ventral tract and an ipsilaterally ascending dorsal tract in frogs and in salamandrid salamanders. The contralateral tract reaches the level of the mesencephalon; the ipsilateral tract terminates at the level of the cerebellum in salamandrids and sparsely innervates mesencephalic and diencephalic structures in frogs. In plethodontid salamanders projections from the DCN and LCN ascend in three tracts, i.e., a contralateral ventral, a contralateral and an ipsilateral lateral one (spinal lemniscus), which ipsi- and contralaterally reach the cerebellum, tegmentum, torus, and tectum in the midbrain, posterior tubercle, pretectum, and ventral thalamus by a substantial number of fibers (Muñoz *et al.*, 1997; Dicke and Muhlenbrock-Lenter, 1998).

11.3.2 Medulla Oblongata and Cerebellum

11.3.2.1 Medulla oblongata

11.3.2.1.(i) The longitudinal zones In amphibians, as in other vertebrates, the medulla oblongata is an anatomically and functionally heterogeneous part of the brain. It contains primary and secondary relay stations for somato- and viscerosensory information as well as sensory input from the inner ear, and – when present – form the lateral line organs and ampullary organs (electroreception). Networks exist for the control of vital body functions such as respiration and blood circulation; the reticular formation is involved in the control of vigilance and attention. Finally, the medulla oblongata is the convergence zone of numerous descending pathways from all parts of the brain (for a synopsis of the sensory and motor cranial nerves and the reticular formation of vertebrates, see Butler and Hodos, 1996).

The division of the medulla oblongata of amphibians into four longitudinal zones is based mainly on the density and arrangement of cell masses and size and shape of somata, and this likewise holds for the division of the reticular formation into median, medial, and lateral zones. The existence of such longitudinal zones was demonstrated by characteristic differences in the distribution of neurotransmitters such as serotonin (raphe nuclei) or noradrenaline (locus coeruleus). Nuclei of the reticular formation were investigated by means of immunohistochemistry and tracer techniques in ranid frogs and assumed to be homologous to the classical distinction of reticular nuclei established in mammals (Marín *et al.*, 1996; Adli *et al.*, 1999; Stuesse *et al.*, 2001; Zhao and Debski, 2005). In salamanders, somata – except for giant cells such as Mauthner neurons – are more or less equal in size and rather evenly distributed throughout the cellular layer. However, tracer and immunohistochemical investigations of viscero- and somatomotor nuclei, sensory afferents, and transmitter-specific nuclei demonstrate that the longitudinal zones in the medulla oblongata of salamanders match those of other amphibian species (Dicke *et al.*, 1997; Landwehr and Dicke, 2005).

11.3.2.1.(ii) Motor nuclei The efferent system is situated in the basal plate of the medulla oblongata and consists of somatomotor and branchiomotor nuclei (Roth *et al.*, 1988b; Székely and Matesz, 1993) (Figures 3a–3c and 3f–3h). The hypoglossal nucleus and the motor nuclei innervating the external eye muscles (abducens nucleus, and the trochlear and oculomotor nuclei situated in the mesencephalon) constitute the somatomotor nuclei. Branchiomotor nuclei comprise the trigeminal and facial motor nuclei, the nucleus ambiguus (glossopharyngeal and vagal nucleus), and the accessory nucleus. The basic organization of the frog ambiguus nucleus is comparable to that of the rat, and differences in nuclear organization reflect differences in peripheral structures (Matesz and Székely, 1996). Motor neurons subserving different functions in tongue movements disclose characteristic morphological differences. Motor neurons innervating different groups of muscles involved in the movements of the tongue (protractor, retractor, and inner muscles) could be separated on the basis of the shape of dendritic arborization in the horizontal, frontal, and sagittal planes of the brainstem (Matesz *et al.*, 1999; Birinyi *et al.*, 2004).

11.3.2.1.(iii) Primary sensory afferents Cranial nerves V–XII terminate and/or originate in the rhombencephalon. The alar plate receives somatosensory fibers of the head, general visceral sensory and gustatory fibers, as well as afferents from the inner ear and the lateral line system (Fritzsch *et al.*, 1984; Kuruvilla *et al.*, 1985; Roth and Wake, 1985a; Fritzsch, 1989; Muñoz *et al.*, 1994b). The sensory fibers of the trigeminal nerve extend in the descending tract of the trigeminal nerve to the first and second spinal segment, where they cross to the contralateral side. Fibers and collaterals terminate continuously along the tract in an area containing small cells, i.e., the nucleus of the spinal trigeminal tract; gap junctional coupling was observed between fibers of the descending limb and their postsynaptic targets (Bacskai and Matesz, 2002). Primary afferents of the trigeminal nerve also terminate at the level of the obex in the principal sensory nucleus and in the mesencephalic nucleus of the trigeminal nerve. The latter nucleus is situated in the tectum mesencephali and is characterized by large, unipolar somata dispersed in the cellular layers. The solitary tract comprises mainly general and gustatory viscerosensory fibers of the IXth and Xth cranial nerves. This tract extends from the level of the trigeminal motor nucleus to the spinomedullary border and is accompanied by small, densely packed cells of the nucleus of the solitary tract. Afferents of the inner ear terminate at the level of the entrance of the VIIIth nerve in the dorsolateral nucleus, which is the first relay nucleus of the auditory system and homologous to the cochlear nucleus of mammals. Projections from sensory epithelia of the canal ampullae, utriculus, sacculus, and lagena of the inner ear reach the nucleus of the ventral octavus column, which in ranid frogs has been divided into four vestibular nuclei. GABA and glycine are the major inhibitory transmitters of neurons in the vestibular nuclear complex in frogs as well as in mammals (Reichenberger *et al.*, 1997). The lateral line system is present in fully aquatic species and in those with biphasic lifestyle during larval stages; it is lacking in direct-developing or life-bearing taxa. It is involved in both directional current detection and current-related postural adjustments in *Xenopus* (Simmons, 2004). Afferents enter via the VIIth and Xth cranial nerves (also via the Vth cranial nerve in urodeles) and bifurcate into descending and ascending branches within the medulla oblongata; collaterals terminate on a medially situated lateral line (octavolateral) column of cells that accompany the afferent tracts.

11.3.2.1.(iv) Afferents from other brain regions In frogs and salamanders, descending projections to the rostral medulla oblongata arise from at least 30 major cell groups situated in the telencephalon, diencephalon, synencephalon, mesencephalon, and cerebellum. The majority of afferent fibers originate from ipsilateral nuclei of these brain parts, and the white matter of the lateral and the medial medulla oblongata is reached by afferent fibers of different brain regions. Main afferents exclusively reaching the lateral white matter comprise fibers of the dorsal and ventral striatum and amygdalar nuclei of the telencephalon, the magnocellular nucleus of the preoptic area, the large neurons of the superficial pretectal nucleus, the mesencephalic nucleus of the trigeminal nerve, the red nucleus, and the cerebellar nucleus, while a substantial number of axons of the dorsal thalamus, lateral posteroventral thalamic nuclei, the dorsal hypothalamus, the nucleus of the longitudinal medial fascicle, and the superior and isthmic reticular nuclei exclusively descend to the medial white matter of the medulla oblongata. The lateral and the medial white matter receive extensive descending projections from the preoptic area, ventral thalamic and lateral posterodorsal thalamic nuclei, the deep pretectal nucleus, the tectum (see Section 11.3.4.9.(i)), the torus and tegmental nuclei, and the middle and lateral reticular nucleus. Fewer neurons of the ventral (ventral lateral) pallium, the central thalamic nucleus, the posterior tubercle, the nucleus Darkschewitsch and Edinger–Westphal likewise project to the white matter of the medulla oblongata (Naujoks-Manteuffel *et al.*, 1988; Dicke *et al.*, 1998).

11.3.2.1.(v) Ascending pathways of the medulla oblongata The raphe nuclei of the brainstem display extensive ascending projections to all brain parts, although the exact contribution of the different raphe nuclei, using combined immunohistochemistry and tracing, has mainly been studied in the mesencephalon and is lacking for targets in the forebrain. This also holds true for other reticular nuclei of the medulla oblongata (Dicke *et al.*, 1997; Stuesse *et al.*, 2001; Landwehr and Dicke, 2005; Zhao and Debski, 2005).

Second-order projections of the descending trigeminal nucleus reach the cerebellum, ventral mesencephalon, pretectum, and thalamus. The nucleus of the solitary tract projects to the nucleus visceralis secundarius (NVS) situated in the isthmic region and homologous to the parabrachial nucleus of mammals. The NVS/parabrachial nucleus in turn projects to the preoptic area, the amygdala, and ventral pallium (Moreno and González, 2004; Roth *et al.*, 2004). Second-order neurons of the auditory

pathway are situated in the contralateral dorsolateral nucleus, bilaterally in the nucleus of the superior olive and the torus semicircularis. Projections from the superior olive extend to the nucleus of the lateral lemniscus and to the torus, and some axons reach the posterior thalamus. The vestibular input is transmitted onto the vestibular nucleus complex with a remarkable specific convergence pattern, and a number of fundamental organization principles common to most vertebrates is found in the amphibian vestibular system (Straka and Dieringer, 2004). Ascending efferents from vestibular nuclei travel via the medial longitudinal fascicle to the cerebellum and to brainstem nuclei involved in oculomotor function. Projections from the lateral vestibular nucleus interconnect vestibular nuclei and reach tegmental nuclei, the nucleus of medial longitudinal fascicle and the anterior, central, and ventromedial thalamic nuclei (Matesz *et al.*, 2002). Neurons of the lateral line nucleus mainly project to the torus; in the aquatic toad *Xenopus*, the principal and magnocellular nuclei of the torus receive their major input from the lateral line nucleus (Edwards and Kelley, 2001).

11.3.2.2 Cerebellum Compared to that of most other vertebrates, the amphibian cerebellum is small, but exhibits the basic cerebellar circuitry typical of vertebrates. It is composed of the corpus cerebelli and the auricular lobes. The corpus cerebelli is the central part of the cerebellum and consists of a transverse plate, which contains a molecular and a granular layer; the Purkinje cells are aligned at the boundary between these two layers. The cerebellar nucleus, which is considered homologous to the deep cerebellar nuclei of mammals, is situated ventral to the corpus cerebelli. The granular layer contains the afferent fibers to and efferent fibers from the cerebellum. The mossy fiber-granule cell-parallel fiber system and the climbing fiber system are present and constitute excitatory input onto Purkinje cells. Somata of Purkinje cells and of stellate cells in the molecular layer are immunoreactive for GABA, and most of GABA-positive neurons in the granular layer appear to be Golgi cells. True basket cells are missing, stellate cells are fewer in number, and co-localization of GABA and glycine in Golgi neurons is encountered less frequently in frogs compared with mammals. In the bullfrog, Calbindin immunoreactivity (-ir) was observed in various populations of cells in the auricular lobe and interauricular granular band of the cerebellum, in the cerebellar peduncle, and in a bundle of interauricular commissural fibers. Cells in the granular layer of the ventral part (i.e., corpus cerebelli) of the cerebellar plate as well as fibers in the molecular layer of this region were not immunoreactive (Uray and Gona, 1999). The pattern of calbindin-ir in the auricular lobes and marginal part of the cerebellar plate differs distinctly in its origin, biochemistry, and connectivity from the corpus cerebelli.

The main input comes from the rhombencephalon and comprises fibers of the trigeminal and trochlear nerve, the vestibular nuclear complex, the glossopharyngeal-taste sensory system, the hypoglossal nerve (mediating sensory information of the tongue), the inferior olive, and primary and secondary afferents of the somatosensory system (Antal *et al.*, 1980; Amat *et al.*, 1984; Montgomery, 1988; Anderson and Nishikawa, 1997). The efferent cerebellar pathways extend to the lateral medulla oblongata and mainly reach the vestibular complex; they also descend to cervical and lumbar root fibers (Dicke *et al.*, 1998; Bacskai and Matesz, 2002). A small, distinct projection also reaches the ventral tegmentum at the level of the oculomotor nerve; reciprocal connections between the red nucleus and the cerebellum were described in frogs and salamanders (Montgomery, 1988; Naujoks-Manteuffel *et al.*, 1988; Larson-Prior and Cruce, 1992).

In *Rana pipiens*, a pathway from the hypoglossal motor nuclei to the cerebellar nucleus as well as an afferent projection from the peripheral hypoglossal nerve to the Purkinje cell layer of the cerebellar cortex was demonstrated by Anderson (2001). Anatomical convergence of these pathways in the medial reticular formation and a reciprocal connection between the trigeminal motor nuclei and the cerebellar nuclei as well as the medulla appear to be the anatomical basis for feeding reflex modulation. The neuronal circuitry for optokinetic responses includes both visual centers (thalamus, pretectum, BON) and the auricular lobe of the cerebellum (Fite *et al.*, 1992). In general, the cerebellum of amphibians, like that of other vertebrates, appears to be involved in sensorimotor integration and motor coordination.

11.3.3 Mesencephalon

11.3.3.1 Isthmic region and tegmentum The isthmic region (Figures 13c and 13d) is separated from the tegmentum by the sulcus isthmi, and the isthmic nucleus is situated ventral to the dorsocaudal end of the tegmentum, immediately rostral to the cerebellar corpus. The isthmic nucleus is a compact prominent nucleus; its dendrites extend laterally and form a conspicuous dendritic neuropil (see below for a more detailed description). The nucleus

visceralis secundarius, considered homologous to the parabrachial nucleus, is found dorsal to the isthmic nucleus, and neurons of the cholinergic laterodorsal tegmental nucleus are situated ventral to the nucleus isthmi (Marín *et al.*, 1997d). Neurons of the noradrenergic locus coeruleus are dispersed medially, ventrally, and/or caudally to the isthmic nucleus (Marín *et al.*, 1996). They have long processes directed ventrally or ventrolaterally and arborizing in the lateral reticular formation.

The tegmentum is divided into a dorsal and ventral tegmentum. The nucleus of the medial longitudinal fascicle is situated in the rostral dorsal tegmentum, while the dorsal tegmental nucleus is found throughout the rostrocaudal extent. The ventral tegmentum includes the oculomotor and trochlear motor nucleus and the accessory oculomotor nucleus Edinger–Westphal. The pedunculopontine tegmental nucleus is situated at the border of the dorsal and ventral tegmentum; its cholinergic part is present in frogs and plethodontid salamanders, but is absent in salamandrid salamanders (frogs and salamandrids: Marín *et al.*, 1997a; plethodontids: U. Dicke, unpublished data). The ventral tegmental nucleus is bordered by the ventrally located nucleus ruber, and the interpeduncular nucleus is situated in the median basal tegmentum.

The dorsal tegmental and the pedunculopontine tegmental nucleus have reciprocal connections with the tectum; neurons of the nucleus of the medial longitudinal fascicle and the nucleus ruber give rise to descending pathways to the medulla oblongata and rostral spinal cord (Naujoks-Manteuffel *et al.*, 1988; Dicke *et al.*, 1998). The interpeduncular nucleus receives olfactory input via the fasciculus retroflexus that descends from the habenula. Ascending and descending pathways of the brain run in the fiber layer of the dorsal and/or ventral tegmentum, and neurons of tegmental nuclei with their laterally directed dendrites are likely to receive input from a variety of brain regions. In general, the tegmental relay stations are poorly studied even though the tegmentum most likely constitutes a complex anatomical zone of interfaces, where the sensory, motor, and limbic systems of the brain meet.

11.3.3.2 Torus semicircularis The torus semicircularis is situated below the tectum. In frogs, it consists of three major auditory nuclei, the principal, laminar, and magnocellular nucleus (Potter, 1965). The laminar nucleus forms nearly a hemisphere and occupies the entire dorsal and rostral surface of the torus bordering the tectal ventricle (Figure 4). The somata of this nucleus are arranged in laminae (hence the name of the nucleus). The principal nucleus is situated caudally and ventrally of the laminar nucleus, which caudoventrally includes the magnocellular nucleus. In *Salamandra salamandra*, the subtectal dorsal tegmentum is divided into a dorsally located torus semicircularis and a ventrally situated dorsal tegmental nucleus. The torus of this species processes auditory and vibratory signals, and the hearing capabilities are comparable to those of anurans with extra-tympanic sound transmission (Manteuffel and Naujoks-Manteuffel, 1990).

The torus semicircularis is the center of convergence of ascending auditory (terminating primarily in the principal nucleus), vestibular (entire torus), somatosensory (lateral laminar nucleus), lateral line (if present, lateral principal nucleus) pathways and descending pathways from the forebrain, i.e., the central, ventromedial, and posterior thalamic nuclei, the anterior entopeduncular nucleus and suprachiasmatic nucleus terminating predominantly in the laminar and principal nucleus (Wilczynski, 1981; Feng and Lin, 1991; Matesz and Kulik, 1996; Edwards and Kelley, 2001). Weak descending afferents originate in the lateral septum and in the caudal striatopallidum (Marín *et al.*, 1997a; Endepols *et al.*, 2005).

Efferents from all toral nuclei run to the tectum, tegmentum, and isthmic nucleus; the principal nucleus projects to the posterior and central dorsal thalamic nuclei and the laminar and magnocellular nucleus to the telencephalon, predominantly to the striatopallidum (primarily the nucleus laminaris). All toral nuclei have descending projections to auditory brainstem nuclei (Neary, 1988; Feng and Lin, 1991; Luksch and Walkowiak, 1998; Endepols and Walkowiak, 2001).

The torus contains numerous neuromodulatory substances such as dopamine, bombesin, noradrenaline, enkephalin, substance P, somatostatin, neuropeptide Y, as well as steroid hormones (Endepols *et al.*, 2000). It contains neurons that either exhibit weak or no tonotopy and either simple or complex tuning curves (Walkowiak, 1980; Feng *et al.*, 1990). Whereas the majority of neurons can be driven by relatively simple auditory stimuli, some of them respond preferentially to complex sounds. In brief, the data underline the essential role of the torus semicircularis as the major audiomotor interface.

11.3.4 Neuroanatomy of the Retino-Tecto-Pretectal System

11.3.4.1 Retina The retina of amphibians, like that of other vertebrates, exhibits a typical five-layered

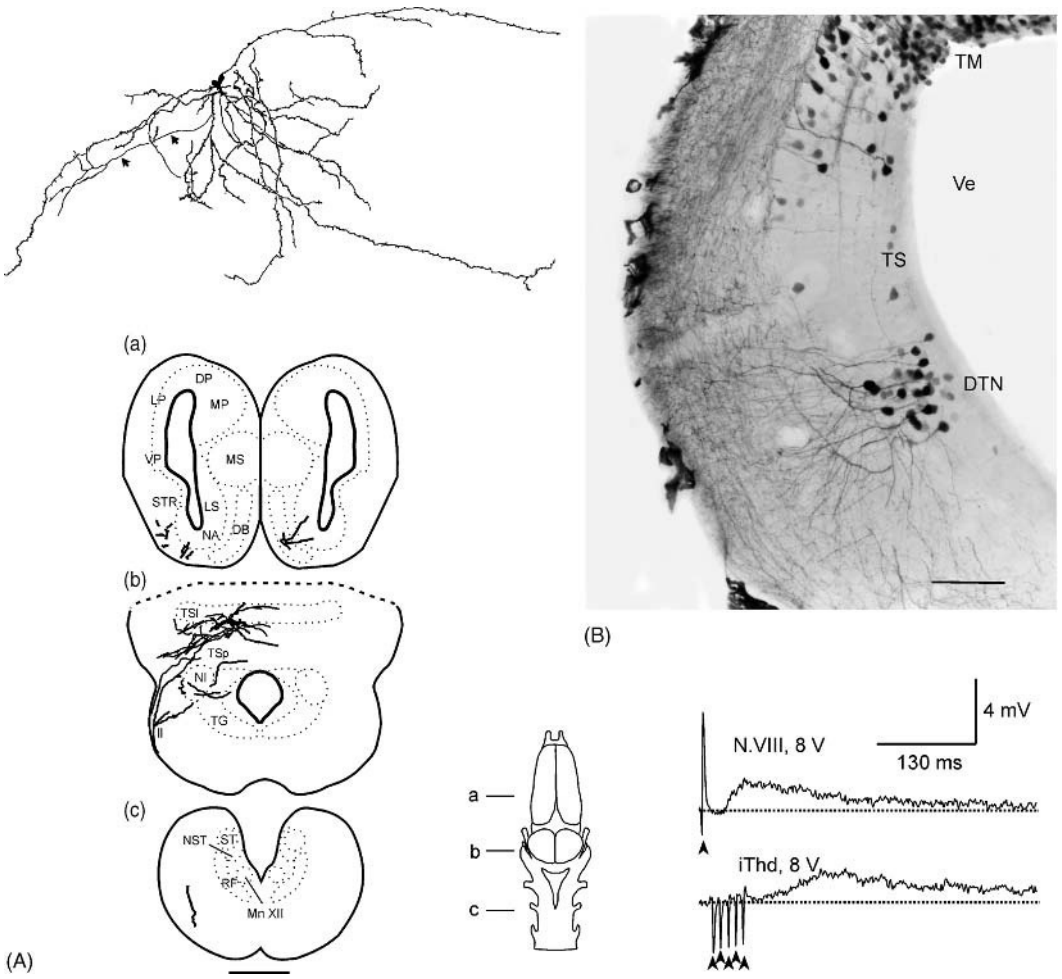

Figure 4 A, Reconstruction of two neurons in the torus of *Discoglossus pictus* recorded and subsequently labeled with neurobiotin. The reconstruction of the dendritic tree is shown at the top left; arrows point to the proximal part of an axon. Scheme of transverse sections (a c): a, telencephalon showing the termination sites of two axons in its ipsi- and contralateral ventral part; b, location of the two neurons in the caudal laminar nucleus of the torus; the dorsal part of the tectum is cut off (broken outline); c, one axon descends laterally in the fiber layer down to the level of the obex. Responses at single stimulation of the contralateral auditory nerve (VIII) and repetitive stimulation of the ipsilateral dorsal thalamus (iThd) are given at the bottom right. Scale bar: 500 μm. B, Microphotograph of dorsal tegmental neurons of *Plethodon teyahalee* retrogradely labeled after tracer application to the tectum and forming a band extending rostrocaudally throughout the tegmentum. DP, dorsal pallium; LP, lateral pallium; VP, ventral pallium; STR, striatum; NA, nucleus accumbens; DB, diagonal band; LS, lateral septum; MS, medial septum; MP, medial pallium; TSl, laminar nucleus of the torus semicircularis; TSp, principal nucleus of the torus semicircularis; NI, isthmic nucleus; TG, tegmentum; II, XII, cranial nerves; NST, nucleus of the solitary tract; RF, reticular formation; Mn, hypoglossal motor nucleus; TM, mesencephalic tectum; Ve, ventricle; TS, torus semicircularis; DTN, dorsal tegmental nucleus. Scale bar: 100 μm. A, Reproduced from *J. Comp. Physiol. A*, Vol. 186, 2001, pp. 1119 1133, Integration of ascending and descending inputs in the auditory midbrain of a nurons, Endepols, H. and Walkowiak, W. With kind permission of Springer Science and Business Media.

structure. The outer and the inner nuclear layer and the layer of retinal ganglion cells (RGCs) are separated by the outer plexiform layer and the much thicker inner plexiform layer. The two plexiform layers are the main site of synaptic contacts between the five major types of retinal cells (photoreceptors, amacrine cells, bipolar cells, horizontal cells, and ganglion cells). The outer nuclear layer contains the inner segments of the photoreceptors and their nuclei. In most frog and salamander species, the rod nuclei in the outer nuclear layer are aligned at the distal side, and the cone nuclei are more proximal (Gordon and Hood, 1976). This is the contrary of the situation in most other vertebrates, in which the rod nuclei are vitread to the cone somata. Amacrine cells are concentrated at the vitread side of the inner nuclear layer, bipolar cells in the middle, horizontal cells at the sclerad side, and a few displaced RGCs at the vitread side. In most amphibians, the layer of RGCs contains more than one row of cells, and

axons of the RGCs bundle and constitute the optic nerve.

Amphibians have no specialized intraretinal structure like the fovea of primates or birds. However, in the frogs *Hyla raniceps* (Bousfield and Pessoa, 1980), *Heleioporus eyrei* (Dunlop and Beazley, 1981), and *Bufo marinus* (Nguyen and Straznicky, 1989), a streak of high cell density exists in the RGC layer along the nasotemporal meridian of the retina. The same is found in the inner nuclear layer (Zhu *et al.*, 1990) and the outer nuclear layer (Zhang and Straznicky, 1991). The increase in cell density is comparable to that of the visual streak in the reptilian retina (Wong, 1989; Wilhelm and Straznicky, 1992). In salamanders, differences in intraretinal cell density have not been found so far.

In plethodontid salamanders (Linke and Roth, 1989), four types of RGCs have been identified, while in frogs the number of RGCs varies among three major types (with 12 subtypes based on morphology of dendritic trees) in *Xenopus laevis* (Straznicky and Straznicky, 1988) and seven types in *R. pipiens* (Frank and Hollyfield, 1987).

The optic nerve contains myelinated and unmyelinated fibers. The highest number of optic nerve axons (and thus of RGCs) are found in anurans. In *Rana pipiens*, 470 000 fibers were counted, and the lowest number presently known among anurans was found in *X. laevis* with 68 000–80 000 fibers (Maturana, 1959; Dunlop and Beazley, 1984). On average, salamanders have 5–10 times fewer optic fibers. They range from 26 000 in *Batrachoseps attenuatus* (Linke and Roth, 1990) to 75 000 in the salamandrid *Notophthalmus viridescens* (Ball and Dickson, 1983). The percentage of myelination in adult amphibians is low compared to that of mammals; in *X. laevis*, the percentage of myelination is 11% (Dunlop and Beazley, 1984), whereas the lowest percentage is found in the plethodontid salamander, *B. attenuatus*, with less than 1% myelinated fibers (Linke and Roth, 1990).

11.3.4.2 Visual afferents to the brain The majority of fibers of the optic nerve cross in the optic chiasm and reach targets in the opposite diencephalon and mesencephalon (frogs: Lázár and Székely, 1969; Fite and Scalia, 1976; Montgomery and Fite, 1989; salamanders: Fritzsch, 1980; Rettig and Roth, 1986). Ipsilaterally projecting RGCs in *R. pipiens* include not more than 2.3% of the overall population in the ganglion cell layer and exist in the monocular as well as binocular parts of the retina (Singman and Scalia, 1990, 1991). Within the optic chiasm, a sequence of positional transformations occurs that result in the formation of multiple optic pathways (Montgomery *et al.*, 1998).

Staining of retinal afferents in frogs and salamanders reveals four thalamic neuropils: the neuropil of Bellonci (NB), the corpus geniculatum thalamicum (CGT) (Figures 5 and 6), the preoptic area, and the posterior thalamic neuropil. The latter neuropil is divided into a laterally situated pretectal neuropil and a medially situated uncinate field; in frogs the presence of such a division was reported in a study on *Rana* (Fite and Scalia, 1976). In the mesencephalon, the superficial and part of the deeper fiber layers of the tectum receive extensive visual afferents (Figure 7). A smaller number of RGCs projects to the basal optic neuropil (BON) situated in the tegmentum rostral to the root of the third cranial nerve. In salamanders, the neuropil of Bellonci can be clearly divided into a medial part and a lateral part (Figure 6) (Fritzsch, 1980; Rettig and Roth, 1986; Wiggers, 1999), and plethodontid salamanders have a substantial number of ipsilaterally projecting RGCs compared to other urodeles and anurans (cf. Figure 7a).

11.3.4.3 Organization of retinal projections In amphibians, the projection of RGCs is topographically organized onto diencephalic and mesencephalic targets. In the diencephalon, the topography of projections appears to differ in frogs and salamanders (Rettig and Roth, 1986; Montgomery and Fite, 1989; Montgomery *et al.*, 1998).

In frogs, contralateral projections of the retina distribute as follows: the anterior CGT, NB, and pretectal neuropils receive afferents from the ventral and nasal quadrants of the retina. Axons from the ventral quadrant terminate in the dorsal and those from the nasal quadrant terminate in the ventral portion of the thalamic targets. In the posterior CGT, NB, and pretectum, retinal axons of the temporal and dorsal quadrants terminate in the dorsal and ventral portion of the targets, respectively. The rostral and central tectum receives retinal afferents from the temporal quadrant, the medial tectum is reached by retinal axons of the ventral quadrant, and afferents from the dorsal and nasal quadrant of the retina project to the lateral and caudal tectum, respectively. The BON obtains afferents from the entire retina; the major retinal projection is contralateral, but a small, ipsilateral component was described in *R. pipiens* (Montgomery *et al.*, 1981). The basal optic root consists of a lateral and a medial fascicle. In *R. pipiens*, the lateral fascicle innervates the entire terminal field of the BON, while the medial fascicle innervates only the central and mediodorsal portions. The ventrolateral portion of the BON is innervated only by the lateral

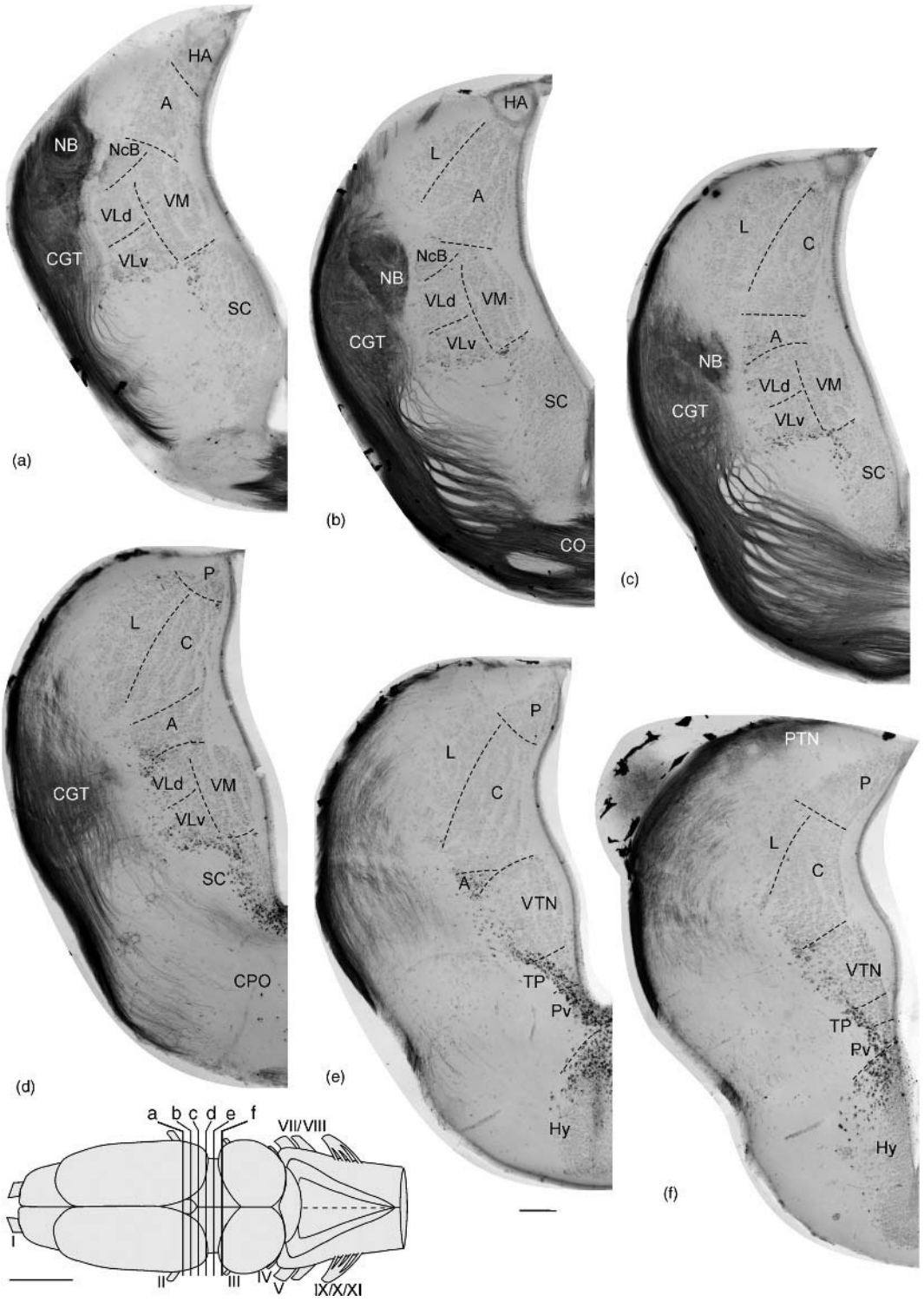

Figure 5 Microphotographs of transverse sections through the diencephalon of *Bombina orientalis* showing retinofugal neuropils (NB and CGT) revealed by anterograde tracing after application of biocytin to the optic nerve. Sites of sections are indicated in the inset. I, II, III, IV, V, VII, VIII, IX, X, XI, cranial nerves; CPO, postoptic commissure; HA, habenula; A, anterior dorsal thalamic nucleus; NB, neuropil of Bellonci; NcB, nucleus of Bellonci; VM, ventromedial thalamic nucleus; VLv, ventral portion of the ventrolateral nucleus; SC, suprachiasmatic nucleus; CGT, corpus geniculatum thalamicum; L, lateral dorsal thalamic nucleus; CO, optic chiasm; C, central dorsal thalamic nucleus; P, posterior dorsal thalamic nucleus; VTN, ventral thalamic nucleus; TP, posterior tubercle; Pv, paraventricular organ; Hy, hypothalamus; VLd, dorsal portion of the ventrolateral nucleus. Scale bar: 100 μm. From Roth, G., Grunwald, W., and Dicke, U. 2003. Morphology, axonal projection pattern and responses to optic nerve stimulation of thalamic neurons in the fire-bellied toad *Bombina orientalis*. *J. Comp. Neurol.* 461, 91 110.

fascicle and the medial region by both fascicles (Fite *et al.*, 1988).

In salamanders, contralateral projections of RGCs are arranged such that afferents from the nasal quadrant terminate in thalamic and pretectal neuropils, while those from the ventral quadrant reach the medial portion of the CGT, lateral NB, and pretectal neuropils. RGCs in the dorsal quadrant of the retina project to the lateral portion of the CGT, lateral NB, and pretectum; in the latter, the entire uncinate field is reached. Afferents originating from RGCs of the temporal retina terminate in the caudal portions of the CGT and lateral NB, in the entire medial NB, the caudal pretectal neuropil and the entire uncinate field. The topography of retinal projections to the contralateral tectum is identical with that found in frogs. Also, the BON receives afferents from all quadrants of the retina. Ipsilateral projections of RGCs reach the rostral tectum; more extensive projections are found in bolitoglossines (Rettig and Roth, 1986). The RGCs project ipsilaterally to all targets in the thalamus and pretectum, but the topic arrangement has not been investigated with modern tracers, which label neuronal structures more intensely.

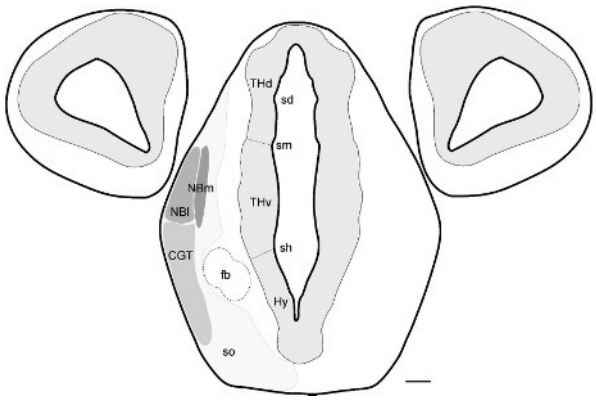

Figure 6 Drawing of a transverse section through the middiencephalon of *Plethodon jordani* showing the sites of retinofugal neuropils (NB pars medialis and pars lateralis and CGT). THd, dorsal thalamus; sd, dorsal thalamic sulcus; sm, medial thalamic sulcus; NBm, neuropil of Bellonci, medial part; NBl, neuropil of Bellonci, lateral part; CGT, corpus geniculatum thalamicum; fb, forebrain bundle; so, stratum opticum; Hy, hypothalamus; sh, hypothalamic sulcus; THv, ventral thalamus. Scale bar: 100 μm. Modified from Roth, G. and Grunwald, W. 2000. Morphology, axonal projection pattern and responses to optic nerve stimulation of thalamic neurons in the salamander *Plethodon jordani*. *J. Comp. Neurol.* 428, 543 557.

11.3.4.4 Projection specificity of retinal ganglion cells and morphology of terminal arbors In amphibians, the morphology of single terminal arbors was studied by means of intracellular labeling of RGCs and anterograde staining of the optic nerve (frogs: Stirling and Merrill, 1987; Hughes, 1990; salamanders: Wiggers, 1999). Axons of RGCs often have multiple terminal structures in the tectum and in the thalamic neuropils, while projections to the BON appear to originate from another type of RGC in plethodontid salamanders (Wiggers, 1999).

In frogs, terminal arbors of intracellularly HRP-labeled RGCs responding to the extinguishing of light were situated in the deep tectal fiber layers with a size of 400 and 200 μm in rostrocaudal and mediolateral axis, respectively. The axon gives off few branches to the pretectum before entering the tectum (Stirling and Merrill, 1987). In the Hughes (1990) study, HRP-labeled axons of RGCs were found in all superficial tectal fiber layers, but the

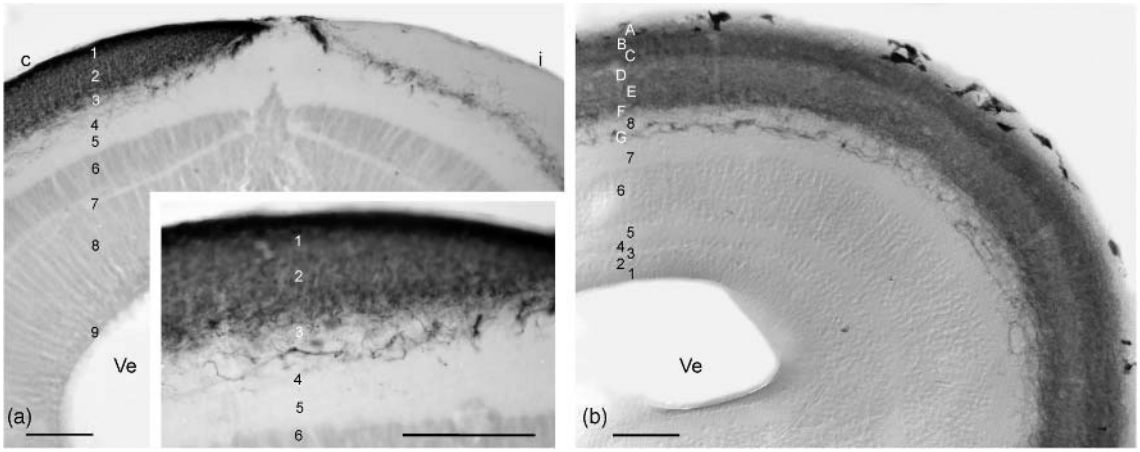

Figure 7 Microphotographs of transverse sections through the tectum of *Plethodon jordani* (a) and *Bombina orientalis* (b) showing retinal afferents after application of biocytin to the stump of the optic nerve. In (a), the contralateral afferents to the superficial tectal layers are shown on the left side and the ipsilateral afferents, mostly restricted to layer 3, on the right side. The inset shows the contralateral afferents at greater magnification. In (b), the contralateral afferents forming laminae A G of layer 9 are shown. Ve, ventricle. Scale bars: 100 μm.

extent and morphology of arbors differed from layer to layer. Small and dense arbors with thin and beaded fibers were found in the superficial layer, whereas in the deep tectal fiber layer large arbors with sparse branching were labeled.

In a study on intracellularly biocytin-labeled RGCs of plethodontid salamanders by Wiggers (1999), the following types of projection pattern were frequently found:

1. RGCs with large terminal arbors and dense branching in thalamic neuropils (mainly in the medial NB) project to the deep tectal fiber layers with axons that are sparsely beaded and reveal no obvious terminal structures; pretectal neuropils are formed by sparse fields of axon collaterals.
2. RGCs with dense terminal arborization in the pretectal neuropils have additional sparse fields of collaterals in the thalamus, especially in the CGT and lateral NB; a projection to tectal fiber layers was not found.
3. RGCs with axons forming dense terminal arbors in the superficial fiber layer of the tectum reveal only few beads in the pretectal neuropils; neuropils in the thalamus were not found.
4. RGCs that have dense terminal fields in one of the two layers underneath the superficial tectal layer reveal additional sparse terminals in thalamic and pretectal neuropils. Axons of RGCs form terminals with moderate or sparse branching of collaterals in the CGT and in the lateral NB.

11.3.4.5 Cytoarchitecture of the tectum mesencephali In the tectum of frogs, nine layers are distinguished beginning from the ventricle (Potter, 1969) (cf. Figures 7b and 8a). Layer 1 contains ependymal glial cells with long processes extending toward the tectal surface, where they form the external limiting membrane. Cellular layers 2, 4, and 6 (stratum griseum periventriculare) together constitute the periventricular gray matter. These cellular layers are divided by deep fiber layers 3 and 5, consisting of unmyelinated afferent and efferent fibers and basal dendrites of the periventricular neurons. Fiber layer 7 (stratum album centrale) contains the bulk of efferent tectal fibers and a few scattered neurons. Layer 8 (stratum griseum centrale) consists of loosely arranged neurons embedded in a meshwork of dendrites of tectal neurons and afferent fibers. Layer 9 (stratum fibrosum et griseum superficiale + stratum opticum) contains relatively few neurons dispersed in the meshwork of retinal afferents and dendrites of tectal neurons. Layer 9 is further divided into seven laminae A–G. Lamina A (occurring only in the rostral tectum) and laminae B, D, and F plus lamina G in layer 8 contain myelinated and unmyelinated fibers (mostly retinal afferents), and C and E are cellular layers.

The tectum of salamanders, like that of caecilians and lepidosirenid lungfishes (Northcutt, 1977), shows an essentially two-layered structure consisting of a periventricular cellular layer and a superficial white matter consisting of dendrites of tectal neurons and tectal afferent and efferent fibers, in which only a few migrated neurons are dispersed. However, based on

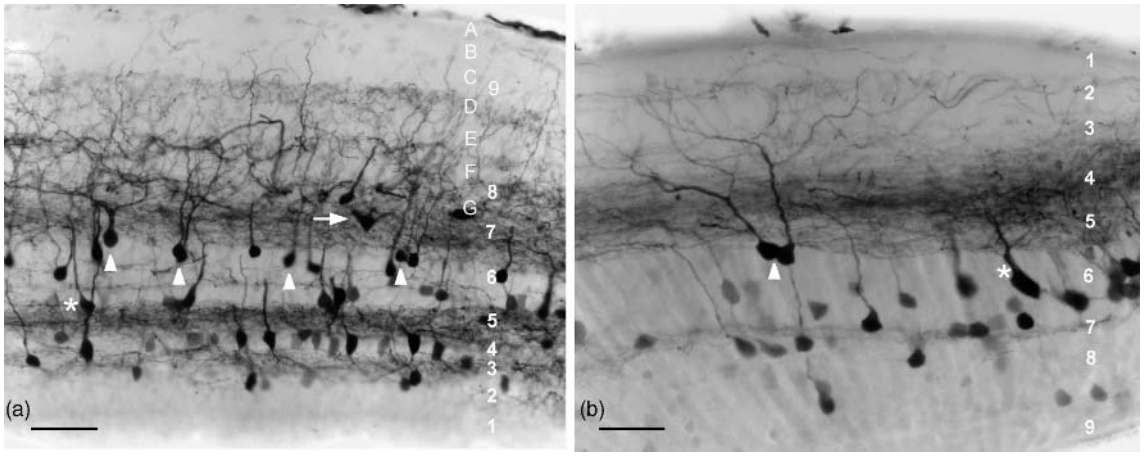

Figure 8 Microphotographs of tectal neurons type 2, 3, and 5 in *Discoglossus pictus* (a) and *Plethodon jordani* (b) labeled after injection of biocytin into the lateral medulla oblongata. The dendritic trees of neurons arborize in the middle layer of retinal afferents (arrowheads point to somata of the homologous type 3 of frogs and type 2 of salamanders) or in the deep, nonretinal afferent layers (asterisk indicates soma of type 5 in frogs and type 3 in salamanders). In (a), the arrow points to a spindle-shaped soma in layer 7; this type of neuron (type 2 of frogs) is only present in the frog tectum. Axons of the different types of neurons constitute the lateral uncrossed tectobulbospinal tract, and give rise to a nontopographic tectothalamic projection. From Dicke, U. and Roth, G. 1996. Similarities and differences in the cytoarchitecture of the tectum of frogs and salamanders. *Acta Biol. Hung.* 47, 41 59.

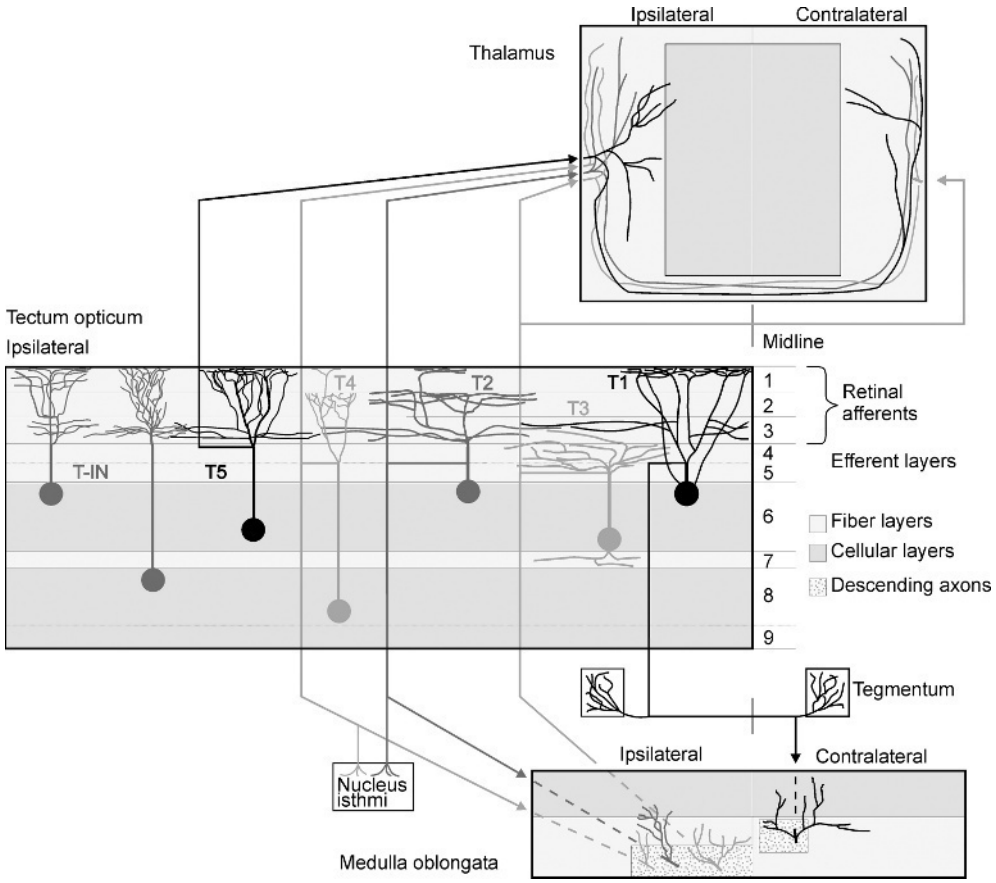

Figure 9 Schematic diagram of ipsilaterally and contralaterally ascending and descending tectofugal pathways constituted by different types (T1 T5) of neurons in salamanders. For further explanation see text. From Roth, G., Dicke, U., and Grunwald, W. 1999. Morphology, axonal projection pattern and response types of tectal neurons in plethodontid salamanders. II: Intracellular recording and labeling experiments. *J. Comp. Neurol.* 404, 489 504.

tracer experiments, the salamander tectum is divided from the surface to the ventricle into nine layers (Roth, 1987) (Figures 7a, 8b, and 9). Layers 1–3 contain retinal afferent fibers as well as afferents from other visual centers such as pretectum, thalamus, and isthmic nucleus; layers 4 and 5 contain efferent fibers and afferents from other senses, e.g., somatosensory, vestibular, and lateral line (if present). Layer 6 consists of the superficial cellular layer, while layer 7 (absent in miniaturized plethodontid salamanders) contains deep unmyelinated fibers. Layer 8 is the deep cellular layer, and layer 9 contains periventricular ependymal (glial) cells. In some salamanders, for example *Ambystoma mexicanum*, the periventricular gray matter regionally exhibits two to three sublayers.

On the basis of data from tracer studies on neuronal types (see below), tectal layers in salamanders and frogs can be homologized. Periventricular cellular and fiber layers 6–8 in salamanders are homologous to periventricular layers 2–6 in frogs, with cellular layer 6 being the most superficial of the periventricular layers in both groups. Dorsally, layer 6 is followed by the main efferent fiber layer(s), layers 4 and 5 in salamanders and layer 7 in frogs. Whereas in salamanders, only a few migrated neurons are found in the superficial part of the tectum (layers 1–3), in frogs such cells form a cellular band in layer 8 and are loosely arranged in layer 9. Retinal afferents terminate in layers 1–3 in salamanders and in laminae A–G of layers 8 and 9 in frogs.

11.3.4.6 Morphology and location of neuron types in the tectum A comparison of the morphology of dendritic trees of projection neurons and their targets in frogs and salamanders reveals that in both orders the same set of tectal projection neurons exists (frogs: Lázár *et al.*, 1983; Antal *et al.*, 1986; Dicke and Roth, 1996; salamanders: Roth *et al.*, 1990, 1999; Dicke and Roth, 1996; Dicke, 1999) (cf. Figures 8, 10, and 11). Types with descending axons are presented first and are ordered by their type of arborization from the superficial to the deeper fiber layers.

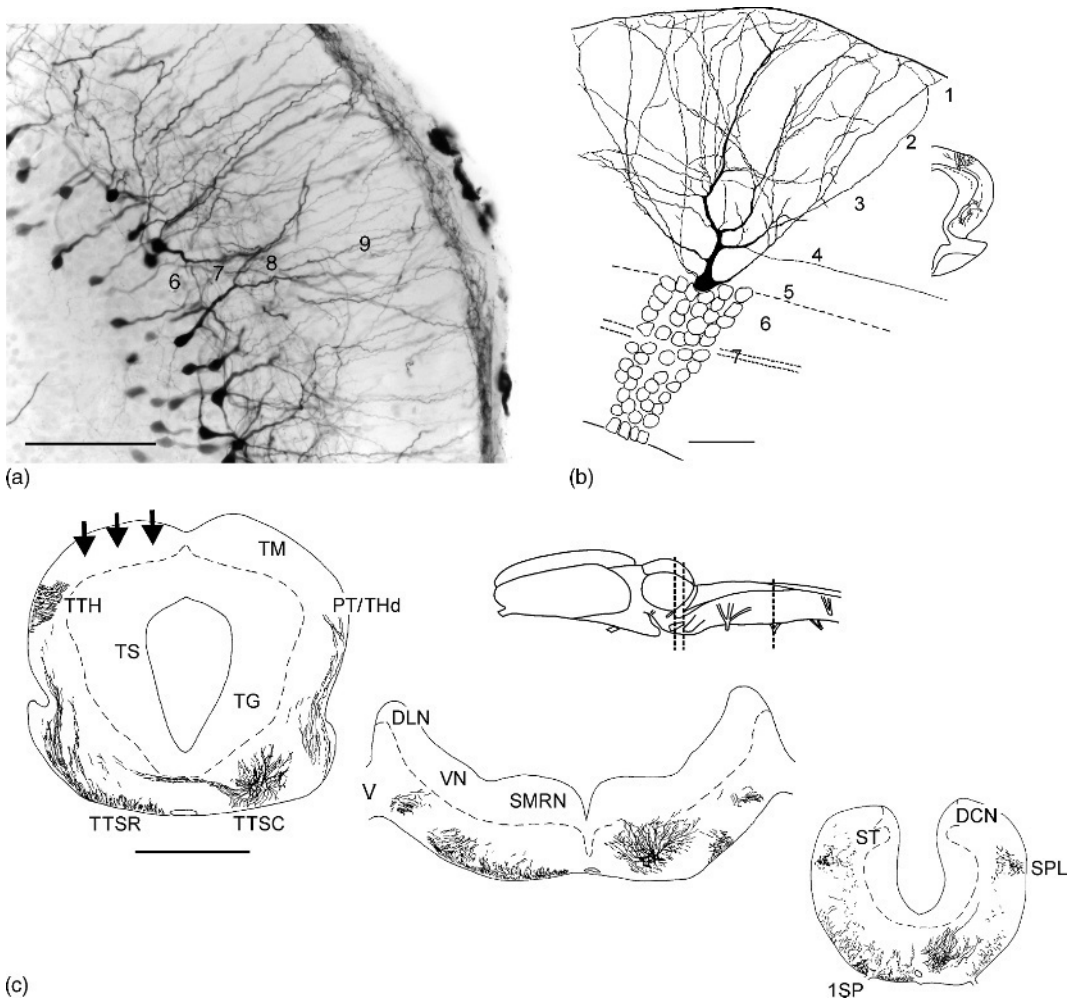

Figure 10 a, Microphotograph of the tectum of *Discoglossus* showing type-1 neurons labeled after application of biocytin into the medial medulla oblongata and projecting to the contralateral medulla oblongata. b, Camera lucida drawing of a type-1 neuron in *Plethodon jordani* labeled after application of biocytin into the medial medulla oblongata and projecting to the contralateral medulla oblongata. c, Drawing of descending tectobulbospinal tracts at levels indicated in the inset (tectum, level of Vth cranial nerve and 1st spinal nerve). Black arrows indicate the site of tracer application. 1, 2, 3, 4, 5, 6, 7, 8, 9, ventricles; TM, mesencephalic tectum; TTH, tecto-thalamic tract; TS, torus semicircularis; TTSR, uncrossed tecto-bulbo-spinal tract; TTSC, crossed tecto-bulbo-spinal tract; TG, tegmentum; PT/THd, descending tract of pretectum and thalamus; DLN, dorsolateral nucleus; VN, vestibular nucleus; SMRN, superior middle reticular nucleus; 1SP, first spinal nerve; SPL, spinal lemniscus; DCN, dorsal column nucleus. Scale bars: 100 μm in (a, b) and 500 μm in (c). Modified from Dicke and Roth (1996); Dicke (1999), Roth *et al.* (1999).

In frogs, type-1 neurons with large pear-shaped or pyramidal somata situated in layer 6 and occasionally in layers 7 and 8 have candelabrum-shaped dendritic trees that arborize predominantly in lamina A. They closely resemble type-1 neurons of salamanders with somata situated in the superficial cellular layer 6 or in efferent layers 4 and 5; their likewise candelabrum-shaped dendritic trees extend into layers 1–3. Axons descend contralaterally to the medulla, where they constitute the crossed tectobulbospinal tract (Figures 10a and 10b).

Type-2 and type-3 neurons of frogs have the same pattern of descending and ascending axonal projections, but the former type of cells have spindle-shaped somata situated in or immediately above the large efferent layer 7 (Figure 8a). Two to several thick dendrites originate directly from the soma and branch into thick secondary dendrites, which extend obliquely to the surface. Smaller dendrites either terminate in lamina F and less frequently in laminae B and D or terminate in lamina B. This type of tectal neuron, with ipsilaterally descending axons, was not found in salamanders.

Type-3 neurons (pear-shaped or pyramidal cells) in frogs and type-2 neurons in salamanders (Figures 8a and 8b) can be regarded as homologous, because their somata are situated in the superficial layer of the periventricular gray. Both types have

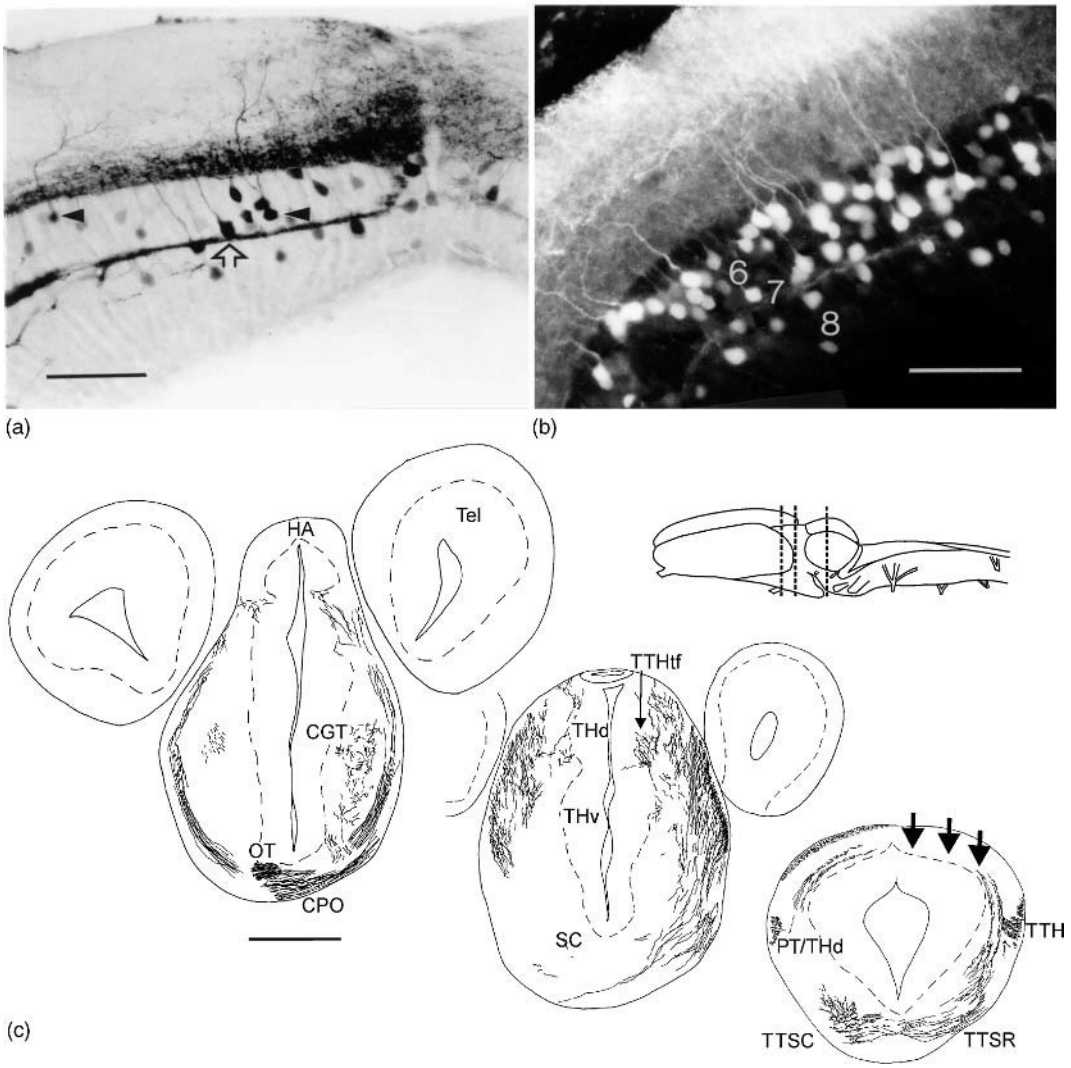

Figure 11 a, b, Microphotographs of transverse section showing tectal neurons labeled after tracer application to the postoptic commissure in *Plethodon jordani*. a, Type-3 tectal neuron (open arrow) and type-5 tectal neuron (black arrowhead) ipsilateral to the application site of biocytin. b, Type-5 neurons constituting the bulk of the ascending tectothalamic tract labeled ipsilateral to the application site of tetramethylrhodamine. c, Drawings of the ascending tectothalamic tract at levels indicated in inset. 6, 7, 8, ventricles; HA, habenula; CGT, corpus geniculatum thalamicum; OT, optic tract; CPO, postoptic commissure; Tel, telencephalon; TTHtf, terminal fields of tecto-thalamic tract; THd, dorsal thalamus; THv, ventral thalamus; SC, suprachiasmatic nucleus; PT/THd, descending tract of pretectum and thalamus; TTSC, crossed tecto-bulbo-spinal tract; TTSR, uncrossed tecto-bulbo-spinal tract; TTH, tecto-thalamic tract. Scale bars: 100 μm (a, b), and 500 μm (c). Modified from Dicke, U. 1999. Morphology, axonal projection pattern, and response types of tectal neurons in plethodontid salamanders. I: Tracer study of projection neurons and their pathways. *J. Comp. Neurol.* 404, 473 488.

wide to very wide dendritic trees, which arborize predominantly in the deeper retinorecipient laminae (lamina C or D in the frog, and layers 2 and 3 in the salamander tectum). In frogs, the somata are located in periventricular layers 2 and 4, a larger number in layer 6, and fewer in layers 7 and 8. In salamanders, somata are found in the upper part of layer 6. The axons of this type descend ipsilaterally, some of them forming contacts with the isthmic nucleus; in the medulla, axons run in a ventrolateral superficial position. They constitute the lateral part of the uncrossed tectobulbospinal tract. Axons ascend to the ipsi- and contralateral thalamus.

Type-5 neurons (pear-shaped cells) of frogs and type-3 neurons of salamanders can be considered homologous, because their somata are usually situated in the deeper part of the periventricular gray (Figures 8a and 8b). Their dendritic tree is flat and T-shaped and mostly confined to the efferent fiber layers (layer 7 in frogs, layers 4 and 5 in

salamanders). With their descending axons they either contribute to the lateral part of the uncrossed tectobulbospinal tract or constitute its medial part; they have ascending ipsilateral or bilateral projections to the pretectum and thalamus.

In frogs, the somata of the type-4 neuron (pear-shaped) are located in the deep cellular layer; their slender primary dendrite arborizes in lamina C. They resemble the rarely labeled type-4 neurons of salamanders with narrow dendritic trees arborizing predominantly in layer 2. Axons of this type descend ipsilaterally to the ventrolateral part of the rostral medulla oblongata; other axons or axon collaterals ascend to the ipsilateral thalamus.

In the frog tectum, cells with small pear-shaped somata situated in layer 8 and slender dendritic trees and arborizing either in lamina B or D were identified as rostrally projecting cells. They strongly resemble the slender type-5 neurons found in salamanders (Figures 11a and 11b). The somata of the latter are situated in layer 6 and less often in 8; the narrow dendritic trees arborize in fiber layer 1 or 2. Axons only ascend ipsi- and/or contralaterally to the pretectum and thalamus.

Interneurons are similar in the salamander and frog tectum. Their pear-shaped somata are situated mainly in the deeper part of the periventricular gray matter, and their dendritic trees are mostly very slender (Figure 9). The dendritic trees arborize in different retinorecipient laminae of the tectum. However, because of a much higher degree of cell migration in the anuran compared to the urodele tectum, in frogs many interneurons, i.e., small pear-shaped cells, occupy a superficial position. During ontogeny of the frog tectum, these neurons (like other tectal cells) originate in the periventricular germinal zone and then migrate toward the surface. In salamanders, this late ontogenetic migration process is either strongly reduced or completely abolished, with the consequence that cells remain within the periventricular cellular layer 6 (Schmidt and Roth, 1993).

11.3.4.7 The number of tectal neurons The salamander tectum comprises on average 100 000 cells; frogs possess 2–17 times more tectal cells (Roth *et al.*, 1995). The bulk of them is formed by interneurons; projection neurons make up roughly 5% independent of the absolute number of neurons (Dicke, 1999).

11.3.4.8 Nonretinal afferents The tectum mesencephali of amphibians, like that of other vertebrates, is a center for multisensory integration. It receives afferents from the thalamus and pretectum, which are also targets of primary visual afferents. In frogs, thalamic neurons projecting to the tectum were described recently in *Bombina* (Roth *et al.*, 2003). They are located in the dorsal portion of the ventral nucleus, the NB, and the central and posterior dorsal nucleus. Most of these neurons are characterized by a dendritic tree oriented dorsolaterally, sometimes also ventrolaterally entering the NB or the CGT neuropils. Their axons terminate in layer 7 of the tectum, the layer of tectal efferents. They are either restricted to the medial portion or extend throughout that layer and form collaterals. In the salamander *Plethodon*, the situation corresponds with that found in *Bombina* (Roth and Grunwald, 2000). Two groups of cells send axons to the tectum: one group of cells has its somata in the posterior dorsal thalamus around the sulcus medialis (corresponding to the central dorsal and posterior dorsal nucleus of frogs) and possesses a wide and flat dendritic tree that arborizes mostly in the medial white matter; dorsalmost dendrites reach the lateral surface. The other group consists of cells with somata in the ventral thalamus (ventral nucleus and NB in frogs), which forms relatively narrow dendritic trees extending laterally or ventrolaterally toward the surface. Neurons of the latter group constitute two nuclei, one at the level and another one ventrally to the sulcus medialis (Dicke, unpublished data) (Figure 12). In the three groups, terminals in the tectum are confined mostly to deeper tectal fiber layers 3–5 within the medial, intermediate, or lateral zone of the tectum. Furthermore, neurons of the pretectum project to the tectum (Marín *et al.*, 1997b); in salamanders, axons run ipsilaterally in the deep fiber layers of the tectum (Luksch *et al.*, 1998). Finally, afferents originate from a small number of neurons in the contralateral tectum.

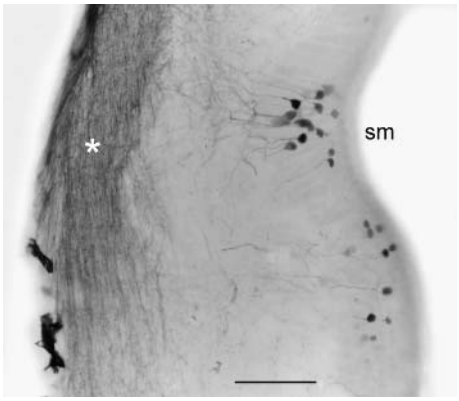

Figure 12 Microphotograph of ipsilaterally labeled ventral neurons in the mid thalamus of the salamander *Plethodon teyahalee* after application of biocytin to the tectum. Medial is to the right, dorsal is to the top. Asterisk indicates anterogradely labeled axons of tectal neurons as well as retinofugal axons of retinal ganglion cells that collateralize and run to the tectum and thalamus. Scale bar: 100 μm. sm, medial thalamic sulcus.

Neurons of the vomeronasal amygdala, dorsal tegmentum, isthmic nucleus, and medulla oblongata and spinalis project into tectal fiber layers; only few striatal neurons project to the deep fiber layers of tectum (Marín *et al.*, 1997c; Roth *et al.*, 2004). In *Rana*, there are mainly indirect connections of the striatum to the tectum constituted by pathways via the amygdala/entopecuncular nucleus, pretectum, and tegmentum (Marín *et al.*, 1997b; see also below).

A substantial tectal innervation arises in the dorsal tegmental nucleus, which projects ipsilaterally (entire nucleus) and contralaterally (only the posterior portion) to the tectum (Figure 4b); there is no information on the site of termination inside the tectum. In addition to retinal afferents from the retina, in amphibians as well as in other vertebrates, a major input to the superficial tectum originates in the isthmic nucleus (homologous to the parabigeminal nucleus in mammals). In frogs, neurons in the dorsal part of this nucleus project to the ipsilateral fiber layers 9 and 8 and those of the ventral part to the superficial contralateral fiber layer (Gruberg and Udin, 1978; Gruberg *et al.*, 1994). In salamanders, two subnuclei were likewise reported in a double-labeling study of Wiggers and Roth (1991), but intracellular labeling revealed that the majority of isthmic neurons project to both tectal hemispheres (Wiggers, 1998). However, an ipsilateral projection to several retinorecipient layers and a contralateral projection to only the superficial layer of retinal afferents is commonly found in all vertebrate tecta. The isthmotectal projection is topographically ordered and in register with the retinal map (Gruberg and Lettvin, 1980; Gruberg *et al.*, 1989; Wiggers 1998).

In amphibians as well as in all vertebrate species studied so far, neurons of the isthmic nucleus comprise acetylcholine as a major transmitter and are the principal source of cholinergic input to the tectum (Ricciuti and Gruberg, 1985; Wallace, *et al.*, 1990; Marín *et al.*, 1997a; Marín and González, 1999). In plethodontid salamanders and in the caecilian, *Dermophis mexicanus*, part of the cholinergic input to the tectum stems from tectal cells revealing ChAT-like immunoreactivity (-ir); their dendritic trees also reach superficial fiber layers (Wallstein and Dicke, 1998; Gonzalez *et al.*, 2002) (Figure 13). In frogs, the isthmic nucleus also contains GABA-ir somata, but the distribution varies among species. GABA-ir cells are evenly distributed in the isthmic nucleus of *R. pipiens* (Li and Fite, 1998), while in *Rana esculenta* the majority of them are found in the anterior one-third of the nucleus (0.5% of the total population), and a meshwork of GABA-immunostained fine-beaded axons fills the entire isthmic nucleus (Pollak *et al.*, 1999). Based on lesion experiments, it is assumed that the majority of GABA-positive fibers derives from local GABA-positive cells and the rest from tegmental GABAergic cells. In *Rana catesbeiana*, staining of GABA-ir cells is moderate to dense in the anterior and posterior part of the nucleus and absent in the central part, and the isthmic nucleus is sparsely GABA-immunoreactive in *X. laevis* (Hollis and Boyd, 2005).

Afferents from the medulla to the tectum include the auditory dorsal nucleus, the vestibular nucleus, the middle reticular nucleus and the raphe nuclei of the rostral medulla oblongata as well as nuclei of the medulla oblongata and spinal cord that mediate somatosensory information. Axons from sensory nuclei mainly innervate the contralateral tectum and run in the deep fiber layers containing efferent fibers of tectal neurons (layers 7 in frogs, and 4 and 5 in salamanders), whereas the majority of axons of the reticular nucleus ascend to the ipsilateral tectum and extend in all deep fiber layers of the tectum (3, 5, 7 in frogs; 3–5, 7 in salamanders). The distribution of transmitters in the nuclei with ascending projection was recently studied in the salamander, *Plethodon* (Landwehr and Dicke, 2005). Projection neurons of the dorsal and vestibular nucleus are glutamate-ir, and in the latter nucleus often reveal additional GABA- and/or glycine-ir. Projection neurons of the middle reticular nucleus reveal predominantly gly-ir, often co-localized with glu-ir; this nucleus appears to be homologous to the mammalian gigantocellular reticular nucleus (Figure 14b).

In salamanders, the serotonergic raphe nuclei strongly innervate the retinorecipient layers 2 and 3, and some axons of the raphe nuclei reach the efferent fiber layers 4 and 5 (Dicke *et al.*, 1997) (Figure 14a). In *Rana*, 5-HT is located in tectal layers 3, 5, 6, 7, and 9 (Liu and Debski, 1995), but the source of serotonergic innervation differs from that found in salamanders. The 5-HT-ir cells are labeled in cellular layers 2, 4, and 6 of the tectum and are assumed to contribute to the serotonergic innervation of the deeper fiber layers. The distribution of serotonergic neurons is most prominent in the lateral tectum and decreases significantly medially, but is largely constant in the rostrocaudal dimension (Debski *et al.*, 1995). The 5-HT-ir fibers in lamina A of layer 9 are mainly of retinal origin, while serotonergic fibers in the other laminae most likely originate from neurons in the midbrain tegmentum and the isthmic nucleus. The raphe nuclei of the reticular formation of the medulla project nontopographically to midtectal layers (Zhao and Debski, 2005). Neurons of the DCN and LCN situated in the caudal medulla oblongata and cervical spinal cord receive predominantly somatosensory

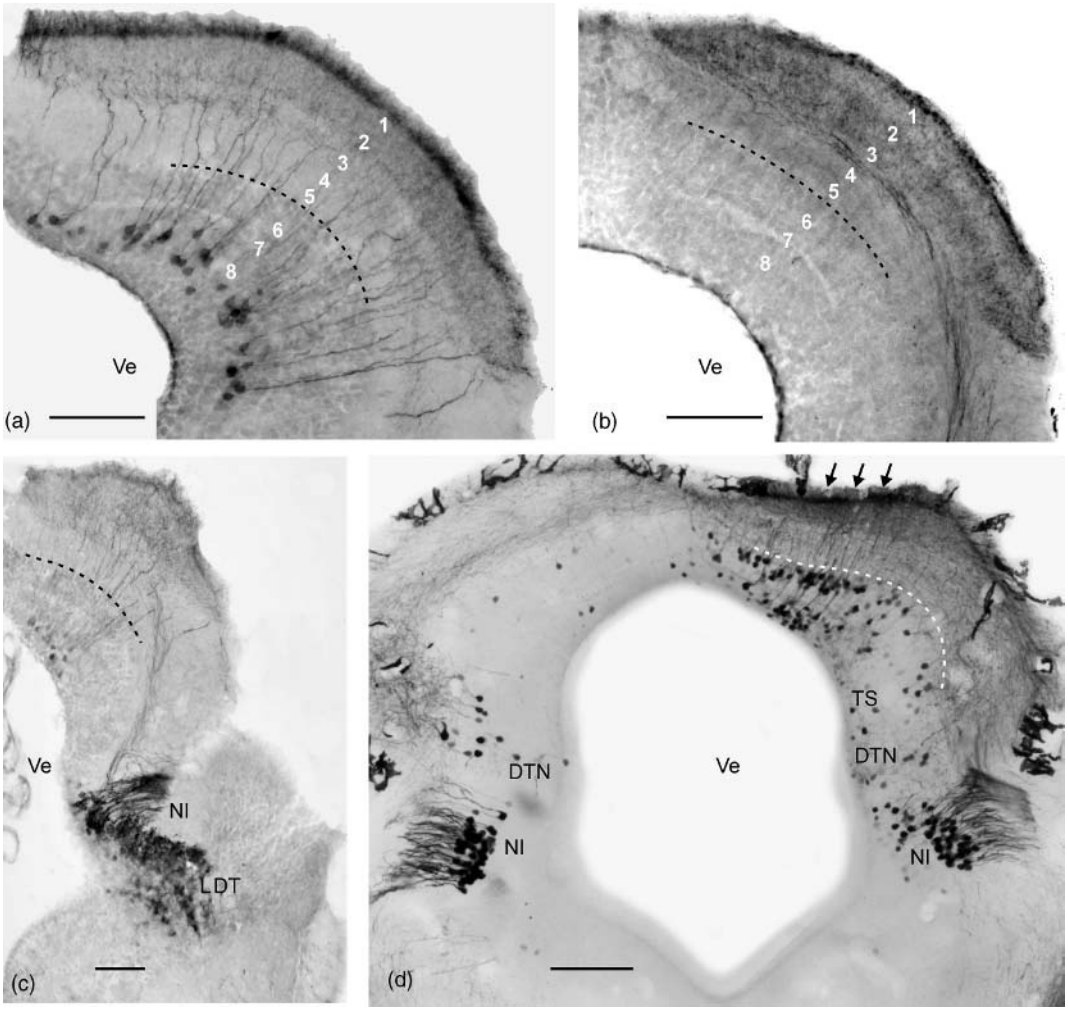

Figure 13 Microphotographs of ChAT-immunoreactive structures in the plethodontid salamander *Desmognathus ochrophaeus* ((a), (c)) and in the salamandrid *Salamandra salamandra* (b). In (d), retrograde labeling of bilateral tegmental and isthmic neurons after unilateral application of biocytin (black arrows) to the tectum is shown. Note that in *Desmognathus*, ChAT-ir tectal neurons are present that extend processes into the superficial layers, whereas in *Salamandra* only the isthmic neurons provide the tectal layers with cholinergic fibers. 1, 2, 3, 4, 5, 6, 7, 8, ventricles; Ve, ventricle; NI, isthmic nucleus; LDT, laterodorsal tegmental nucleus; DTN, dorsal tegmental nucleus; TS, torus semicircularis. Scale bars: 100 μm.

input. These neurons carry different sensory modalities and project to the tectum and the torus semicircularis. The pattern of ascending somatosensory projections differs between plethodontid salamanders on the one hand and *Pleurodeles waltl* and frogs on the other (Muñoz *et al.*, 1994a, 1994b, 1995, 1997; Dicke and Muhlenbrock-Lenter, 1998) (see Section 11.3.1.5.(*ii*) above). In the salamander, *Ambystoma tigrinum*, ipsi- and contralateral projections to the tectum have been reported (Gruberg and Solish, 1978; Gruberg and Harris, 1981).

In plethodontids, the tectum appears to be the main center for multimodal integration, while in frogs the torus is the main target of somatosensory afferents. The presence of a more elaborate secondary somatosensory system in terrestrial plethodontids compared to salamandrid salamanders with a larval stage as well as ranid and pipid frogs may be due either to phylogeny, differences in development (direct vs. biphasic), or functional adaptation (e.g., differences in quantity and distribution of transmitters in the tectum in the context of visuomotor and visual functions).

11.3.4.9 Tectal efferent pathways

11.3.4.9.(i) Descending pathways In order to elucidate descending tectal pathways, tracer studies have been carried out in frogs and salamanders using HRP or cobaltic lysine applied to the entire half of the medulla spinalis (ten Donkelaar *et al.*, 1981; Tóth *et al.*, 1985; Naujoks-Manteuffel and Manteuffel, 1988), and the existence of a crossed

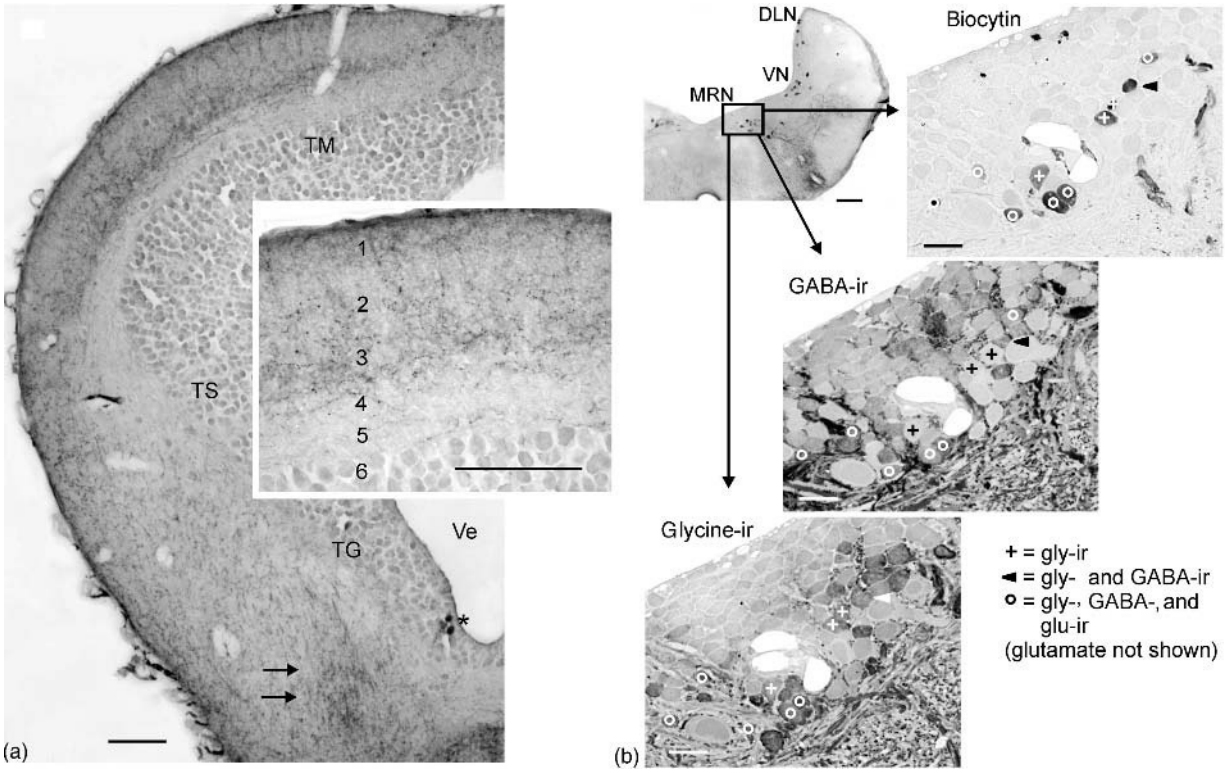

Figure 14 a, Microphotographs of transverse sections through the midbrain revealing 5-HT-immunoreactivity in the salamander *Plethodon jordani*. Arrows point to the ascending bundle and asterisk indicates labeled somata of the rostral raphe nucleus. Inset shows part of the tectum at higher magnification. b, Glycine-ir and GABA-ir in neurons of the middle reticular nucleus retrogradely labeled after biocytin application to the tectum. Consecutive semithin sections with cell bodies stained for the two transmitters and biocytin reveal that co-localization of transmitters occurs more frequently than previously assumed. TM, mesencephalic tectum; TS, torus semicircularis; TG, tegmentum; Ve, Ventricle; 1 6, ventricles; DLN, dorscelateral nucleus; VN, vestibular nucleus; MRN, middle reticular nucleus. Scale bar in (a) 100 μm and in (b) 50 μm for the section above left and 20 μm for semithin sections. a, Modified after Dicke, U., Wallstein, M., and Roth, G. 1997. 5-HT-like immunoreactivity in the brains of plethodontid and salamandrid salamanders (*Hydromantes italicus, Hydromantes genei, Plethodon jordani, Desmognathus ochrophaeus, Pleurodeles waltl*): An immunohistochemical and biocytin double-labelling study. *Cell Tissue Res.* 287, 513 523. b, Modified from Landwehr, S. and Dicke, U. 2005. The distribution of GABA, glycine and glutamate in neurons of the medulla oblongata and their projections to the midbrain tectum in plethodontid salamanders. *J. Comp. Neurol.* 490, 145 162.

and uncrossed tectal tract was consistently reported. Locally restricted application of biocytin to the ventromedial or ventrolateral medulla oblongata revealed that different populations of tectal neurons give rise to a crossed and one or two uncrossed descending tracts. In plethodontid salamanders, neurons in the ipsilateral tectum are labeled after ventromedial and ventrolateral tracer application, whereas in *Discoglossus* they are labeled only after ventrolateral tracer application (Dicke *et al.*, 1998; Dicke, 1999).

In plethodontid salamanders, the course of the descending axons and sites of collaterals to the medulla were investigated by biocytin application into the tectum as well as by intracellular biocytin injection into tectal neurons (Dicke and Roth, 1994; Dicke, 1999; Roth *et al.*, 1999) (Figures 9 and 10c). The fibers of the crossed tectobulbospinal tract run to the ipsilateral ventral tegmentum and cross the midline in the rostral to caudal tegmentum. They further descend in the contralateral ventral tegmentum, where fibers form a neuropil extending inside the ventral white matter. In the medulla oblongata and spinalis, fibers run close to the midline within the ventral white matter below the surface. The tract thins out during its course to the spinal cord; axons terminate in the rostral medulla oblongata or reach at least the level of the third spinal nerve.

Axons of the uncrossed tract descend in the caudal tectum to the ventral tegmentum. During their course from the tegmentum to the ventral medulla, axons are distributed broadly in the medial white matter. More laterally, dense axon bundles are formed, which remain close to the lateral edge of the uncrossed tract inside the medulla; they originate from other types of neurons than the medially descending axons. In the medulla oblongata and spinalis, axons run in a superficial ventral position;

in the transverse plane, they are distributed from the midline to the ventrolateral part. The uncrossed tracts extend to rostral spinal cord levels, but only one-half to one-third of axons reach the level of the third spinal nerve.

During their course within the medulla oblongata and spinalis, axons of the descending tracts give rise to many fine collaterals that often carry boutons. Axons of single neurons give rise to a substantial amount of axon collaterals inside the medulla oblongata. The majority of collaterals extend inside the white matter. At the level of the IXth and Xth cranial nerves, prominent axon collaterals of the crossed tract extend dorsally and dorsolaterally into the gray matter; they often twist around each other. In the caudal medulla oblongata, some axon collaterals extend along the border of the gray matter to the dorsal side.

In salamanders as well as in frogs, the descending fibers of the crossed and uncrossed pathways show strong branching, but unlike in salamanders (and in other tetrapod vertebrates), in frogs only few contralaterally descending axons reach spinal levels. This may be a consequence of the condensation of the neck region and consequently of bulbar and cervical spinal motor nuclei that has occurred during the evolution of anurans.

11.3.4.9.(ii) Ascending pathways Systematic studies of the ascending axons of tectal neurons have been carried out in the frog *R. pipiens* and in the salamander *Plethodon jordani* (Montgomery and Fite, 1991; Dicke, 1999; Roth *et al.*, 1999). In *Rana*, the ascending projections from the dorsal mesencephalon to the thalamus and pretectum were investigated by means of HRP tracing. Axons of small pear-shaped neurons (type-5 in salamanders) in the superficial portion of tectal layer 8 exit the tectum through layer 9, travel in the superficial portion of the dorsal and ventral tectothalamic tracts, and innervate the nucleus lentiformis mesencephali (the large-celled part of the pretectal nucleus), the posterior lateral dorsal nucleus, and the CGT. Axons of small pear-shaped neurons in the lateral and caudal tectum ascend via the ventral tectothalamic tract, while those of neurons in the rostral and medium tectum run in the dorsal tectothalamic tract. Axons from pear-shaped neurons (type-5 of frogs homologous to type-3 in salamanders) in layer 6 and pyramidal neurons (type-3 of frogs homologous to type-2 in salamanders) in layer 8 leave the tectum through layer 7, travel in the dorsal or ventral tectothalamic tracts and are located medial to the axons of the pear-shaped neurons of superficial tectal layer 8. The majority of type-3 neurons project to the posterior lateral ventral nucleus and the anterior lateral nucleus, but terminals do not display a tectotopic organization. Another major projection to the thalamus originates from the pretectal gray and innervates the pretectal nucleus lentiformis mesencephali, the posterior lateral dorsal nucleus, the anterior lateral nucleus, dorsal and ventral divisions of the ventral lateral thalamus, and the nucleus of Bellonci. Other axons from the pretectal gray terminate in the contralateral medial portions of the posterior lateral dorsal thalamus, the ventral lateral thalamus, and the anterior lateral nucleus.

In *P. jordani*, ascending tectal projections were studied by tracer application and intracellular injection of biocytin into neurons (Figures 9 and 11c). Tracer application to the tectal hemisphere likewise labels retinal afferent fibers, and double labeling of the optic nerve and the tectum using different tracers reveals that the retinofugal axon bundles and ascending axons of tectal neurons take partially overlapping courses. The ascending axons of tectal neurons leave the tectum through fiber layers 3–5 and ascend superficially at the lateral edge of the tectum. During their course, axons give rise to many collaterals extending in the ipsilateral dorsal white matter. In the ipsilateral pretectum, two dense neuropils, a dorsolateral and a lateral one, are formed. In the ipsilateral thalamus, axons run laterally in the fiber layer, and collaterals are distributed in the entire white matter of the dorsal and ventral thalamus. They form a distinct neuropil at the border between the gray and white matter in the dorsal thalamus, where the neuropil and NB are situated. Many axons cross in the postoptic commissure to the contralateral thalamus, where they branch inside the ventral and dorsal white matter and again form a neuropil in the dorsal thalamus. Axon collaterals are labeled in the ipsi- and contralateral preoptic area, and only few extend to the ventral fiber layer of the ipsi- and contralateral caudal telencephalon.

In summary, the ascending pathways of amphibians are constituted by small-field neurons with only ascending projections and by two types of wide-field neurons with ascending and descending projections. The former neurons constitute the majority of ascending tectal projection neurons: they are regularly distributed throughout the tectum and give rise to a retinotopic tectal projection to the thalamus in the frog *Rana* and most likely in the salamander *Plethodon*. In contrast, the latter two types of neurons either are very low in number or are unevenly distributed in the tectum. They appear to give rise to a nonretinotopic tectal projection to the thalamus. Accordingly, in amphibians the ascending pathways may be divided into two functional systems, a retinotopically and a nonretinotopically

organized system, which is comparable to the situation found in reptiles and birds (see Evolution of the Nervous System in Reptiles).

In the tecta of reptiles and birds, efferent cells constitute ipsilaterally and contralaterally descending tracts as well as ipsi- and contralaterally ascending tracts (Reiner and Karten, 1982; Sereno, 1985; Sereno and Ulinski, 1985; Dacey and Ulinski, 1986a, 1986b; ten Donkelaar, 1990; Reiner, 1994). The morphology of neurons of origin of these tracts and their axonal projection patterns reveal similarities with the situation found in amphibians. One ascending pathway arises from neurons that possess wide dendritic fields in the retinorecipient layer; this pathway terminates in a nonretinotopic manner in the nucleus rotundus, the possible homologue to the pulvinar of mammals. The other pathway arises from radial neurons with long dendrites ascending to the stratum griseum superficiale (SGF); it ascends to the dorsal and ventral lateral geniculate nucleus (LGN) of the thalamus. In amphibians, we likewise find wide-field as well as small-field tectal neurons that give rise to pathways ascending to the thalamus. Axons of small-field neurons form neuropils in the dorsal thalamus, whereas wide-field neurons predominantly project to the ventral thalamus. In the thalamus, in turn, a large group of neurons project to the striatum, and a small group to the medial and dorsal pallium. Thus, a tectothalamopallial pathway exists in salamanders, but its thalamopallial part is formed by relatively few neurons. This will be discussed in greater detail below.

11.3.4.10 The distribution of transmitters and neuropeptides in the retinotectal system

11.3.4.10.(i) Retinal ganglion cells In amphibians, a large number of RGCs use glutamate as transmitter. Polysynaptic responses of tectal cells in *Rana* were found to be mediated by NMDA and non-NMDA receptors, and examination of monosynaptic currents revealed that retinotectal synapses express functional NMDA receptors that were voltage dependent and not responsible for the bulk of normal excitatory transmission (Hickmott and Constantine-Paton, 1993). GABA is likewise used as transmitter in retinotectal transmission; the number of GABAergic ganglion cells synapsing on tectal neurons differs across species. In *B. marinus*, roughly 3% of retrogradely labeled RGCs contained GABA; 88% of retinal axon terminals in the tectum revealed glutamate-ir, 6% GABA-ir, and 6% were negative for both GABA and glutamate (Gabriel *et al.*, 1992; Gabriel and Straznicky, 1995), while in *R. pipiens*, 15% of back-filled RGCs contained GABA (Li and Fite, 1998). Over 50% of cells in the retinal ganglion cell layer of *R. esculenta* contained calretinin, and many optic fibers were also labeled; a co-localization of calretinin and GABA was rarely observed in RGCs (Gabriel *et al.*, 1998). In *Ambystoma*, small populations of retrogradely labeled RGCs contained substance P (2%) or GABA (less than 1%); substance P and GABA were not co-localized in RGCs (Watt *et al.*, 1994). In the salamandrid salamanders *P. waltl* and *Triturus alpestris*, axons in the retinal radiation in the diencephalon are mainly GABA-negative (Naujoks-Manteuffel *et al.*, 1994).

11.3.4.10.(ii) Tectum mesencephali In *R. catesbeiana* and *X. laevis*, GABA-ir cell bodies are distributed throughout all tectal cellular layers, and form dense GABAergic populations in layers 2, 4, and 6, that in layer 6 probably has the densest population in the entire brain. In *Xenopus*, the laminar organization was less distinct and fewer labeled cells were present compared to *Rana* (Hollis and Boyd, 2005). In the tectum of *R. pipiens*, the synthesizing enzyme GAD is distributed with punctate structures in several laminae of the optic tectum, and the highest concentrations are found in layers 9 and 8 (Tyler *et al.*, 1995). GABA immunochemistry in this ranid species reveals that perikarya and fibers are labeled in superficial layers 8 and 9, but densely packed immunoreactive perikarya occur in deep tectal layers 2, 4, and 6 (Li and Fite, 1998). In the tectum of *R. esculenta*, nearly one-third of the total population of cells appears to be GABA-immunoreactive (Antal, 1991); the stained population consists of neurons with small perikarya, and the proportion is highest in layer 9 (61%), and lower in layers 7 (21%) and 6 (27%).

A substantial proportion of retinal axons terminate on GABA-containing tectal neurons. In *B. marinus*, 57% of retinal axons synapsed on GABA-ir and 5% on glutamate-ir tectal elements, while the remaining axons synapsed on dendrites revealing neither GABA- nor glutamate-ir (Gabriel and Straznicky, 1995). In *Xenopus*, an ultrastructural analysis of tectal layers 8 and 9 using labeling of retinotectal axon and GABA immunohistochemistry revealed that GABA-ir neurons participate in serial synaptic arrangements, in which retinotectal axons are the first element (Rybicka and Udin, 1994). Furthermore, retinotectal and isthmotectal axons do not synapse close to each other on the same dendrites. Surprisingly, axons of isthmotectal neurons relaying ipsilateral eye input to tectal cells mainly synapse onto GABA-ir interneurons (Rybicka and Udin, 2005).

In *R. esculenta*, a substantial portion of axons of RGCs that terminate in laminae B, C, and F of tectal layer 9 contain the calcium-binding protein

calretinin. Also, approximately 10% of the tectal cells were found to be immunoreactive for calretinin. Tectal neuron populations in layers 4, 6, 8, and 9 were labeled, and a few calretinin-positive cells were detected also in layer 2. Cells in layers 4, 6, and 8 belonged to projection neurons. Co-existence of GABA and calretinin was characteristic of cells in upper tectal layers, but was absent in neurons of deep layers of the tectum (Gabriel *et al.*, 1998). Several Met-enkephalin immunoreactive perikarya were found in tectal layer 6 of *R. esculenta*, and a third of these neurons showed GABA-ir in addition. Also, GABA and neuropeptide Y (NPY) were co-localized in half of NPY-immunopositive cells in layer 6, while only a few cells were double-stained in layers 9 and 4 (Kozicz and Lázár, 2001).

In salamanders, GABA-ir neurons are scattered in all cellular layers of the tectum in *Triturus cristatus* and *P. waltl*, and a rich GABergic innervation characterizes tectal fiber layers (Franzoni and Morino, 1989; Naujoks-Manteuffel *et al.*, 1994). In *P. jordani* and *Hydromantes italicus*, GABA-ir somata were found in one-third of tectal neurons, with the majority located in the deep cellular layer 8 and to a lesser degree in cellular layer 6. They were either immunoreactive for GABA only, for GABA and glutamate, or for GABA and glycine. About 80% of tectal somata revealed glutamate-ir, including those with GABA-ir in addition (one-fourth), and were situated in both cellular layers (Wallstein and Dicke, 1996). On the basis of immunochemical detection of co-localization of transmitters in tectal neurons with descending projections, the majority of the latter neurons appears to contain glutamate and a substantial part of them GABA in addition (S. Landwehr, unpublished data). It is unclear, however, to which degree glutamate appears as precursor of GABA.

In addition to the cholinergic and serotonergic innervation of the tectum already described above, catecholamines were investigated in the tectum of *Rana perezi* and *P. waltl* (Sanchez-Camacho *et al.*, 2002). Dopaminergic fibers were found primarily in deeper tectal layers of *Rana* (5–8) and in all tectal layers of *Pleurodeles*, whereas noradrenergic fibers predominated in superficial layers (7–9 in *Rana*; 1–5 in *Pleurodeles*); catecholaminergic somata were not found in the tectum. The catecholaminergic fiber input originates mainly from the pretectal area (*Pleurodeles*) and juxtacommissural nucleus (*Rana*), a smaller component from the suprachiasmatic nucleus, and in *Rana* also from the posterior tubercle as well as from the noradrenergic locus coeruleus. In plethodontid salamanders, the dopaminergic fiber input to the midbrain tectum appears to be less pronounced, since only few ir-fibers were found in the deep fiber layers 4 and 5 (Wallstein and Dicke, 1997). Furthermore, histochemistry of NADPH-diaphorase revealed labeled fibers and cell bodies in the tectum of *R. perezi* (Muñoz *et al.*, 1996).

The lamination of tectal cells and fibers is paralleled by another lamination established by peptides (cf. review of Lázár, 2001). A variety of peptides (23 of 28 peptides including those already mentioned above) is present in the frog tectum, and a substantial number of peptides was also localized in the lateral geniculate complex and pretectal area (13 and 15 of 28 peptides, respectively). Immunoreactivity of some peptides overlap tectal laminae or layers, while others partly overlap. Again other peptides share one lamina, but may be sharply separated within the lamina.

11.3.4.10.(iii) Pretectum and BON The BON contains a small number of lightly labeled GABA-ir perikarya, mostly located in its dorsal half (Li and Fite, 1998). GABAergic afferents extend from the retina to the contralateral tectum, from the BON to the ipsilateral pretectal nucleus lentiformis mesencephali, and a second-order pathway from the isthmic nucleus bilaterally to the optic tectum (Li and Fite, 2001). In *S. salamandra*, the pretectal and the accessory optic system display many GABAergic neurons (Naujoks-Manteuffel *et al.*, 1994). Labeled fibers and cell groups stained for the enzyme NADPH-diaphorase were observed in the pretectal area. NADPH-diaphorase and catecholamines were co-distributed in these areas; however, restricted co-localization in the same neurons was found (Muñoz *et al.*, 1996).

11.3.5 Neurophysiology of the Retino-Tecto-Pretectal System

11.3.5.1 Response properties of RGCs Based on recordings from the optic nerve and in the superficial layers of the tectum, five classes of RGCs have been identified in ranid frogs and three classes in toads and in salamanders (frogs: Maturana *et al.*, 1960; Grüsser and Grüsser-Cornehls, 1970, 1976; Ewert and Hock, 1972; Grüsser-Cornehls and Langeveld, 1985; salamanders: Cronly-Dillon and Galand, 1966; Norton *et al.*, 1970; Grüsser-Cornehls and Himstedt, 1973; 1976). The classes differ in the size of the excitatory and inhibitory receptive field (RF), the response to the on- and/or offset of illumination, the response to stationary or moving stimuli of different velocity, size, shape, or contrast, and the response to stimulation with light of different wavelengths. In brief, three universal classes of RGCs exist, which appear to constitute (1) a shape/color pathway, (2) a motion pathway, and (3) an ambient illumination pathway

(for an extended summary on the properties of RGCs, see Roth *et al.*, 1998).

Neurons of the first class of RGCs respond either to moving or nonmoving objects and require relatively high visual contrast. They can be classified as small-field edge-detector cells. They exhibit low conduction velocity due to unmyelinated fibers. Their axons terminate in the uppermost layer of the optic tectum. Class-1 and class-2 cells of frogs, R2 cells of toads, and layer-1 cells of salamanders belong to this class. They may include several subclasses differing in color sensitivity, responses to light ON or OFF, and erasability or nonerasability of stimulation by stationary edges. These cells are most probably involved in the detection of small, high-contrast objects such as prey. They are comparable to X-cells in cats and to P-cells in primates (Spillmann and Werner, 1990).

The second class of RGCs comprises neurons that respond to small changes in contrast and small dislocations of edges. They do not respond to nonmoving objects and are considered medium-field motion-detector or ON–OFF cells. They exhibit high-conduction velocity due to myelination of fibers. Axons run to the thalamus, pretectum, and tectum in parallel; in the tectum, they terminate in the intermediate layer of retinal afferents. They are represented by class-3 RGC in frogs, R3 cells in toads, and layer-2 cells in salamanders. The RGCs of this class are predominantly involved in motion and movement pattern detection. They correspond to Y-cells in cats and M-cells in primates.

RGCs of the third class respond well to large objects and to changes in illumination in larger parts of the visual field, but do not respond well to small- or medium-sized objects. They are classified as large-field dimming-detector or OFF cells. They show high-conduction velocity and have thick myelinated fibers, which project in parallel to the thalamus, pretectum, and tectum. Inside the tectum, they terminate in the deepest layer of retinal afferents. Class-4 and R4 cells of frogs and toads, respectively, and layer-3 cells of salamanders form this class. They may be involved in predator detection, optomotor behavior, or respond to changes in overall illumination.

11.3.5.2 Tectum

11.3.5.2.(i) Topic organization Retinal afferents from each retina form a contralateral and an ipsilateral two-dimensional representation or map in the tectum. In the plethodontid salamander *Hydromantes*, these maps were identified on the basis of data from single-cell recording and neuroanatomy (Wiggers and Roth, 1991; Wiggers *et al.*, 1995). Each visual hemifield is projected completely onto the contralateral tectal hemisphere. The ipsilateral retinotopic projection covers roughly the rostral two-thirds of the tectal surface. This representation is rotated 180° compared to that of the contralateral retinotopic projection such that the contralateral and ipsilateral retinotectal projection run in opposite directions. The ipsilateral tectal representation from one eye and the contralateral representation from the other eye are in register, whereas the left and right contralateral and the left and right ipsilateral representations are arranged opposite to each other. An object that moves from lateral to frontal in the visual hemifield shifts from rostral to caudal in the ipsilateral tectum, i.e., in a direction opposite to the contralateral projection from the same eye. When objects move along the *z*-axis, i.e., straight toward or away from the animal, the two contralateral tectal representations move in the same direction and opposite to the two ipsilateral representations. This topographic arrangement apparently is the basis for a precise localization of objects and is also important for depth perception based on retinal disparity (Wiggers and Roth, 1994; Eurich *et al.*, 1995; Wiggers *et al.*, 1995).

11.3.5.2.(ii) Neurons The visual field covers an area of 360°–400° in different frog species. Several classes of tectal neurons have been defined by the size and shape of their RFs as well as their location in the visual field, by their responses to stationary or moving objects of different velocity, size, shape, or contrast, and their responses to the direction of movement. The RFs of tectal neurons range from 1° to the size of the entire visual field, and seven different types of tectal neurons have been identified in various frog species (cf. Grüsser and Grüsser-Cornehls, 1976). Ewert and von Wietersheim (1974) and Roth and Jordan (1982) studied the response properties of tectal neurons in *Bufo bufo*, and Schürg-Pfeiffer and Ewert (1981) those of *Rana temporaria*. A detailed comparison of response properties of tectal neurons in frogs and salamanders is given in Roth (1987).

In salamanders, tectal neurons were classified in *S. salamandra* (Grüsser-Cornehls and Himstedt, 1973; Himstedt and Roth, 1980; Finkenstädt and Ewert, 1983a, 1983b; Himstedt *et al.*, 1987), in *H. italicus* (Roth, 1982), and in *Hydromantes* and *Bolitoglossa subpalmata* (Wiggers *et al.*, 1995). In plethodontid salamanders, the RFs of tectal neurons are not evenly distributed across the tectum, but concentrate in the frontal area of the visual field (Wiggers *et al.*, 1995). More than half of recorded neurons are situated in the rostral tectum, and their RFs are located in the binocular visual field. The size and shape of RFs does not differ between in the monocular contralateral visual field and in the

binocular field. In *H. italicus*, the size of the RFs ranges from 10° to 360°, with an average of about 40° (Wiggers *et al.*, 1995); in *Plethodon shermani* (formerly *P. jordani*) the RFs in the binocular visual field range from 6° to 200° with an average of 22° (Schuelert and Dicke, 2005).

A comparison between tectal response types in urodeles and anurans shows that similar types can be found in *Salamandra* and *Bufo*, on the one hand, and plethodontid salamanders and *Rana*, on the other. In the two former species, a worm-preference type was found, i.e., neurons responded best to the presentation of a horizontal rectangle moving in direction of its long axis inside the RF, with lower frequencies to the presentation of a square and with lowest frequencies to a rectangle moving perpendicular to its long axis. Such a preference for worm-like stimuli was absent in tectal neurons of *Rana* and plethodontid salamanders. This difference fits nicely with natural prey preferences of the species under consideration (cf. Roth, 1987).

In these studies on the response properties of tectal neurons, the relationship between neuronal responses elicited by a given object and the response of the behaving animal to such a stimulus remained unclear. In toads, a strong correspondence between the configural features of elongated visual objects and the response selectivity of one type of neuron was proposed (Ewert and von Wietersheim, 1974; Ewert *et al.*, 1978; Schürg-Pfeiffer and Ewert, 1981). Other studies demonstrated that a combination of stimulus features rather than a single feature determines the responses of tectal neurons (Himstedt and Roth, 1980; Roth, 1982; Roth and Jordan, 1982). From these latter studies it was concluded that visual features are encoded only ambiguously by the spike rate of a single neuron, and that object recognition is based rather on population coding (an der Heiden and Roth, 1987).

In a recent extracellular recording and labeling study of tectal neurons in *Plethodon* (Schuelert and Dicke, 2005), prey dummies differing in size, contrast, velocity, or movement pattern were presented either singly inside the excitatory RF or paired with one stimulus inside and another stimulus outside the excitatory RF (Figure 15). The authors found that

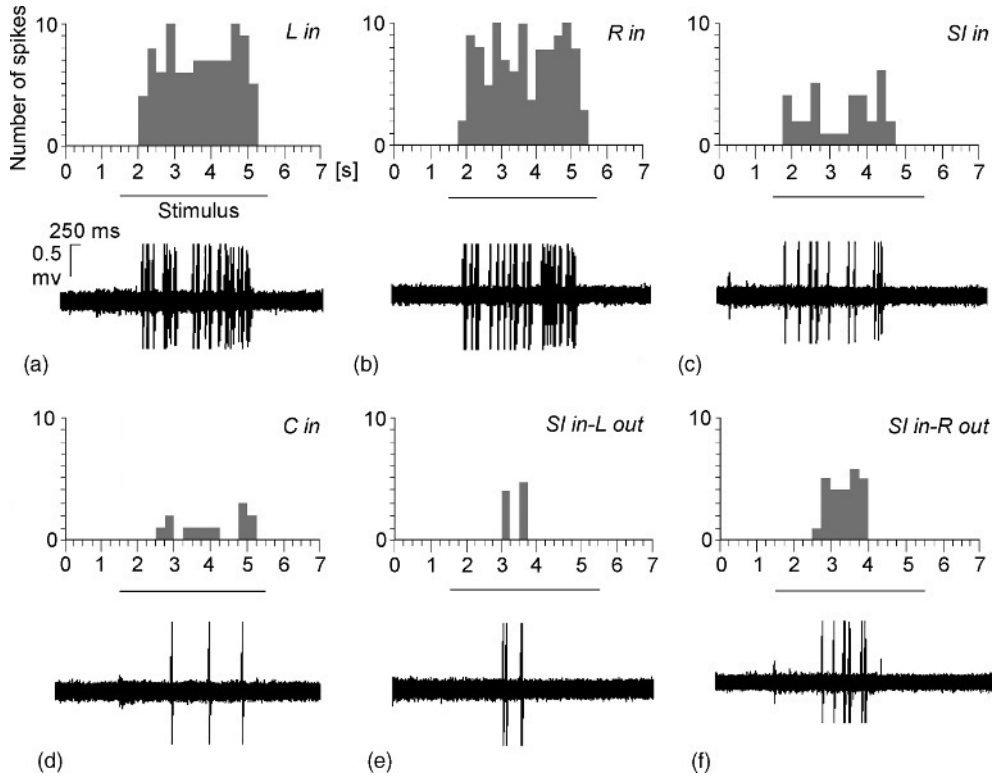

Figure 15 Responses of a tectal neuron in *Plethodon shermani* to the following stimuli: single presentation of a large-sized cricket (a; *L in*), a rectangle (b; *R in*), a still-image cricket (c; *SI in*) and a contrast-reduced cricket (d; *C in*) inside the excitatory receptive field (ERF). Although the number of spikes is comparable at single presentation of the L and the R, at paired presentation of the SI inside and the L (e; *SI in-L out*) outside the ERF, the spike number is much lower at presentation of *SI in-L out* compared to *SI in-R out*. Histograms of spike number at presentation of stimuli (n 3 for each stimulus type) are shown above the recording traces; the black line below the trace indicates the duration of stimulus presentation. From Schuelert, N. and Dicke, U. 2005. Dynamic response properties of visual neurons and context-dependent surround effects on receptive fields in the tectum of the salamander *Plethodon shermani. Neuroscience* 134, 617 632.

tectal neurons are not merely tuned to simple stimulus properties; rather, their responses are heavily influenced by the type of stimulus, i.e., the combination of single features, the familiarity of an object, and the context in which a visual stimulus occurs.

It became evident that in amphibians, visual object recognition involves much more complex spatial and temporal processing than previously assumed and concerns changes in spike number, temporal pattern, and dynamic changes in the size of the RF. Also, the response properties of tectal neurons indicate that these neurons integrate information across a much larger part of visual space than covered by the RF. For example, an inhibitory surround effect resulting in a decrease in the number of spikes and a reduction in RF size occurred at paired presentation, when a large-sized cricket or a rectangle was located outside the RF. However, this inhibition was significantly greater for the large-sized cricket stimulus than for the rectangle and indicates the biological relevance of the prey-like stimulus in object selection. This dynamic processing corresponds with the selection of stimuli in the visual orienting behavior of *Plethodon*, in which orientation toward a stimulus depended on the precise combination of different features (Schulert and Dicke, 2002). These findings suggest that prey recognition is guided by a number of visual features instead of a single feature and supports the idea of population coding as the basis for object recognition.

11.3.5.3 Nucleus isthmi Gruberg and co workers found that unilateral lesions of the nucleus isthmi resulted in a scotoma to visually presented prey and threat stimuli in the contralateral monocular visual field (Gruberg *et al.*, 1991). A correlation exists between the size of the scotoma and the amount of nucleus isthmi ablated. Electrophysiological recording from positions within the area of the optic tectum including the scotoma reveal a roughly threefold increase in the size of the multi-unit RFs compared to mirror-image positions in the contralateral optic tectum. Across the entire extent of the isthmic nucleus, two superimposed maps exist, one representing the entire visual field of the contralateral eye, the other one representing the binocular visual field of the ipsilateral eye (Winkowski and Gruberg, 2002).

The role of the isthmic nucleus in enhancing intracellular calcium concentrations in retinotectal fibers was studied *in vitro*; results suggest that the input of the isthmic nucleus can facilitate retinotectal neurotransmission. This mechanism could be used to allow the frog to attend to a single prey stimulus in an environment of several prey stimuli (Dudkin and Gruberg, 2003). Recent recordings from the intermediate layer 7 of *Rana* revealed the existence of superimposed topographic maps of the monocular visual fields in the caudolateral tectum. The ipsilateral eye monocular visual field representation can be abolished by electrolytic ablation of the contralateral isthmic nucleus (Winkowski and Gruberg, 2005).

In the salamander *H. italicus*, the representation of the visual field and the properties of isthmic neurons were studied by Wiggers and Roth (1991). The RFs of isthmic neurons are centered in the frontal 100° field. The visual hemifield covered by neurons of each nucleus extends horizontally from 50° contralateral to 30° ipsilateral to the nucleus, and vertically from 36° below to 50° above the horizon. Thus, the projection fields of the isthmic nucleus overlap by 60°. About two-thirds of recorded neurons had their RFs within the upper part of the visual field, above eye level. The majority of neurons preferred stimulus sizes with edge lengths between 4.6° and 9.1°. The highest impulse rates, up to 25 impulses s^{-1}, were recorded at velocities between 20 and 36° s^{-1}. Half of isthmic neurons responded to stimuli from both eyes, whereas 35% responded only to contralateral stimulation. The remaining 14% responded weakly to ipsilateral stimulation and strongly to contralateral stimulation.

In summary, neurons of the isthmic nucleus reveal response properties that are similar to those found in tectal neurons; however, the representation of the visual space in the isthmic nucleus is not a simple copy of the tectal representation of visual space. The findings support the view of the isthmic nucleus as an essential structure involved in object localization and selection.

11.3.5.4 Pretectum In the pretectum of frogs and salamanders, a superficial nucleus (nucleus lentiformis in frogs) consisting of migrated, large-celled neurons and a deep subnucleus are found (Figure 16). Neurons in the pretectum in *R. pipiens* and *R. esculenta* are directionally selective and particularly sensitive to horizontal optokinetic patterns moving at velocities of 5–10° s^{-1} (Katte and Hoffmann, 1980). Neurons in the nucleus lentiformis mesencephali are involved in horizontal optokinetic nystagmus in *R. pipiens* (Fite *et al.*, 1989). At presentation of a large-field patterned stimulus at eight directions and three velocities of movement, all recorded units were spontaneously active and motion sensitive. Directional information appears to be encoded in the activity of a large population of motion-sensitive units, which includes both narrowly and broadly tuned individual response profiles. In contrast, neurons recorded in the nucleus/neuropil of the basal optic root respond more selectively to slowly

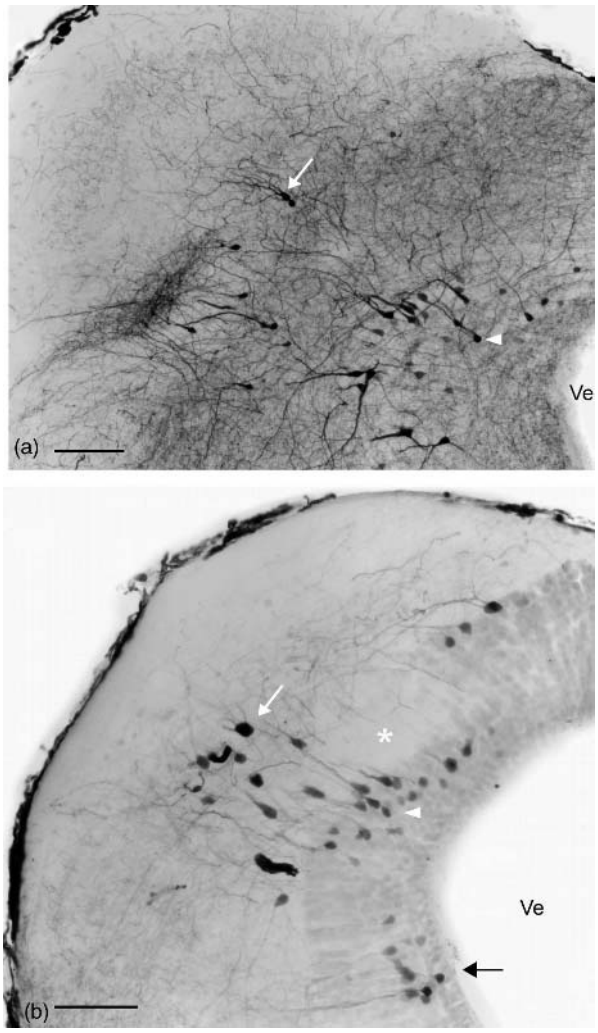

Figure 16 Microphotographs of transverse sections through the superficial and deep pretectal nucleus of *Discoglossus pictus* (a) and *Plethodon jordani* (b). Neurons were retrogradely labeled after application of biocytin to the ventral rostral medulla oblongata. Asterisk indicates the posterior commissure, white arrows and arrowheads point to neurons in the superficial and deep pretectal nuclei, respectively, and black arrow in B to ventral thalamic neurons. Ve, Ventricle. Scale bars: 100 μm. From Dicke, U., Roth, G., and Matsushima, T. 1998. Neural substrate for motor control of feeding in amphibians. *Acta Anat.* 163, 127 143.

moving vertical patterns, although horizontally sensitive neurons also have been reported (Katte and Hoffmann, 1980; Gruberg and Grasse, 1984).

Recordings of pretectal cells were also carried out in *S. salamandra* (Manteuffel, 1984a, 1984b, 1989; Sperl and Manteuffel, 1987). Two-thirds of recorded cells were sensitive to a temporonasal movement of stimuli; the majority preferred velocities between 1° and 5° s ^{1}. These neurons mostly possessed large RFs with a diameter of 82° and 34° in horizontal and vertical direction, respectively, and the RF center was situated always within the contralateral visual hemifield. Nearly half of pretectal cells were binocular and did not respond to ipsilateral stimulation. Cells in the BON of *Salamandra* and *T. cristatus* were also sensitive to temporonasal direction of stimulus movement, except one cell which was sensitive to the opposite direction (Manteuffel, 1982, 1984b). All neurons recorded from the BON or the nucleus of the oculomotor nerve were strictly monocular. Deeper and more caudal sites close to the BON cells that respond preferentially to vertical movement tend to be more numerous.

According to lesion experiments, the amphibian pretectum has proven to be essentially involved in control of gaze-stabilizing reflexes (Lázár, 1972; Montgomery *et al.*, 1982; Manteuffel *et al.*, 1983). In summary, the findings of recording and lesion experiments provide evidence that pretectal and accessory neurons are involved in oculomotor and optokinetic behaviors.

11.3.6 Diencephalon

The diencephalon of amphibians forms the walls surrounding the third ventricle and is, like that of all tetrapod vertebrates, traditionally divided into three parts, i.e., epithalamus, thalamus, and hypothalamus (Figures 17h–17j). The pretectum is considered to form either the posterior part of the thalamus or – together with the anterior portion of the tectum mesencephali – to represent a transition zone between diencephalon and mesencephalon surrounding the posterior commissure and called synencephalon (Kuhlenbeck, 1977).

11.3.6.1 Epithalamus and pineal complex The rostral part of the epithalamus contains the dorsal and ventral habenular nuclei and the habenular commissure. The ventral habenular nucleus is divided by the fasciculus retroflexus. Caudal to the habenular nuclei, the pineal gland or epiphysis, the posterior commissure, and below it the subcommissural organ (a gland) are found. The pineal gland is connected, via the pineal nerve, with the light-sensitive frontal organ located in the skin between the two eyes. Pineal gland and frontal organ project, among others, to the amygdala, pretectum, ventrolateral thalamic nucleus, and ventrolateral mesencephalic tegmentum.

11.3.6.2 Dorsal and ventral thalamus Dorsal and ventral thalamus are separated by the sulcus medialis, and the ventral thalamus is separated from the hypothalamus by the sulcus hypothalamicus (Figures 17h–17j, 5, and 6). In anurans, a number of thalamic nuclei can be distinguished morphologically. The dorsal thalamus is comprised of three

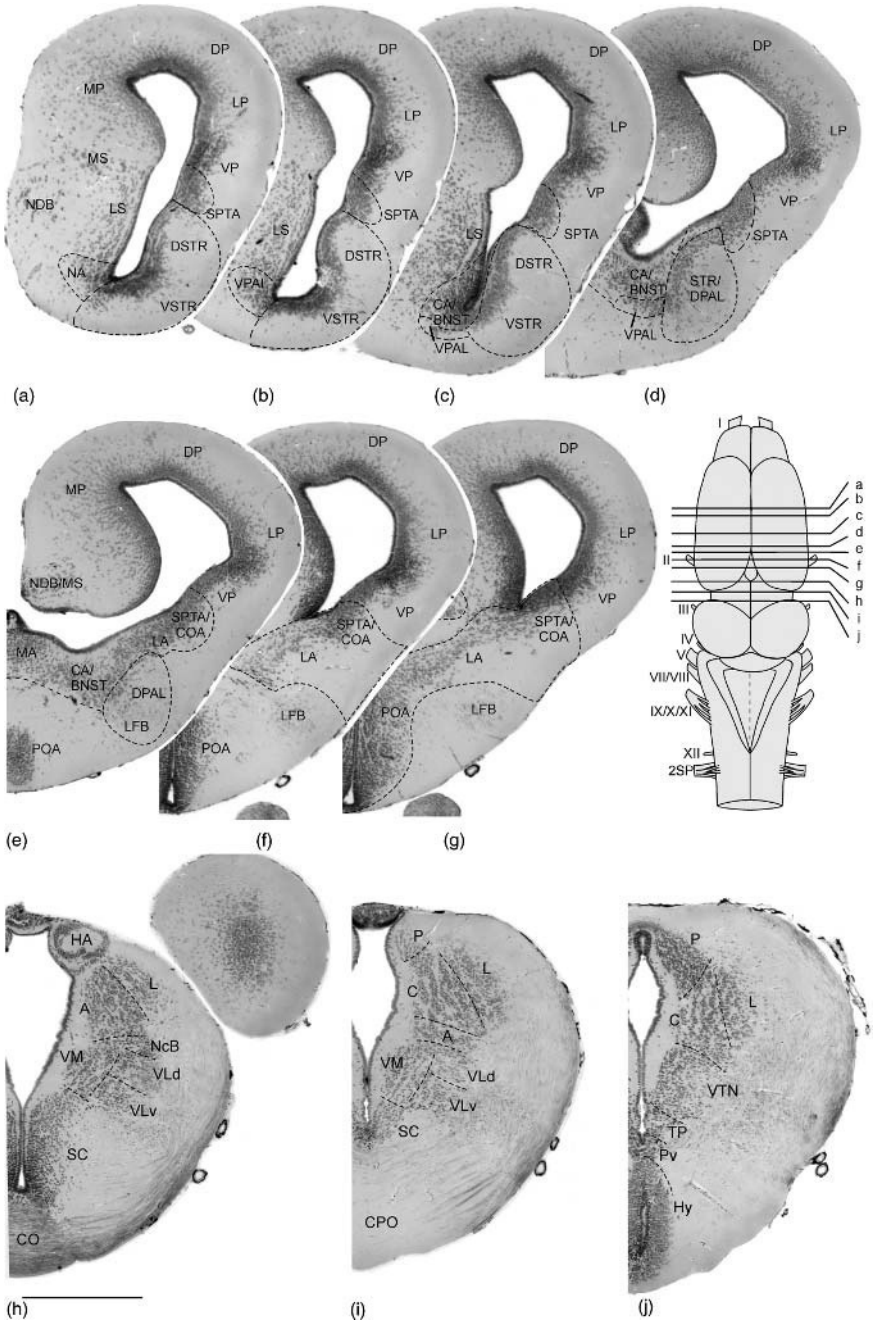

Figure 17 a j, Transverse sections (Kluver Barrera staining) through the telencephalon and diencephalon of *Bombina orientalis* at levels indicated in inset. Anterior mid-telencephalon (a), intermediate mid-telencephalon (b), posterior mid-telencephalon (c), anterior caudal telencephalon (d), mid-caudal telencephalon at the level of the foramen interventriculare (e), posterior caudal telencephalon at the level of the telencephalic commissures (f), posterior caudal telencephalon at the level of the magnocellular preoptic nucleus (g), anterior thalamus at the level of the optic chiasm and habenula (h), central thalamus at the level of the posterior commissure (i), and caudal thalamus at the level of the postoptic commissure (j). I, II, III, IV, V, VII, VIII, IX, X, XI, XII, cranial nerves; 2SP, second spinal nerve; MP, medial pallium; MS, medial septum; NDB, nucleus of the diagonal band of Broca; LS, lateral septum; NA, nucleus accumbens; VSTR, ventral striatum; DSTR, dorsal striatum; SPTA, striatopallial transition area; VP, ventral pallium; LP, lateral pallium; DP, dorsal pallium; CA, central amygdala; BNST, bed nucleus of the stria terminalis; VPAL, ventral pallidum; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala; STR, striatum; DPAL, dorsal pallidum; LFB, lateral forebrain bundle; MA, medial amygdala; POA, anterior preoptic area; HA, habenula; VLd, dorsal portion of the ventrolateral nucleus; VLv, ventral portion of the ventrolateral nucleus; VM, ventromedial thalamic nucleus; SC, suprachiasmatic nucleus; L, lateral dorsal thalamic nucleus; A, anterior dorsal thalamic nucleus; CO, optic chiasm; CPO, postoptic commissure; C, central dorsal thalamic nucleus; VTN, ventral thalamic nucleus; TP, posterior tubercle; Pv, paraventricular organ; Hy, hypothalamus; P, posterior dorsal thalamic nucleus; NcB, nucleus of Bellonci. Scale bar: 500 μm. Modified from Roth *et al.* (2003, 2004).

periventricular nuclei, i.e., an anterior, a central, and a posterior (pretectal) nucleus, as well as of a migrated lateral nucleus with an anterior and posterior subdivision. The ventral thalamus consists of a periventricular ventromedial nucleus and a number of migrated nuclei, i.e., a ventrolateral nucleus subdivided into a dorsal and a ventral portion, a dorsally situated NB, and a superficial ventral nucleus (Neary and Northcutt, 1983; Roth *et al.*, 2003).

In salamanders, the dorsal and ventral thalamus consists – like the entire diencephalon – of a more or less compact cellular layer surrounding the third ventricle, with very few, if any, cells found in the white matter, and nuclei cannot be distinguished by morphological boundaries. However, on the basis of retrograde and anterograde tracing and intracellular labeling experiments, anterior, central, and posterior zones can be distinguished, with projection patterns that correspond to those found in anurans, but show great overlap. In the ventral thalamus, neurons with different projection patterns can be distinguished, which likewise correspond with the ventral thalamic divisions of anurans, but do not form distinct nuclei (Roth and Grunwald, 2000).

Sensory afferents to the thalamus include (1) primary visual afferents from the optic nerve and secondary visual afferents from the tectum, (2) secondary auditory afferents from the midbrain torus semicircularis, and (3) somatosensory and vestibular afferents from the spinal cord and the Vth and VIIth cranial nerves. As described above, the optic nerve/tract forms two neuropils in the thalamus, i.e., the NB and the CGT (Scalia *et al.*, 1968; Scalia and Gregory, 1970; Scalia, 1976; Levine, 1980; Roth *et al.*, 2003) (Figures 5 and 6). These two neuropils occupy most of the lateral white matter leaving a narrow zone between them and the gray matter of the thalamic nuclei. Here and in the entire lateral zone occupied by the retinofugal fibers, afferents from the optic tectum terminate (Dicke, 1999).

In the thalamus of *R. pipiens*, intensely labeled GABA-immunoreactive neurons and fibers were observed within the NB and CGT. In the pretectum, the posterior thalamic nucleus contained the most intensely labeled GABA-immunoreactive perikarya and fibers in the entire brain (Li and Fite, 1998). In *R. catesbeiana* and *X. laevis*, GABA-ir somata were distributed throughout the different areas of the thalamus. At mid-thalamic level, GABA-ir somata were arranged in columns that extended through the anterior, ventrolateral, and ventromedial thalamic nuclei (Hollis and Boyd, 2005). In the pretectum of *S. salamandra*, the transition zone of the dorsal to the ventral thalamus is almost completely devoid of GABA-ir cells (Naujoks-Manteuffel *et al.*, 1994), whereas the ventral thalamus contains a substantial number of GABAergic cells.

Numerous neurons stained for the enzyme NADPH-diaphorase were localized in the dorsal anterior, lateral anterior, central, and lateral posteroventral thalamic nuclei of *R. perezi*, and highly labeled terminal fields were found in the NB, the CGT, and the superficial ventral thalamic nucleus. The distribution of labeled cells did not correspond to any single known neurotransmitter or neuroactive molecule system. Abundant co-distribution of NADPH-diaphorase and catecholamines was found; double labeling techniques revealed restricted co-localization in the same neurons (Muñoz *et al.*, 1996).

Anterograde and retrograde tracing and intracellular labeling studies (Roth and Grunwald, 2000; Roth *et al.*, 2003; G. Roth *et al.*, unpublished data) reveal the projection pattern of dorsal thalamic neurons in anurans and urodeles (summary in Figures 18a–18c). These studies confirm earlier findings that the anterior nucleus or zone of the dorsal thalamus projects bilaterally or ipsilaterally via the medial forebrain bundle to the ventromedial, medial, dorsal, and dorsolateral part of the telencephalon (Kicliter, 1979; Neary, 1984) (Figures 19 and 20). Inside the anterior dorsal thalamic nucleus, the majority of neurons innervate the entire medial, dorsal and lateral pallium and most strongly the rostral portion of the medial and dorsal pallium. Many fibers form a dense terminal neuropil around the cellular prominence at the level of the sulcus rhinalis dividing the lateral and ventral pallium (Figure 15a, right). This thalamopallial tract as part of the medial forebrain bundle sends collaterals to the central and medial amygdala, nucleus accumbens, and septal nuclei (Figure 15a, right). A subpopulation of anterior dorsal thalamic neurons targets only the superficial neuropil in the ventral portion of the rostral medial pallium. These two pathways appear to supply sensory information to the pallium (with the exception of olfaction), because stimulation of the optic nerve (visual), spinal nerve (somatosensory), and torus semicircularis (auditory) elicits evoked potentials with shorter latency and higher amplitude in the rostral two-fifths of the medial pallium, from where responses flow into the caudal medial pallium and the dorsal and lateral pallium (F. Laberge, unpublished results).

An additional thalamotelencephalic pathway originating in the anterior dorsal thalamus and to a lesser extent from a region below runs, via the lateral forebrain bundle, to the caudal lateral pallium – a region that receives input from the main olfactory bulb (Moreno and Gonzalez, 2004).

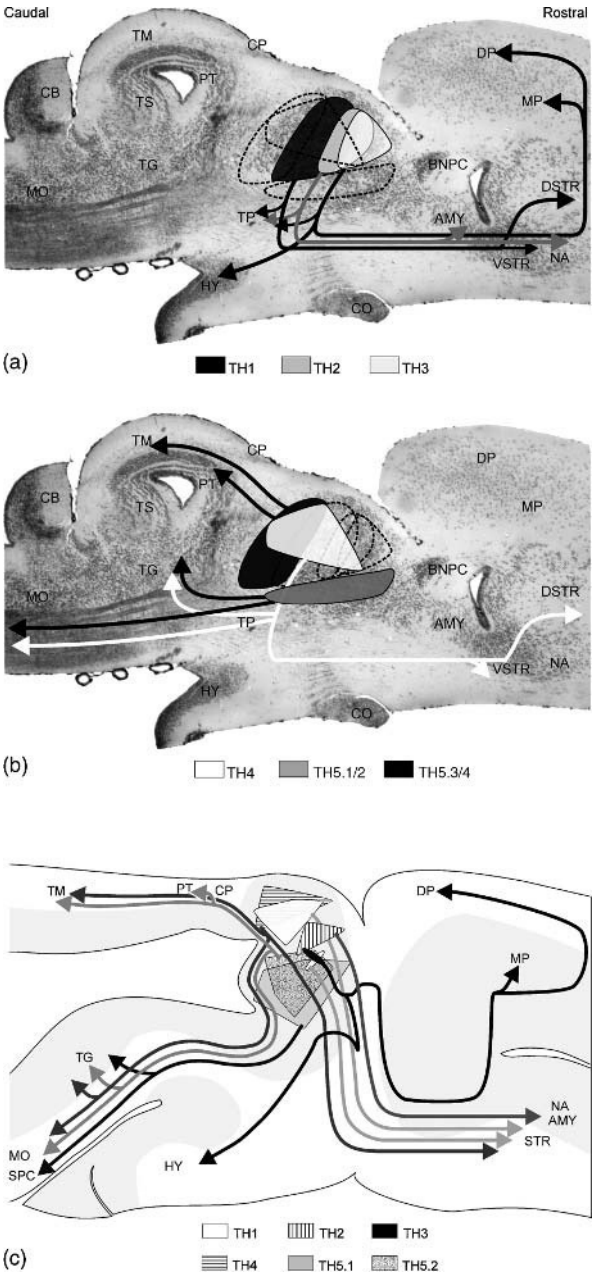

Figure 18 a, b, Diagrams of projection patterns of thalamic neurons combined with a lateral view of the caudal telencephalon, diencephalon, and mesencephalon in *Bombina orientalis*. c, Same in *Plethodon jordani*. CB, cerebellum; MO, medulla oblongata; TG, tegmentum; TM, mesencephalic tectum; TS, torus semicircularis; TP, posterior tubercle; CP, posterior commissure; DP, dorsal pallium; MP, medial pallium; BNPC, bed nucleus of the pallial commissure; DSTR, dorsal striatum; AMY, amygdala; NA, nucleus accumbens; VSTR, ventral striatum; PT, pretectum; CO, optic chiasm; Hy, hypothalamus; SPC, spinal cord. Modified from Roth and Grunwald (2000) and Roth *et al.* (2003).

The central dorsal nucleus/zone receives auditory input from the torus semicircularis (Hall and Feng, 1986; Mudry and Capranica, 1987; Feng *et al.*, 1990) and projects, via the lateral forebrain bundle, to the lateral amygdala and striatopallidum (Figure 21), where it contributes to the dense striatal neuropil (Figure 21B, right) (Wilczynski and Northcutt, 1983; Neary, 1988; Marín *et al.*, 1997a; Endepols *et al.*, 2004a). The ventromedial thalamic nucleus (probably homologous to the mammalian zona incerta), which receives auditory input from the torus semicircularis, likewise projects to the striatopallidum (frogs: Vesselkin *et al.*, 1980; Marín *et al.*, 1997a; Endepols *et al.*, 2004; salamanders: U. Dicke, unpublished data). The ventrolateral thalamic nucleus projects to the ventral pallium/SPTA. The lateral and posterior nuclei of the dorsal thalamus have no ascending but only descending projections to the pretectum, tectum, and tegmentum. (Roth and Grunwald, 2000; Roth *et al.*, 2003).

A long-standing question is whether the anterior and central nuclei/zones of the dorsal thalamus that project to the telencephalon receive direct or only indirect sensory afferents. Stimulation of the optic nerve in the salamander, *Plethodon*, and the frog, *Bombina*, elicited intracellular responses in the anterior dorsal thalamic nucleus, which showed either inhibition or excitation, but always at long latencies (about 35 ms on average). In contrast, short-latency, probably monosynaptic, responses could be recorded only from the ventral thalamus (Roth and Grunwald, 2000; Roth *et al.*, 2003) (Figure 22). Likewise, stimulation of the trigeminal and facial root (somatosensory and vestibular afferents) produced field potentials at long latencies in the anterior and central dorsal thalamus (Westhoff *et al.*, 2004), and the same holds for stimulation of the auditory pathway including the torus semicircularis (Mudry and Capranica, 1987; Hall and Feng, 1986; Feng *et al.*, 1990). Responses of neurons in the central dorsal thalamic nucleus at stimulation of auditory afferents show extreme habituation (Mudry and Capranica, 1987). This corroborates the view that the dorsal thalamus of frogs and salamanders does not receive substantial direct visual, auditory, somatosensory, and vestibular input. Rather, primary (only visual) or secondary (auditory, somatosensory) sensory afferents terminate in the ventral thalamus, which in turn projects to the dorsal thalamus (Dicke and Muhlenbrock-Lenter, 1998).

11.3.6.3 Response properties of thalamic neurons In anurans, thalamic and pretectal neurons were studied electrophysiologically in the toad, *Bufo americanus* and *B. bufo* (Ewert, 1971; Ewert and von Wietersheim, 1974). Ten classes of neurons were distinguished; eight of them responded to visual stimulation. One type of neuron responded best to large moving stimuli and had RF sizes between 30°

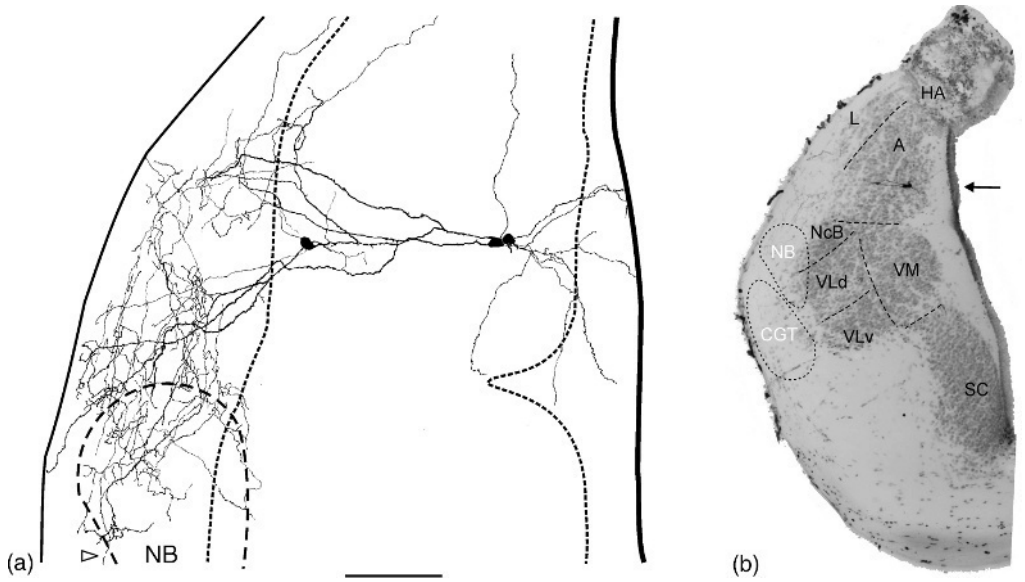

Figure 19 Camera lucida drawings of intracellularly labeled neurons in the dorsal thalamus of *Bombina orientalis*. a, Three neurons in the anterior dorsal thalamus, two of them in the periventricular anterior dorsal nucleus, with axons running to the medial and dorsal pallium, and one in the lateral anterior dorsal nucleus, without ascending projections. Only the dendrites of the latter enter the NB. The site of the neurons is indicated by a black arrow in (b). NB, neuropil of Bellonci; CGT, corpus geniculatum thalamicum; HA, habenula; A, anterior dorsal thalamic nucleus; L, lateral dorsal thalamic nucleus; NcB, nucleus of Bellonci; VLd, dorsal portion of the ventrolateral nucleus; VLv, ventral portion of the ventrolateral nucleus; VM, ventromedial thalamic nucleus; SC, suprachiasmatic nucleus. Scale bar: 100 μm. Modified from Roth, G., Grunwald, W., and Dicke, U. 2003. Morphology, axonal projection pattern and responses to optic nerve stimulation of thalamic neurons in the fire-bellied toad *Bombina orientalis*. *J. Comp. Neurol.* 461, 91 110.

and 46°. Another type responded selectively to motion; its RFs covered the contralateral or the entire visual field. Some of these neurons adapted quickly to stimuli, while others did not. Furthermore, one type of neuron responded best to stimuli approaching the toad along the *z*-axis, while another type represented luminance or darkness detectors.

Himstedt *et al.* (1987) studied the response characteristics of thalamic visual neurons in *S. salamandra* using a square, a horizontal, and a vertical rectangle as well as a random-dot pattern at different velocities. Neurons in the rostral thalamus, at the level of the NB and CGT, mostly had RF sizes between 36° and 50° and responded preferentially to the horizontal or vertical rectangle moved horizontally at low stimulus velocity. At intermediate velocity, most neurons responded best to the horizontal rectangle or square, and some responded best to the random-dot pattern. At high velocity, most neurons responded only to the horizontal rectangle or responded best to the square; one neuron responded best to the random-dot pattern. In the caudal dorsal thalamus, rostral to the pretectal neuropil and to the posterior commissure, RF sizes of neurons were comparable to those measured in the rostral thalamus. Likewise, in the majority of neurons the preferences of responses were similar to those of neurons in the rostral thalamus, but neurons did not respond to the random-dot pattern.

In summary, a topographically ordered representation of the visual space has not been reported in thalamic neurons. In general, the physiological properties of thalamic visual neurons differ from tectal neurons by their larger RFs and/or by profound adaptation to visual stimuli.

11.3.6.4 Hypothalamus The hypothalamus is divided into a preoptic and an infundibular region divided by the chiasmatic ridge (Neary and Northcutt, 1983). The preoptic region consists of the anterior preoptic area and the magnocellular preoptic nucleus. This nucleus is part of the cholinergic basal forebrain and also contains neurons belonging to the lateral, vomeronasal amygdala (see below). Therefore, it should be considered part of the telencephalon (secondary prosencephalon *sensu* Puelles, 1996). The suprachiasmatic nucleus is separated from the anterior preoptic area by a cell-free zone and is situated below the anterior thalamus and above the optic chiasm. Laterally, the posterior entopeduncular nucleus is found. The infundibular hypothalamus consists of a periventricular dorsal, ventral, and lateral nucleus composed of scattered neurons. Between the ventral hypothalamus and the ventromedial thalamic nucleus, the small posterior tuberculum and the equally small nucleus of the periventricular

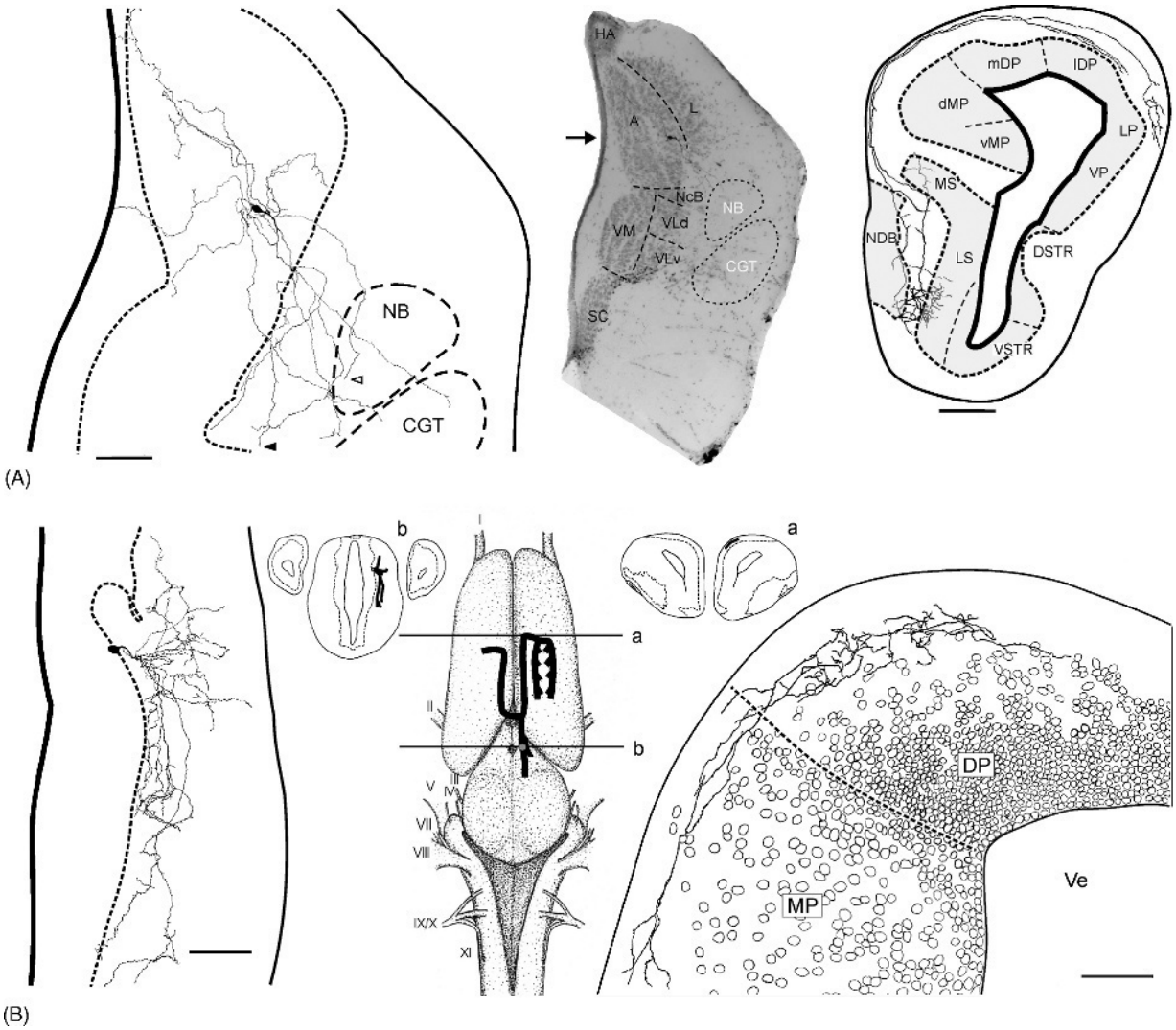

Figure 20 Camera lucida drawings of intracellularly labeled neurons in the anterior dorsal thalamus projecting to the medial and dorsal pallium of *Bombina orientalis* (A) and *Plethodon jordani* (B). In (A), middle, the site of the neuron is indicated by a black arrow; in (A), right, the course and terminal arborization of the axon in the septum, medial, dorsal, and lateral pallium is shown. In (B), middle, the site of the neuron (b) and the projection pattern of the neuron are indicated; in (B), right, the axon terminals in the medial and dorsal pallium at level (a) are shown. I V, VII XI, cranial nerves; NB, neuropil of Bellonci; CGT, corpus geniculatum thalamicum; A, anterior dorsal thalamic nucleus; HA, habenula; L, lateral dorsal thalamic nucleus; NcB, nucleus of Bellonci; VLd, dorsal portion of the ventrolateral nucleus; VLv, ventral portion of the ventrolateral nucleus; VM, ventromedial thalamic nucleus; SC, suprachiasmatic nucleus; NDB, nucleus of the diagonal band of Broca; LS, lateral septum; MS, medial septum; dMP, dorsal medial pallium; vMP, ventral medial pallium; mDP, medial dorsal pallium; lDP, lateral dorsal pallium; LP, lateral pallium; VP, ventral pallium; DSTR, dorsal striatum; VSTR, ventral striatum; Ve, ventricle; MP, medial pallium; DP, dorsal pallium. Scale bars in (A) left and (B) 100 μm, in (B) right 200 μm. Modified from Roth *et al.* (2003) and Roth and Grunwald (2000).

organ are found (Neary and Northcutt, 1983). The posterior tuberculum is considered to be homologous to the substantia nigra of mammals, because of the presence of dopaminergic neurons that show a distinct projection to the striatopallidal complex (Marín *et al.*, 1995).

The preoptic-hypothalamic region exhibits a pattern of wide connections, as revealed by biocytin tract tracing (Roth *et al.*, 2004) (Figure 23). The anterior preoptic area projects to the mediocentral amygdala, medial septum, nucleus of the diagonal band, suprachiasmatic nucleus, infundibular hypothalamus, ventral tegmentum, torus semicircularis, caudal tegmentum, and rostral medulla oblongata. Injection into the entire hypothalamus reveals projections to the periventricular zone of the central amygdala, ventral portion of the nucleus of the diagonal band,

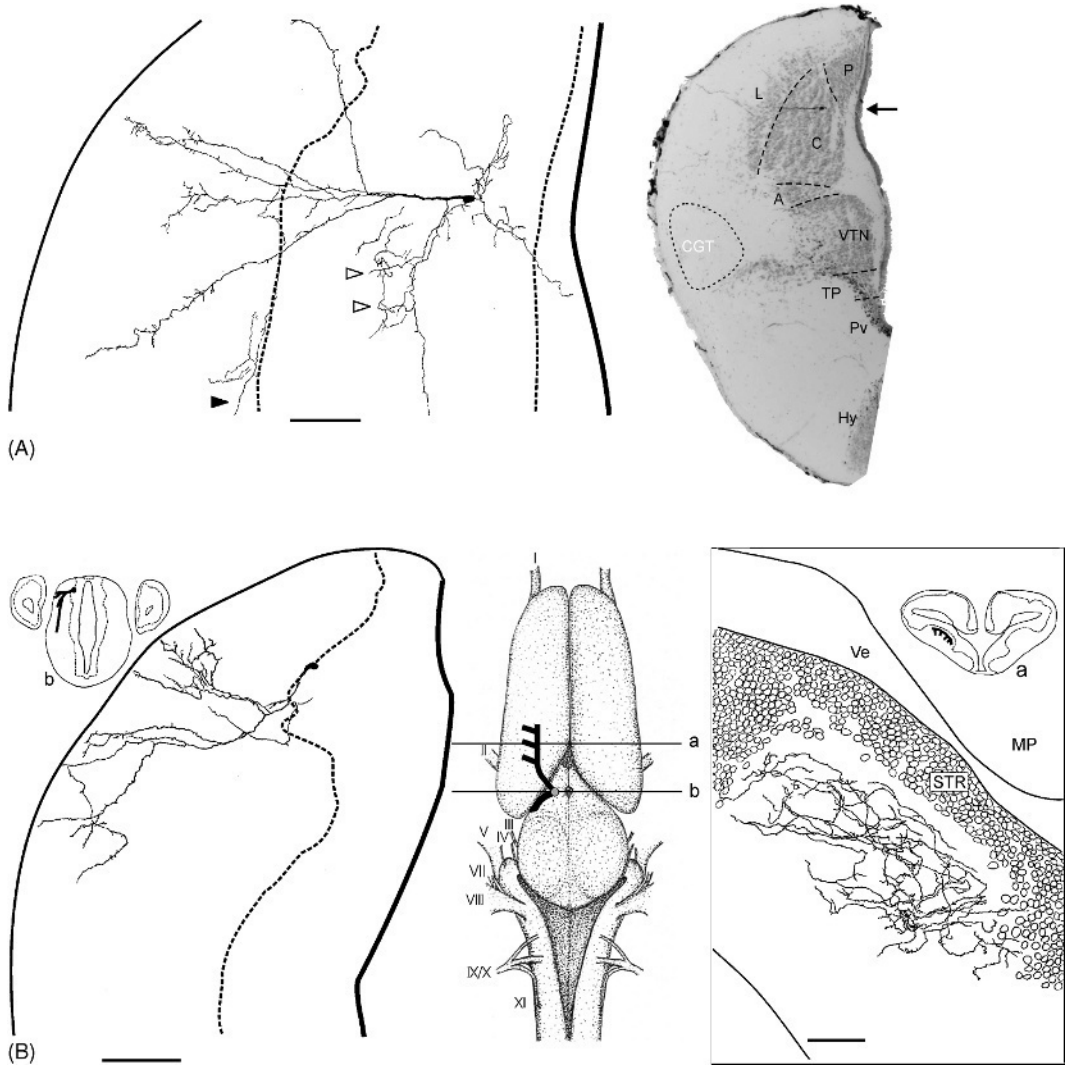

Figure 21 Camera lucida drawings of intracellularly labeled neurons in the central dorsal thalamus with ascending projections to the striatal neuropil in *Bombina orientalis* (A) and *Plethodon jordani* (B). Open arrowheads in (A) point to the ascending, black arrowhead to the descending axon. The site of the neuron is indicated by a black arrow in (A), right side. In (B), the site of the neuron is indicated by the left insert (b) and the projection pattern of the ascending axon in the middle with indications of the site of the striatal neuropil (a) and the neuron (a). Drawing in (B), right side, illustrates the terminal arborization of the axon inside the striatal neuropil at level a. I, II, III, IV, V, VII, VIII, IX, X, XI, cranial nerves; A, anterior dorsal thalamic nucleus; C, central dorsal thalamic nucleus; L, lateral dorsal thalamic nucleus; P, posterior dorsal thalamic nucleus; Pv, paraventricular organ; TP, posterior tubercle; CGT, corpus geniculatum thalamicum; Hy, hypothalamus; VTN, ventral thalamic nucleus; Ve, ventricle; STR, striatum; MP, medial pallium. Scale bars: 100 μm. Modified from Roth *et al.* (2003) and Roth and Grunwald (2000).

medial amygdala, the transition zone between medial pallium and dorsal septum, dorsal medial septum, nucleus accumbens, anterior preoptic area, and lateral (vomeronasal) amygdala; descending fibers run to the medulla oblongata and spinal cord. Injections of biocytin restricted to the dorsal hypothalamus reveals projections to the ventral pallidum, medial and central amygdala, ventral–lateral septum, caudal preoptic area, and suprachiatic nucleus.

Afferents to the anterior preoptic area originate in the nucleus of the solitary tract and the rostral medulla oblongata; the suprachiasmatic nucleus receives a sparse retinal input. The infundibular hypothalamus receives afferents from the ventral pallium, commissural medial pallium, medial and central amygdala, anterior preoptic area, suprachiasmatic nucleus, medial septum, nucleus of the diagonal band, lateral septum, lateral amygdala, magnocellular preoptic nucleus, dorsal and ventral thalamus, posterior tubercle, ventral tegmentum, torus semicircularis, locus coeruleus, and nucleus visceralis secundarius/parabrachial nucleus (Roth *et al.*, 2004).

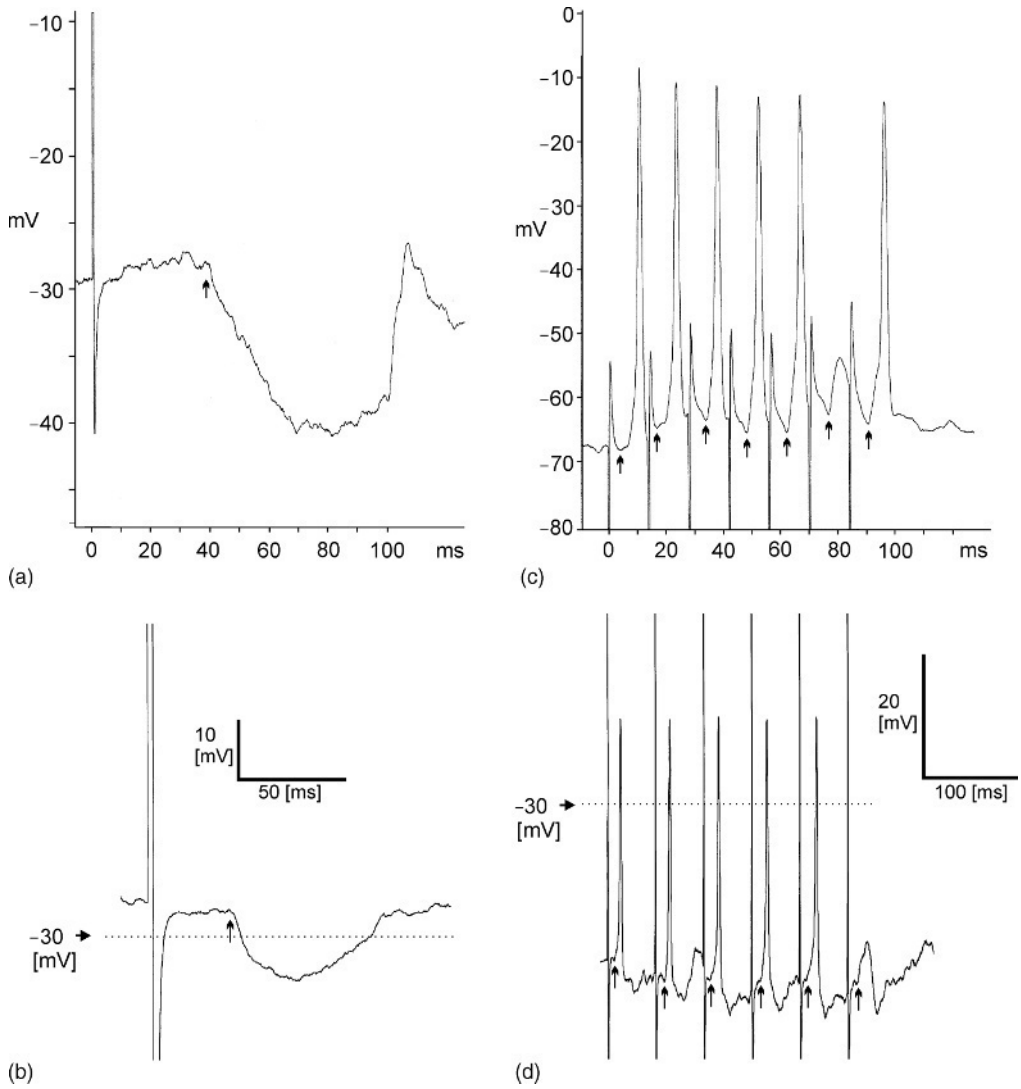

Figure 22 Responses of thalamic neurons to electrical stimulation of the optic nerve. Single sweeps are shown. Arrows indicate the onset of postsynaptic potentials. a, Inhibition in a dorsal thalamic neuron at long latency in *Bombina orientalis*. b, Same in *Plethodon jordani*. c, Short-latency response of a ventral thalamic neuron in *Bombina orientalis*. d, Same in *Plethodon jordani*. From Roth *et al.* (2003) and Roth and Grunwald (2000).

11.3.7 Telencephalon

The amphibian telencephalon, like that of all tetrapods, consists of pallial and subpallial regions (Figures 17a–17g). The pallium was traditionally divided into a medial, dorsal, and lateral pallium, but recent gene expression data led to a subdivision of the lateral pallium into a lateral and ventral pallium as different types of pallium (Puelles *et al.*, 2000; Puelles, 2001). Also, the rostral portion of the pallium appears to be a pallial region of its own. The subpallium includes a septal region below the medial pallium and a nucleus accumbens/ventral striatum situated in the rostral and central part of the ventromedial telencephalon, caudally tapering into a shell-like ventral pallidum situated below the septal region. The caudal ventral telencephalon of anurans is occupied medially and ventrally by the mediocentral amygdala and laterally by the lateral (vomeronasal) and cortical amygdala (Roth *et al.*, 2004). In salamanders, the mediocentral amygdala is situated more rostrally (Laberge and Roth, 2005). The striatopallidal complex is situated in the ventrolateral aspect of the telencephalon, bordered dorsally by the striatopallial transition area (SPTA).

11.3.7.1 Pallium

11.3.7.1.(i) Medial pallium The medial pallium of frogs and salamanders occupies the dorsomedial quadrant of the telencephalon and is characterized by extensive cell migration, which, however, does not

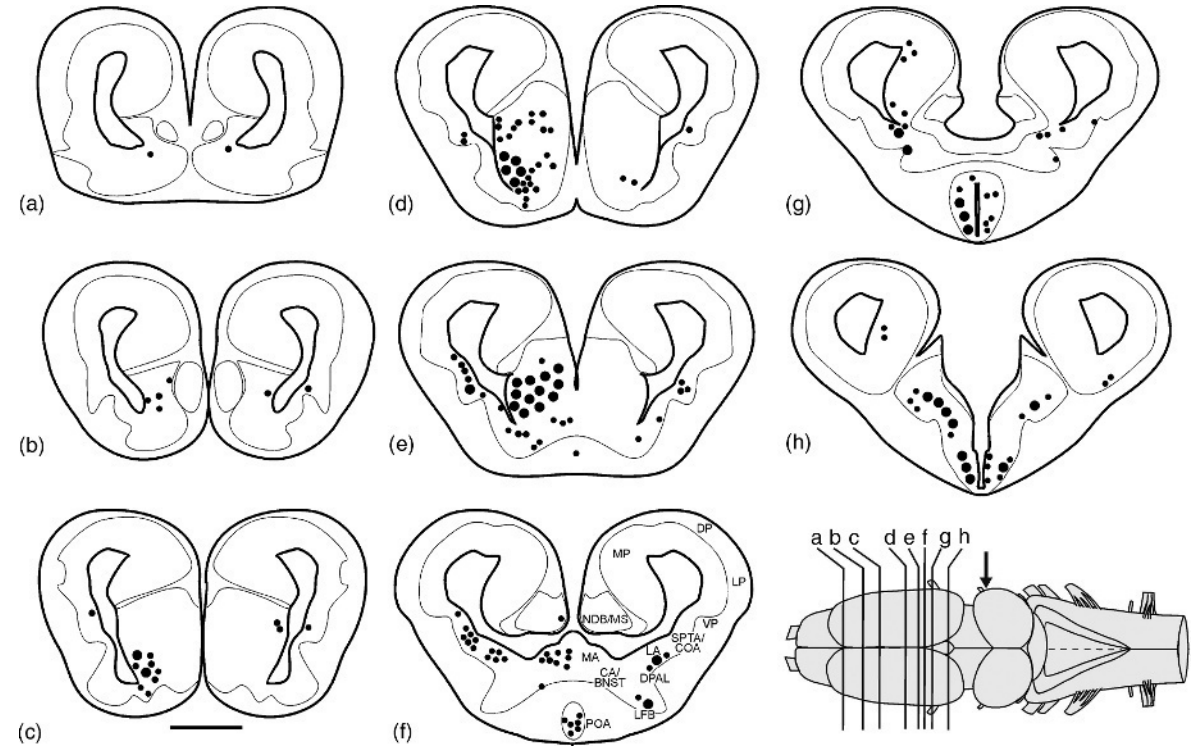

Figure 23 a h, Schematic view of ipsilaterally labeled cell bodies after tracer application to the hypothalamus. Left, neurons retrogradely labeled after application of biocytin to the entire hypothalamus. Right, labeled neurons after application to the dorsal hypothalamus. Levels of sections and of tracer application (black arrow) are indicated in the inset. Large black dots represent 10 cell bodies, small black dots a single cell body. MP, medial pallium; LP, lateral pallium; VP, ventral pallium; DP, dorsal pallium; NDB, nucleus of the diagonal band of Broca; MS, medial septum; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala; DPAL, dorsal pallidum; LFB, lateral forebrain bundle; BNST, bed nucleus of the stria terminalis; CA, central amygdala; MA, medial amygdala; POA, anterior preoptic area. Scale bar: 500 μm. Modified from Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35 61.

show any lamination. Ventrally, the medial pallium is confined by the zona limitans medialis – a cell-free zone separating the pallium from the septal region.

The dorsal border of the medial pallium of anurans is difficult to assess because of the gradual decline in cell migration toward the dorsal pallium. Consequently, some authors draw the border between medial and dorsal pallium more medially, i.e., at the level of the dorsomedial telencephalic sulcus (Hoffman, 1963; Northcutt, 1974; Scalia *et al.*, 1991) and others more laterally (Scalia, 1976; Northcutt and Kicliter, 1980; Neary, 1990; Northcutt and Ronan, 1992; Westhoff and Roth, 2002). After tracer application to the medial pallium, labeled neurons and fibers form a rather sharp border at the level of the dorsal sulcus (S. Mühlenbrock-Lenter, unpublished data). This would speak in favor of a more medial border between medial and dorsal pallium.

In the anuran medial pallium, authors distinguish either two subdivisions, i.e., a ventral small-celled part and a dorsal large-celled part (Röthig, 1912; Hoffman, 1963), or three subdivisions, i.e., a small-celled part, a large-celled part, and a transitional part (Neary, 1990). The medial pallium of salamanders has likewise been proposed to consist of two subdivisions, a ventral small-celled and a dorsal large-celled portion that might correspond to those observed in anurans (Herrick, 1933a; Northcutt and Kicliter, 1980; Neary, 1990).

Intratelencephalic afferents to the medial pallium originate in the dorsal and lateral pallium, mediocentral amygdala, nucleus accumbens, ventral pallidum and dorsal and central septal nuclei, the nucleus of the diagonal band, and the bed nucleus of the pallial commissure (Endepols *et al.*, 2005). Extra-telencephalic afferents come from the anterior dorsal thalamic nucleus, thalamic eminence, preoptic region, hypothalamus, periventricular organ, ventral tegmentum, locus coeruleus, raphe nucleus, and nucleus of the solitary tract (Roth *et al.*, 2004).

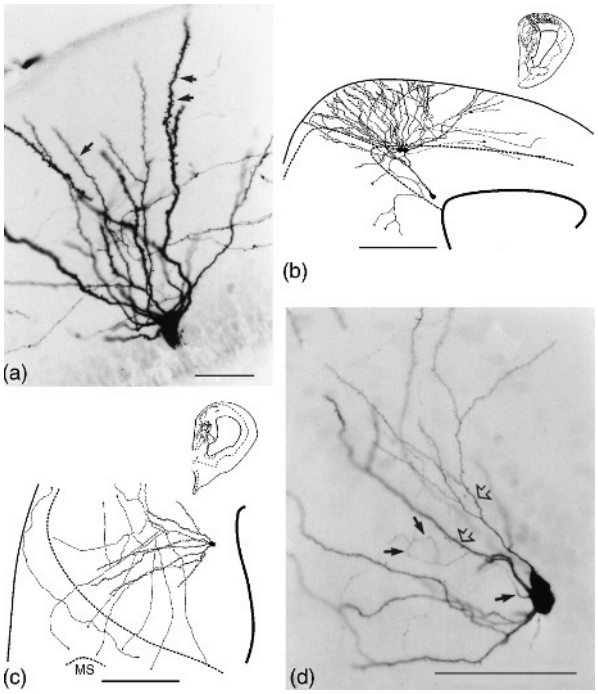

Figure 24 Microphotographs and reconstructions of intracellularly labeled neurons situated in the medial and dorsal pallium of *Discoglossus pictus* (a, c) and *Plethodon jordani* (b, d). a, Microphotograph of a cluster of two neurons situated in the dorsal pallium of *Discoglossus*; note that dendrites are heavily covered with spines (arrows). b, Camera lucida drawing of a cluster of neurons in the medial portion of the dorsal pallium (see inset) of *Plethodon*. c, Drawing of a type-1 neuron situated in the ventral half of the medial pallium of *Discoglossus* (see inset). d, Cluster of two neurons situated exactly at the border between medial and dorsal pallium, with one neuron in the medial and the other in the dorsal pallium. Dendrites of the dorsal pallial neuron are thinner than those of the medial pallial one (open arrows); filled arrows point to the primary axon of the medial pallial neuron. MS, medial septum. Scale bar: 50 μm in (a, d), 200 μm in (b, c). From Westhoff, G. and Roth, G. 2002. Morphology and projection pattern of medial and dorsal pallial neurons in the frog *Discoglossus pictus* and the salamander *Plethodon jordani*. *J. Comp. Neurol.* 445, 97 121.

Efferents of the medial pallium in the frogs *Discoglossus pictus* (Westhoff and Roth, 2002) and *Bombina orientalis* (G. Roth, unpublished data) have been studied by intracellular labeling (Figure 24). In *Discoglossus*, three types of medial pallial neurons were identified:

1. Neurons in the ventral medial pallium with bilateral projections to telencephalic areas including septum, amygdala, and striatum (weak), and diencephalic areas including the preoptic area, hypothalamus, anterior dorsal, and ventral thalamus (Figure 24c).
2. Neurons in the dorsal medial pallium with projections to the contralateral medial pallium and only ipsilateral projections to the dorsal and lateral pallium, septum, nucleus accumbens, amygdala, preoptic area, hypothalamus, anterior dorsal, and ventral thalamic nucleus.
3. Neurons at the border between medial and dorsal pallium with ipsilateral and contralateral projections to the medial and dorsal pallium, ipsilateral projections to the septum, and no extra-telencephalic projections.

In *Bombina*, of the neuron clusters labeled in the dorsal and intermediate medial pallium, all except one exhibited projections to the contralateral medial pallium and septum and ipsilateral projections to the dorsal pallium, septum, and nucleus accumbens. In addition, a substantial number of neurons projected to the dorsal portion of the ipsilateral lateral pallium. Two-thirds of them had extra-telencephalic to the suprachiasmatic nucleus, dorsal or ventral hypothalamus, and rostral tegmentum. Projections to the ventral pallium or to the dorsal portion of the striatopallidum originated only from neurons situated in rostral pallial regions. In contrast to the situation found in *Discoglossus*, neurons with and without extra-telencephalic projections showed no clear spatial separation. Of the 10 clusters labeled in the ventral-most part of the medial pallium, all projected to the dorsal septum and to the intermediate medial pallium, two to the dorsal medial and the medial part of the dorsal pallium, one to the nucleus accumbens, and four to the eminentia thalami. None projected to the contralateral side or to any extra-telencephalic target.

In the salamander, *Plethodon*, medial pallial neurons could be divided into a dorsal and a ventral group (Westhoff and Roth, 2002). Dorsal neurons project bilaterally to all telencephalic areas and to the preoptic area, ventral thalamus, and caudal hypothalamus. Ventral neurons project bilaterally to the medial pallium, medial septum, and nucleus accumbens, ipsilaterally to the dorsal pallium, and contralaterally to the anterior preoptic area and hypothalamus.

11.3.7.1.(ii) Dorsal pallium The delimitation of the amphibian dorsal pallium is likewise debated. While some authors denied the existence of a dorsal pallium in anurans (Kicliter and Ebbesson, 1976) or defined it as a narrow dorsal band between the medial and lateral pallium (Gaupp, 1899; Herrick, 1933b; Ariens Kappers *et al.*, 1936; Hoffman, 1963; Scalia *et al.*, 1991), other authors positioned the dorsal pallium (again as a relatively narrow band) more laterally, occupying the dorsal portion of the earlier lateral pallium (Northcutt, 1974; Northcutt and Kicliter, 1980; Northcutt and Ronan, 1992). Here, we adopt the above view that the border between medial and dorsal pallium is marked by the dorsomedial telencephalic sulcus and the border

between dorsal and lateral pallium by the rhinal sulcus.

The dorsal pallium consists of a periventricular cellular layer of densely packed somata and a number of migrated neurons which are substantial in number medially and decrease toward the lateral pallium. There is no sign of lamination.

Extra-telencephalic afferents to the dorsal pallium come from the anterior dorsal thalamic nucleus, hypothalamus, nucleus parabrachialis, and raphe nuclei (Roth *et al.*, 2003, 2004). Intra-telencephalic afferents originate mostly from the ipsi- and contralateral medial pallium and the ipsilateral lateral pallium, including the cellular prominence. Intracellular labeling experiments in the frogs, *Discoglossus* (Westhoff and Roth, 2002) and *Bombina* (G. Roth, unpublished data), reveal that dorsal pallial neurons in general lack extra-telencephalic and reveal only ipsilateral intra-telencephalic projections. They project ipsilaterally to the medial and lateral pallium and some of them also to the septal region, mostly to its dorsal part, as well as to the nucleus accumbens (Figure 3a).

In the salamander, *Plethodon* (Westhoff and Roth, 2002), neurons in the dorsal pallium are likewise defined by the absence of extra-telencephalic projections. Neurons in the medial part of the dorsal pallium project to the contralateral medial pallium and to the ipsilateral medial pallium, septum, nucleus accumbens, medial amygdala, and internal granular layer of the olfactory bulb (Figure 24b). Neurons in the lateral dorsal pallium have no contralateral projection; they project mainly to the ipsilateral medial pallium and about half of them to the ipsilateral septum. If we consider the medial portion of the dorsal pallium as representing the dorsal-most portion of the medial pallium, then the situation becomes similar in urodeles and anurans.

11.3.7.1.(iii) Lateral and ventral pallium
Traditionally, the lateral pallium has been divided into a dorsal and a lateral portion (cf. Kicliter and Ebbesson, 1976) divided by the rhinal sulcus and the lateral pallial cellular prominence. However, recent gene expression data demonstrate that these parts have to be considered different types of pallium called lateral and ventral pallium (cf. Puelles *et al.*, 2000; Puelles, 2001).

The lateral pallium receives essentially the same input as the dorsal pallium and some input from the ventral pallium as well. Intracellular labeling in *Bombina* (G. Roth, unpublished data) demonstrates that neurons in the precommissural lateral pallium are similar in morphology to those in the dorsal pallium and project to the dorsal and medial pallium and the dorsal septal region, often with extensive arborization, and a few axon collaterals extend to the ventral pallium. Neurons in the caudal, postcommissural portion of the lateral pallium likewise project to the dorsal and medial pallium, septum, and diagonal band of Broca. Neurons in the posterior part send axons rostrally at a subpial position inside the diagonal band of Broca, resembling the perforant path of mammals and terminating in the rostromedial pole of the telencephalon. In addition, neurons in the posterior part send dendrites and axons around the ventral caudal pole of the hemisphere accompanying the course of the lateral olfactory tract (olfactohabenular tract). This tract extends through the fiber layer of the lateral pallium close to the cellular prominence (and ventral to it); in the precommissural portion of the lateral pallium, it gives off only a few and in the postcommissural, caudal portion a substantial number of collaterals.

The ventral pallium is separated from the lateral pallium by the dorsolateral cellular prominence. The accessory (vomeronasal) olfactory tract runs through the periventricular cellular layer of the ventral pallium giving off numerous collaterals on its way to the vomeronasal amygdala. The ventral pallium terminates at the level of the pallial commissures where it merges with the vomeronasal amygdala. It receives intratelencephalic afferents from the dorsally adjacent lateral pallium, the caudal medial pallium, vomeronasal amygdala, nucleus accumbens, and striatopallidum. Extra-telencephalic afferents originate in the preoptic area, hypothalamus, anterior and central dorsal thalamus (weak), and the tegmental parabrachial nucleus (Moreno and González, 2004).

Intracellular labeling (Roth *et al.*, 2004, and unpublished data) reveals that neurons situated in the ventral pallium differ in morphology from those found in the lateral pallium in that they do not, or with only few dendrites, reach into the lateral pallium; rather, they extend their dendrites laterally or ventrolaterally (Figure 25). In most cases, ascending axons reach the accessory or main olfactory bulb; descending axons terminate in the neuropil of the vomeronasal and central amygdala, ventral pallidum, suprachiasmatic nucleus, and dorsal and ventral hypothalamus. Moreno and González (2004) consider the ventral portion of the ventral pallium (called SPTA by Marín *et al.*, 1998) a separate region, which they interpret as anterior amygdala (see below). However, there are no major differences in the morphology and projection pattern between the more dorsal portion of the ventral pallium and the SPTA (Roth *et al.*, 2004).

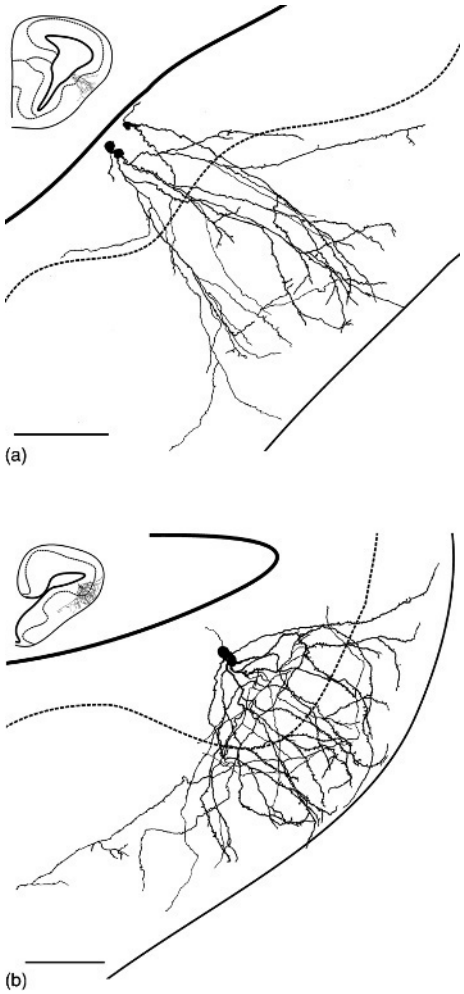

Figure 25 Reconstruction of an intracellularly labeled neuron situated in the ventral pallium/SPTA of *Bombina orientalis* (a) and *Plethodon shermani* (b). This type of neuron projects to the vomeronasal neuropil and the hypothalamus. Scale bar: 100 μm. From Roth *et al.* (2004) and Laberge and Roth (2005).

11.3.7.1.(iv) Rostral pallium Neurons situated in the rostral pole of the medial, dorsal, lateral, and ventral pallium receive the mass of dorsal thalamic afferents (see above) and differ in their projection pattern from neurons in more posterior regions in that all of them project to the main olfactory bulb and some of them to the SPTA and dorsal or dorsolateral edge of the dorsal striatal complex (G. Roth, unpublished data).

11.3.7.1.(v) Summary In summary, based on intracellular labeling, tract-tracing, and (immuno)-histochemical experiments, we can distinguish the following areas of the pallium of frogs and salamanders:

1. A medial pallium, which in many species is divided into a small-celled ventral and a large-celled dorsal portion. Neurons in this region, except those situated in the ventral part, generally have wide intra- and extra-telencephalic projections, the latter predominantly to the hypothalamus and ventral and dorsal thalamus. In frogs, ventral neurons, except those in the ventral-most part, exhibit mostly bilateral projections and dorsal neurons only ipsilateral extra-telencephalic projections. Most authors consider the dorsal portion of the medial pallium as homologous to the mammalian Ammon's horn and the ventral portion to the subiculum; a dentate gyrus seems to be missing.
2. A dorsal pallium separated from the medial pallium by the dorsal pallial sulcus. A dorsomedial portion (which could also be considered the dorsal portion of the medial pallium) contains neurons that have projections to the contralateral medial pallium via the anterior commissure and ipsilateral projections to the olfactory bulb to medial and ventral limbic centers and extra-telencephalic targets, mostly the preoptic region and hypothalamus; and a dorsolateral portion that contains neurons with projections confined to the ipsilateral dorsal septum, medial and lateral pallium, and are lacking extra-telencephalic projections.
3. A rostral-intermediate, or precommissural, lateral pallium, with neurons that project to the medial, dorsal, and ventral pallium and to the main olfactory bulb. The lateral olfactory tract runs through this region around the cellular prominence, but gives off only a few collaterals on its way to the caudal pallium.
4. A caudal, postcommissural, lateral pallium that receives massive input from the lateral olfactory tract and contains neurons that send their dendrites and axons along that tract (here the olfactohabenular tract) to the dorsal and medial pallium and to the entire septum.
5. A ventral pallium, including the SPTA containing neurons that project to the accessory olfactory bulb (AOB), the neuropil adjacent to the vomeronasal amygdala and preoptic region, striatopallidum, suprachiasmatic nucleus, and hypothalamus.
6. A rostral pallium occupying the rostral pole and projecting, unlike the medial, dorsal, and lateral pallium and like the ventral pallium, to the dorsal edge of the striatal complex.

The homologies and functions of the pallial regions mentioned are largely unclear. Most authors agree that the medial pallium is homologous to at least parts of the mammalian hippocampus,

i.e., Ammon's horn and subiculum, and that a dentate gyrus is lacking (for a more extended discussion see Westhoff and Roth, 2002). Extra- and intracellular recordings from neurons of the medial pallium in frogs including *B. orientalis* after stimulation of visual, somatosensory, and olfactory afferent pathways reveal only multimodal response properties (Supin and Guselnikov, 1965; Karamian *et al.*, 1966; F. Laberge and G. Roth, unpublished data). A few studies suggest that the medial pallium is involved in learning and memory formation (Finkenstädt and Ewert, 1988; Wenz and Himstedt, 1990; Papini *et al.*, 1995; Ewert *et al.*, 2001).

The function of the dorsal pallium is unclear. It receives essentially the same sensory and associative afferents as the medial pallium, but lacks extra-telencephalic and, with the exception of the dorsal septum, extra-pallial efferents. The response properties of neurons in the medial portion of the dorsal pallium are indistinguishable from those of the adjacent medial pallial neurons, which means that unimodal sensory areas are lacking. Thus, the dorsal pallium appears to have integrative-associative and limbic but no primary sensory functions.

The lateral pallium in the traditional sense (i.e., including the ventral pallium) is generally considered an olfactory pallium and homologous to the mammalian piriform cortex. However, although the zone around the cellular prominence dividing the lateral and ventral pallium receives collaterals from the lateral olfactory tract (originating in the main olfactory bulb), these collaterals are substantial only in the caudal, postcommissural portion. Thus, at least the caudal portion of the lateral pallium has to be considered olfactory pallium, while the function of the rostral and intermediate (precommissural) portion remains unclear.

The ventral pallium includes, together with the SPTA, the zone between the lateral pallium and the striatopallidal complex stretching from the olfactory bulb to the level of the telencephalic commissures, where it merges with the olfactory and lateral, vomeronasal amygdala. The ventral pallium is crossed by the accessory olfactory tract originating in the accessory olfactory or vomeronasal bulb; this tract gives off numerous collaterals on its way to the vomeronasal amygdala, where it forms a dense terminal neuropil. Thus, it is safe to consider the ventral pallium representing the vomeronasal pallium homologous to the mammalian posteromedial cortical amygdala (of pallial origin). Detailed functional studies are lacking.

11.3.8 Subpallium

11.3.8.1 Striatopallidal complex The dorsal striatum occupies the ventrolateral wall of the telencephalic hemisphere (cf. Figures 17a–17d). It is distinguishable from the ventral pallium/SPTA by neurons that extend their dendritic trees into the striatal neuropil (Roth *et al.*, 2003). The majority of the intracellularly labeled neurons are covered with spines and resemble the medium-spiny neurons of the mammalian caudate-putamen (cf., Heimer *et al.*, 1995) (Figure 26).

The dorsal striatopallidal complex receives afferents from a wide variety of brain regions including the medial pallium, ventral pallium-SPTA, vomeronasal amygdala, a number of mesencephalic and rhombencephalic nuclei, including the raphe nucleus, locus coeruleus, parabrachial nucleus, and the nucleus of the solitary tract, the hypothalamic-preoptic area, and the central dorsal and ventromedial thalamic nuclei (Marín *et al.*, 1997d). Efferents reach the medial and lateral amygdala, dorsal (sparse) and ventral thalamic nuclei, posterior tubercle, pretectum, tectum mesencephali, torus semicircularis, mesencephalic, and rhombencephalic reticular nuclei and the caudal brainstem (Marín *et al.*, 1997a). Endepols *et al.* (2004) as well as Roth *et al.* (2004) demonstrated that neurons with descending projections to the caudal tegmentum and rostral medulla oblongata are mostly found in the intermediate and caudal portion of the striatopallidal complex, and that more rostrally situated neurons project to the caudal portion of that complex (Figure 27). No projections to the medial or dorsal pallium are found.

The striatopallidum of amphibians and of tetrapod vertebrates in general (Reiner *et al.*, 1998) is characterized by the presence and co-localization of certain transmitters and neuropeptides such as glutamate, GABA, acetylcholine, dopamine, substance P, dynorphin, and enkephalin. GABAergic neurons are densely packed close to the ventricle, whereas cholinergic neurons typical of the amniote striatum are absent (Marín *et al.*, 1998; Mühlenbrock-Lenter *et al.*, 2005), but scattered ACh neurons are found in the dorsal pallidum (Marín *et al.*, 1998; Mühlenbrock-Lenter *et al.*, 2005). Thyroxin-hydroxylase-immunoreactive fibers indicating the presence of dopamine or noradrenaline are found in high concentrations in the cellular layer of the striatopallidum. Enkephalin-ir cell bodies are found in the rostral striatopallidum, i.e., the striatum proper. The striatal neuropil exhibits the highest density of Met+Leu-enkephalin-ir fibers, but many immunoreactive axons are found among cell bodies.

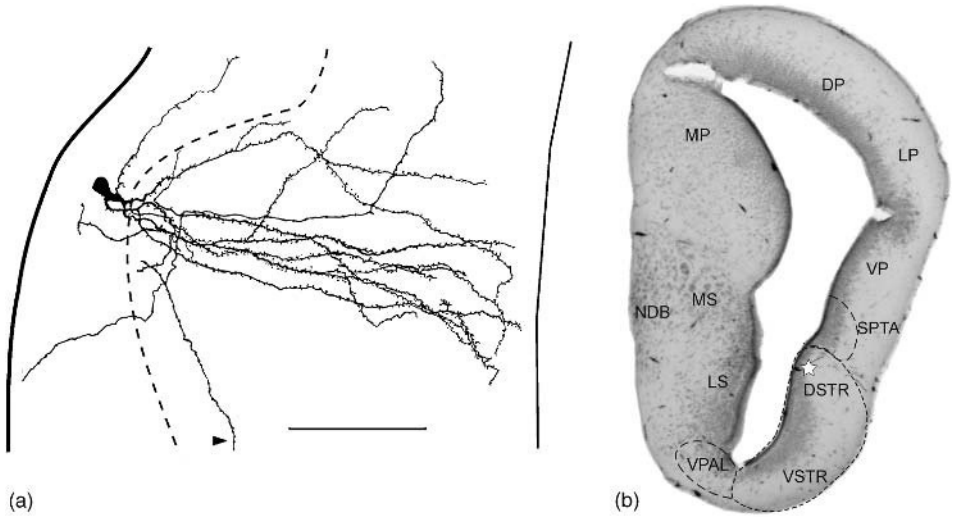

Figure 26 Reconstruction of an intracellularly labeled neuron situated in the striatopallidal complex of *Bombina orientalis*. Dendrites are heavily covered with spines. The site of the neuron is indicated by a white star in (b). This type of neuron has descending projection to the medulla oblongata. MP, medial pallium; MS, medial septum; NDB, nucleus of the diagonal band of Broca; LS, lateral septum; VPAL, ventral pallidum; VSTR, ventral striatum; DSTR, dorsal striatum; SPTA, striatopallial transition area; VP, ventral pallium; LP, lateral pallium; DP, dorsal pallium. Scale bar: 100 μm (a); 500 μm (b). From Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35 61.

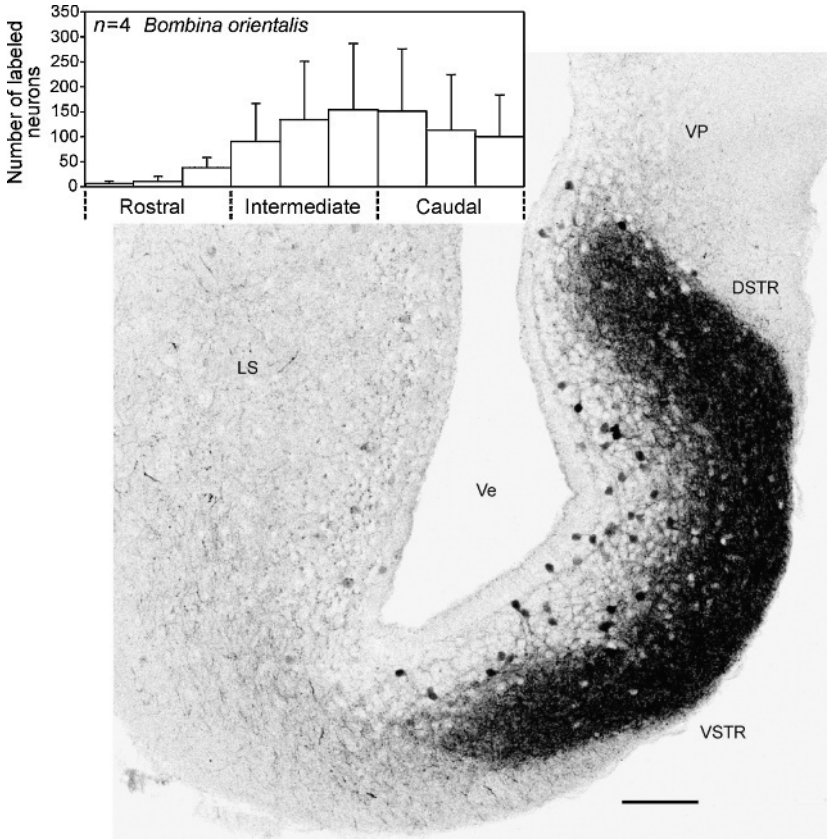

Figure 27 Inverted laser scanning image of labeled structures in the intermediate striatum of *Bombina orientalis* (transverse section) after tracer injection into the ipsilateral forebrain bundle. The inset demonstrates that the number of neurons with descending projections is low in the rostral and high in the intermediate and caudal portion of the striatopallidal complex; each column represents 150 μm. LS, lateral septum; Ve, Ventricle; VSTR, ventral striatum; DSTR, dorsal striatum; VP, ventral pallium. Scale bar: 100 μm. Modified from Endepols, H., Roden, K., and Walkowiak, W. 2004. Dorsal striatopallidal system in anurans. *J. Comp. Neurol.* 468, 299 310.

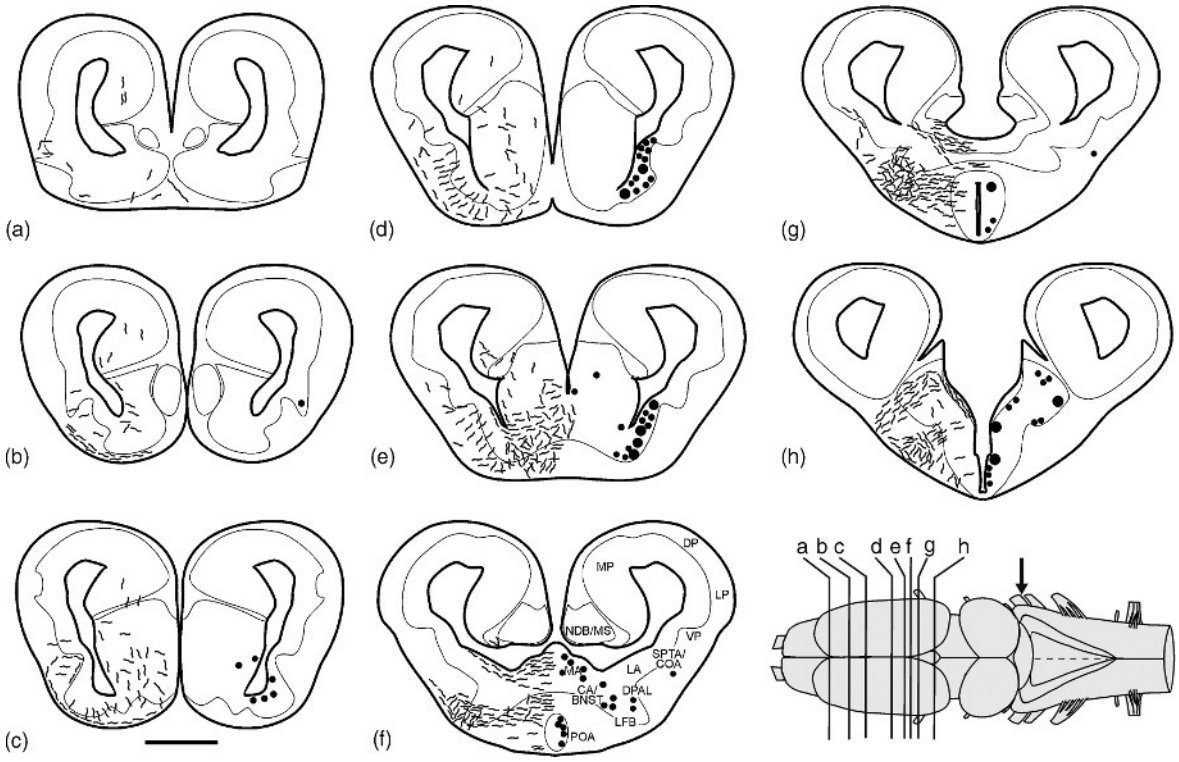

Figure 28 a h, Schematic view of anterogradely labeled fibers (left hemisphere) and retrogradely labeled cell bodies (right hemisphere) in the telencephalon of *Bombina orientalis* after tracer application to the rostral medulla oblongata. Levels of sections and site of tracer application (black arrow) are indicated in the inset. Large black dots represent ten cell bodies, small black dots a single cell body. MP, medial pallium; LP, lateral pallium; VP, ventral pallium; DP, dorsal pallium; NDB, nucleus of the diagonal band of Broca; MS, medial septum; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala; DPAL, dorsal pallidum; LFB, lateral forebrain bundle; BNST, bed nucleus of the stria terminalis; CA, central amygdala; MA, medial amygdala; POA, anterior preoptic area. Scale bar: 500 μm. Modified from Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35 61.

In contrast, Leu-enkephalin-ir fibers are sparse in the striatum proper. Its concentration increases along the rostrocaudal extent of the striatopallidum, with a maximum in the dorsal pallidum. Substance-P neurons are labeled in the rostral part of the striatal complex (Marín *et al.*, 1998; Endepols *et al.*, 2004; Mühlenbrock-Lenter *et al.*, 2005).

These immunohistochemical findings, together with the projection pattern of striatopallidal neurons mentioned, corroborates the view of Endepols *et al.* (2004) that the rostral portion of the dorsal striatopallidal complex can be regarded as dorsal striatum proper and the caudal portion as the dorsal pallidum, with a transition zone in between (Figure 28).In mammals, the striatum consists of functional compartments (striosomes and matrix) which differ in input and output as well as in immunohistological staining patterns (Graybiel and Ragsdale, 1978, 1983; Heimer *et al.*, 1995). Such compartments are absent in amphibians, but neurons and fibers of different immunoreactivity are arranged in layers (Mühlenbrock-Lenter *et al.*, 2005).

11.3.8.2 Ventral striatopallidal complex: nucleus accumbens/ventral striatum and ventral pallidum The ventral striatum *sensu* Northcutt and Kicliter (1980), i.e., the area ventrally adjacent to the dorsal striatopallidal complex (cf. Figures 17a–17d), most probably is not homologous to the ventral striatum of mammals. There is no difference in immunohistochemistry and the projection pattern between the dorsal and ventral parts of the dorsal striatopallidal complex (Mühlenbrock-Lenter *et al.*, 2005) and neurons in the two regions exhibit the same morphology and projection pattern (Roth *et al.*, 2004). Instead, the amphibian nucleus accumbens/ventral striatum is found in the medial rostral ventral telencephalon and extends caudally to what is now considered the ventral pallidum, which is confined to the superficial layer

surrounding the rostral central amygdala–BNST complex (BNST, bed nucleus of the stria terminalis) (Marín *et al.*, 1997a, 1997d, 1998; Roth *et al.*, 2004).

The nucleus accumbens receives afferents from the olfactory bulb, medial pallium, SPTA, medial amygdala, preoptic area and hypothalamus, dorsal and ventral thalamus, and posterior tubercle, i.e., from the same mesencephalic and rhombencephalic reticular nuclei that project to the striatopallidum, and from the anterior dorsal thalamic nucleus. Efferents run to the medial amygdala, preoptic area and hypothalamus, posterior tubercle, and to reticular brainstem nuclei (Marín *et al.*, 1997a, 1997d, 1998; Roth *et al.*, 2004).

In the ventral pallidum of anurans, as in other vertebrate species, a substantial number of numerous cholinergic neurons are found. Also, the nucleus accumbens/ventral striatum and the ventral pallidum are richly supplied by noradrenergic/dopaminergic fibers as well as by fibers containing substance P. In addition, somatostatin-ir fibers are found in the entire complex, and a strong enkephalinergic innervation exists in the rostral nucleus accumbens (Mühlenbrock-Lenter *et al.*, 2005). Nucleus accumbens/ventral striatum and ventral pallidum are closely interconnected (Marín *et al.*, 1997d; Roth *et al.*, 2004), but reveal only weak connections to the dorsal striatopallidum in the ventrolateral telencephalic wall – a situation similar to that found in mammals.

11.3.8.3 Septal region The amphibian septal region occupies the medial aspect of the telencephalon ventral to the medial pallium and dorsal to the nucleus accumbens/ventral striatum, ventral pallidum, and medial amygdala. Traditionally, the amphibian septal region is divided into a dorsally situated medial septum, a lateral septum bordering the ventricle and a nucleus of the diagonal band of Broca situated along the ventromedial surface of the telencephalic hemisphere (Ariens Kappers *et al.*, 1936; Kicliter and Ebbesson, 1976). Scalia (1976) distinguished a dorsal septum as a separate region. Additionally, the postolfactory eminence and the bed nucleus of the pallial commissure were thought to belong to the septum (Northcutt and Kicliter, 1980). More recent tracing studies distinguish (1) a medial complex consisting of a dorsally situated medial nucleus and a ventrally situated nucleus of the diagonal band of Broca, (2) a lateral complex consisting of a dorsolateral and a ventrolateral nucleus, and (3) a central complex consisting of a dorsal and a central nucleus. The postolfactory eminence, the bed nucleus of the pallial commissure, and the ventral portion of the septum are now excluded from the septal region (Endepols *et al.*, 2005; Roden *et al.*, 2005).

These two studies demonstrate that the central and medial septal nucleus receive direct input from the olfactory bulb, amygdala, and nucleus accumbens, whereas input from these regions to the lateral septal nucleus is less abundant or absent. The medial pallium projects to all septal nuclei, as does the anterior dorsal thalamic nucleus. The ventromedial thalamic nucleus/zona incerta of the ventral thalamus projects to the medial and lateral septal nucleus carrying visual, auditory, vestibular, and somatosensory information. The anterior preoptic, suprachiasmatic, and hypothalamic nuclei project to the central and lateral septal nucleus, and only the central septal nucleus receives input from the brainstem, particularly from the raphe nucleus (Roden *et al.*, 2005). All septal nuclei project to the medial pallium, the lateral and central nuclei, and to a lesser degree the medial nucleus projects to the olfactory nuclei, amygdala, nucleus accumbens, and hypothalamus, and the lateral septal nucleus also projects to sensory areas in the diencephalon and midbrain. Studies by Gonzalez and Lopez (2002) demonstrated that a cholinergic projection of the septum to the medial pallium is present in anurans, which the authors interpret as a forerunner of the mammalian cholinergic septohippocampal pathway. It appears that the amphibian septal region has essentially the same structural organization as the mammalian septum, but functional studies are lacking.

11.3.8.4 Amygdaloid complex The ventromedial, ventral, and ventrolateral part of the caudal telencephalon is occupied by the amygdaloid complex (cf. Figures 17c–17g, 22, and 27). Northcutt and Kicliter (1980), in their classical paper on the organization of the amphibian telencephalon, distinguished a medial amygdala caudal to the nucleus accumbens and ventral to the lateral septal nucleus, and a lateral amygdala starting rostrally as a lateral cellular prominence between lateral pallium and striatum, caudalward curving around the dorsal striatum in a C-shaped manner and eventually fusing with the anterior preoptic nucleus. The existence of a central amygdala, a BNST, and a dorsal and ventral pallidum was not discussed by the authors.

A new classification of the amygdaloid complex in anuran amphibians was presented recently by Marín and co workers on the basis of histochemical and immunohistochemical data in the frog *Rana perezi* (Marín *et al.*, 1998). In their opinion, the lateral amygdala occupies the dorsal portion of the ventral pallium situated above an anterior amygdala that occupies the ventral portion of the ventral pallium, previously called SPTA (see above). More caudally,

the ventral part of the ventral striatum *sensu* Northcutt and Kicliter is considered by Marín *et al.* (1998) the dorsal and ventral pallidum and the lateral part of that complex the central amygdala. The medial amygdala now occupies part of the lateral amygdala *sensu* Northcutt and Kicliter, but rostralward curves around the striatum and joins the lateral and the anterior amygdala in the above sense. The medial amygdala *sensu* Northcutt and Kicliter, plus the ventral lateral septum, now becomes the BNST plus the ventral pallidum. Thus, compared to Northcutt and Kicliter, the entire amygdaloid complex is shifted laterally and dorsally in Marín *et al.* (1998).

In order to clarify this situation regarding the components of the amphibian amygdaloid complex and its possible homologies with that of mammals, it is useful to use a functional approach (Swanson and Petrovich, 1998) and look for four different functional parts:

1. A part receiving direct input from the main olfactory bulb (cortical amygdala of mammals).
2. A part receiving direct input from the accessory or vomeronasal olfactory bulb and projecting primarily to the hypothalamus (posteromedial cortical and medial amygdala of mammals).
3. A part with reciprocal connections with visceral-autonomic centers in the mesencephalic tegmentum, brainstem, and spinal cord (central amygdala-BNST of mammals).
4. A part with close connections to the pallium/cortex (basolateral amygdala of mammals).

The vomeronasal amygdala can be identified in the amphibian brain by its massive input from the AOB via the accessory olfactory tract that forms a distinct terminal neuropil in the caudolateral part of the telencephalon and by its projection to the preoptic area and hypothalamus via the ipsilateral stria terminalis (cf. Figure 23) and to the contralateral vomeronasal amygdala via the anterior commissure (commissural portion of the stria terminalis). Neurons in the telencephalon of both frogs and salamanders that exhibit these characteristics form a band of neurons continuous with the SPTA. This band covers the area called lateral amygdala by Northcutt and Kicliter (1980) and then stretches to the magnocellular nucleus of the periventricular preoptic area (Roth *et al.*, 2004; Laberge and Roth, 2005). Neurons in the vomeronasal amygdala extend most of their dendrites into the terminal neuropil of the accessory olfactory tract (Figure 29)

Based on its connections to olfactory structures and to the hypothalamus (Neary, 1990; Bruce and Neary, 1995; Roth *et al.*, 2004; Laberge and Roth, 2005), the region in the ventral part of the caudal pallium dorsolateral to the vomeronasal amygdala can be considered the main olfactory amygdala (Figure 30).

The extended central amygdala (i.e., central amygdala plus BNST) can be identified by reciprocal connections with visceral-autonomic brain centers, e.g., preoptic area, hypothalamus, posterior tubercle, periaqueductal gray, parabrachial nucleus, nucleus of the solitary tract, and DCN (Saper, 1995; Alheid *et al.*, 1995; Pitkänen, 2000). In *Bombina*, neurons fulfilling these criteria occupy the caudal ventral telencephalon around the ventricle medial to the caudal pole of the striatopallidum (Roth *et al.*, 2004) (Figure 23). In *Plethodon*, this visceral-autonomic amygdala is situated more rostrally, extending ventral to the striatopallidum (Laberge and Roth, 2005). Neurons in this zone distinctly differ in the morphology of their dendrites from those belonging to the striatopallidal complex (Roth *et al.*, 2004; Laberge and Roth, 2005). Many of them have a peculiar morphology in that one part of the dendritic tree is directed dorsally toward the ventricle and another one in the opposite, ventral direction (Figure 31).

It is disputed whether amphibians possess an amygdaloid complex homologous to the mammalian basolateral amygdala and it is assumed to be of pallial origin. As mentioned above, Moreno and González (2004, 2006), based on tracing experiments, recently proposed that the ventral pallium above the SPTA (which is believed by the authors to represent the anterior amygdala) is homologous to the mammalian basolateral amygdala.

11.4 Phylogenetic and Evolutionary Considerations

11.4.1 The Nervous System of Amphibians: Primitive or Simplified?

Traditionally, brains are viewed as having increased continuously in functional and morphological complexity during vertebrate evolution (Ariens-Kappers *et al.*, 1936; Romer, 1970; Kuhlenbeck, 1977; Bauchot, 1978; Ebbesson, 1980, 1984). This unilinear view of evolutionary progress has now been replaced by the concept that vertebrates have evolved independently in a radiative manner (cf. Northcutt, 1985; Nieuwenhuys *et al.*, 1998). Thus, it is no longer appropriate to speak of primitive and advanced organisms, arranged along a ladder of increasing complexity. One refers instead to primitive (plesiomorphic) and derived (apomorphic) traits of a taxon and to the shared derived states (synapomorphies) that can be used to infer patterns of genealogical relationship (Hennig, 1966). Nevertheless, it is still widely accepted that within

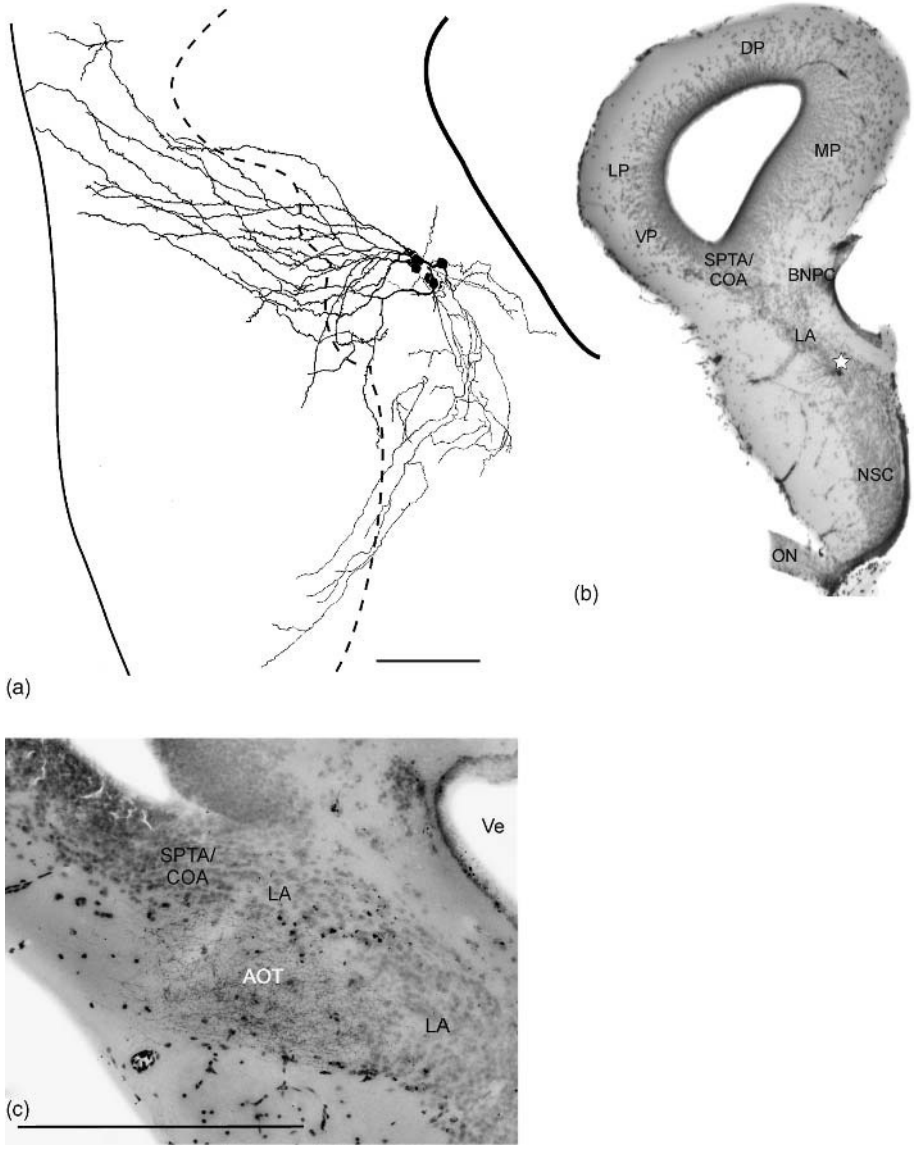

Figure 29 a, Camera lucida drawing of a cluster of intracellularly labeled neurons of the lateral, vomeronasal amygdala neuron of *Bombina* projecting to the rostral medulla. The majority of dendrites extend into the terminal neuropil of the accessory olfactory tract (cf. microphotograph in (c)), a minority into the preoptic region. In (b), the site of neurons is indicated by a white star. c, Microphotograph showing the terminal neuropil of the accessory olfactory tract (AOT) lateral to the vomeronasal amygdala. DP, dorsal pallium; LP, lateral pallium; MP, medial pallium; VP, ventral pallium; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; BNPC, bed nucleus of the pallial commissure; LA, lateral (vomeronasal) amygdala; NSC, suprachiasmatic nucleus; Ve, ventricle; AOT, accessory olfactory tract. Scale bars: 500 μm. Modified after Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35 61.

vertebrate classes more recent taxa (teleosts vs. chondrosteans, mammals and birds vs. reptiles and amphibians) possess relatively more complex brains.

In this context, the evolutionary status of the brains of amphibians has always created difficulties. The brains of frogs, and especially of caecilians (Kuhlenbeck, 1922) and salamanders (Herrick, 1948; Leghissa, 1962), appear to be simpler than those of chondrichthyans and osteichthyans, and even of cyclostomes in some respects. Despite an awareness that amphibians are tetrapods and thus not phylogenetically basal, their brains were viewed by leading comparative neuroanatomists as exemplifying the ancestral state of the vertebrate brain (Leghissa, 1962). However, Herrick (1948) suspected that the seemingly simple brains and sense organs of amphibians as well as many of their bodily characteristics had derived from a more complex

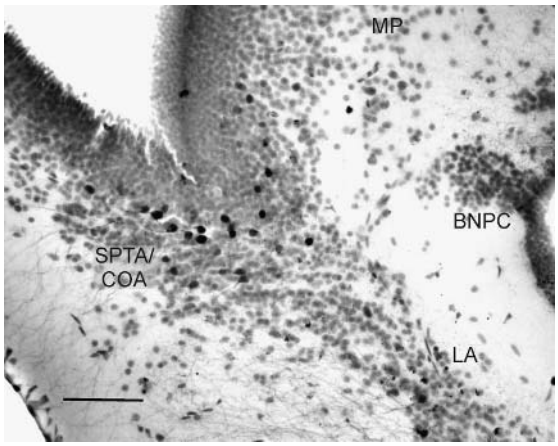

Figure 30 Microphotograph of a transverse section through the caudal telencephalon of *Bombina orientalis* showing retrogradely labeled neurons of the cortical (olfactory) amygdala after tracer application to the hypothalamus. For the level of section see Figure 28g. MP, medial pallium; BNPC, bed nucleus of the pallial commissure; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala. Scale bar: 100 μm. Modified from Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35 61.

ancestral state. This includes the reduction or loss of ossification, limbs, a free-living larval stage, reduction of the inner ear, the electroreceptive, lateral line, and auditory system in a number of frogs, many salamanders, and caecilians. At the same time, among frogs, salamanders, and caecilians, derived traits can be found.

Today, it is widely accepted that lissamphibians have undergone secondary simplification and that secondary simplification arises from 'pedomorphosis', a form of heterochronic evolution in which traits that characterize larvae or juveniles of ancestral taxa are maintained in the adult stage of descendant taxa (cf. Gould, 1977). Pedomorphosis commonly involves different degrees of retardation, reduction, or absence of traits in otherwise fully developed organisms, as compared with phylogenetic outgroups. Thus, a mosaic of fully adult traits, weakly expressed traits, and missing characters appears in terminal ontogenetic stages. Accordingly, amphibian brains are expected to have fewer cells, a lower degree of morphological differentiation of cells, and reduced migration, but retain the plesiomorphic structural, functional, and developmental organization found among other vertebrates. However, this process has affected the three amphibian orders differently: anurans appear to be least and salamanders most

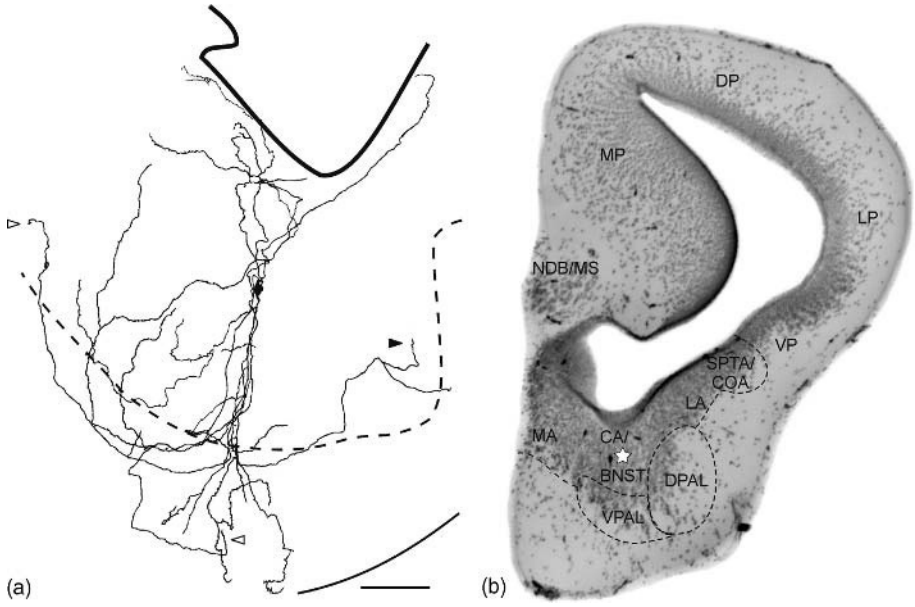

Figure 31 a, Camera lucida drawing of an intracellularly labeled neuron in the central amygdala of *Bombina orientalis* with an ascending projection to the ventral pallidum (open arrowheads) and a descending projection to the medulla oblongata (black arrowhead). Broken line indicates the border between gray and white matter. The site of soma is indicated in (b) by a white star. DP, dorsal pallium; LP, lateral pallium; MP, medial pallium; VP, ventral pallium; SPTA, striatopallial transition area; COA, cortical (olfactory) amygdala; LA, lateral (vomeronasal) amygdala; NDB, nucleus of the diagonal band of Broca; MS, medial septum; DPAL, dorsal pallidum; BNST, bed nucleus of the stria terminalis; VPAL, ventral pallidum; CA, central amygdala; MA, medial amygdala. Scale bar: 100 μm. Modified from Roth, G., Mühlenbrock-Lenter, S., Grunwald, W., and Laberge, F. 2004. Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*. An anterograde, retrograde and intracellular biocytin labeling study. *J. Comp. Neurol.* 478, 35 61.

pedomorphic, while caecilians exhibit an intermediate degree of pedomorphosis (Roth *et al.*, 1993). A similar situation is found in lungfishes, which as a group appear likewise to be pedomorphic; here, the Australian lungfish *Neoceratodus* is less pedomorphic and the lepidosirenid lungfishes profoundly pedomorphic (Northcutt, 1987; Roth *et al.*, 1993).

Among salamanders, the family Plethodontidae is the most specious one (comprising two-thirds of all salamanders in the world), and here the tribe Bolitoglossini (comprising half of salamander species in the world) exhibits many uniquely derived characteristics and has undergone a spectacular radiation in the Neotropics (Wake, 1966, 1987; Wake and Lynch, 1976). Bolitoglossines have developed a spectacular feeding mechanism, i.e., a projectile tongue, which is accompanied by the evolution of specialized characters of the visual system, e.g., in the context of depth perception (overview in Roth, 1987). At the same time, the brain, including the visual system, of plethodontid salamanders and bolitoglossines in particular has the simplest morphology. This includes a greatly reduced auditory system and nonexistent lateral-line system, a small number of large neurons in the sense organs and brain, a small proportion of myelinated fibers in the optic nerve, and a very low degree of cell migration throughout the brain and particularly in the tectum, consisting essentially of a periventricular cellular layer and a superficial fiber layer (Roth, 1987).

A phylogenetic analysis by Roth *et al.* (1993) based on 23 characteristics of the brain and sense organs of all groups of vertebrates came to the conclusion that 19 characters found in the salamander brains and sense organs, including the small number of types of RGCs, the low degree of myelinated fibers and the low degree of cell migration in the tectum, diencephalon, cerebellum, torus semicircularis, medulla oblongata, and spinal cord are most parsimoniously interpreted as secondarily simplified, while only one character appeared to be primitively simple (i.e., cell migration in the medial pallium) and in two cases (i.e., a low number of types of RGCs and a low degree of myelination of the nerve) the question of primitive versus simplified could not be decided. Of all salamander taxa, the Bolitoglossini, believed to be the most derived group, exhibit the most simplified brain and sense organs.

Reductions of brain and sense organs also appear to have occurred within gymnophionans and anurans. As a group, caecilians show reduction in 15 of 23 neuronal characters (Roth *et al.*, 1993). For example, whereas the so-called primitive caecilian *Epicrionops* possesses a multilaminated tectum, tectal lamination is greatly reduced in the derived taxon *Typhlonectes* (Himstedt and Manteuffel, 1985). Most frogs exhibit a multilaminated tectum, but in *B. orientalis* and the Australian frog, *Arenophryne rotunda*, both believed to be pedomorphic species, tectal lamination is substantially reduced (Roth *et al.*, 1994).

The hypothesis presented two decades ago by Roth and Wake (1985b) and now widely accepted is that secondary simplification in the salamander nervous system is related to enlarged genome and cell size (for a recent discussion, see Roth and Wake, 2000; Gregory, 2002a, 2002b). Genome size varies enormously among vertebrates. The smallest genome is found in teleost fishes, with less than 1 pg DNA per haploid nucleus. Some salamanders and all lungfishes have haploid genome sizes between 70 and 90 pg, which are the largest genomes found in any animals (Olmo, 1983). In salamanders, the smallest genome (13.7 pg) is found in the plethodontid *Desmognathus wrighti* (Hally *et al.*, 1986; Sessions and Larson, 1987) and the largest (83 pg) in the neotenic (perennibranchiate) *Necturus maculosus* (Olmo, 1983). The plethodontid salamander *H. italicus* (77 pg) has the largest genome of any terrestrial animal, although several tropical bolitoglossine plethodontids (e.g., *B. subpalmata*, 64 pg) approach this value (Sessions and Larson, 1987). Species of the Bolitoglossini, on average, have larger genome sizes than other plethodontids and than other salamander families, except for the perennibranchiate species (Olmo, 1983; Sessions and Larson, 1987). Caecilians also have relatively large genomes, but the largest known caecilian genome (13.9 pg per haploid nucleus) is equal to the smallest found in salamanders. Among anurans, the mean genome size reported by Olmo (1983) is 3.3 pg. The smallest (approximately 1 pg) is found in *Limnodynastes ornatus*, and the largest is found in *Arenophryne* (19 pg).

There is no universal agreement on the origin and significance of increased genome size in vertebrates (cf. Gregory, 2002a, 2002b). Apparently, genome size tends to increase until the tendency is halted by countervailing selection (Orgel and Crick, 1980). Among plethodontid salamanders, genome size appears to have increased many times independently, especially in the tribes Plethodontini and Bolitoglossini (Sessions and Larson, 1987). However, a phylogenetic analysis of the correlation between genome size and developmental rate concludes that several terrestrial plethodontid species have undergone a secondary reduction of genome size, which counteracts the general increase in genome size seen in terrestrial plethodontids. In the highly miniaturized *Thorius*, for example, the decrease is about 27% from the postulated ancestral bolitoglossine genome size of 34.5 pg (Sessions and Larson, 1987).

An increase in genome size has many important morphological consequences, including: (1) an increase in cell size, (2) a decrease in cell metabolic rate, (3) a decrease in cell division rate, and (4) a decrease in cell differentiation rate (Sessions and Larson, 1987). Compared to other vertebrates, salamanders in general and bolitoglossines in particular have large to very large cells, very low metabolic rates (Feder, 1983), and slow to extremely slow developmental rates (Sessions and Larson, 1987). The ova of plethodontine and bolitoglossine salamanders are large to very large (up to 9 mm in diameter), and they develop very slowly; *Bolitoglossa* may take 10 or more months to hatch (Hanken, 1979; Houck, 1982; Collazo, 1988).

While in amphibians the correlation between genome and cell size on the one hand and metabolic rate on the other is significant only at certain temperatures (cf. Licht and Lowcock, 1991; Gregory, 2002a, 2002b), increased genome and cell size is significantly negatively correlated with anatomical complexity of the brain and sense organs. Species with small genomes have more and smaller nerve cells per volume of gray matter, their neurons are more differentiated morphologically, and the number of migrated nuclei and the degree of lamination (e.g., inside the tectum) is higher than in species with larger genomes (Roth *et al.*, 1988a, 1990, 1994). As a consequence, anurans – with genome and cell sizes much smaller than salamanders (see above) – generally have more differentiated brains than salamanders. Among anurans and salamanders, taxa with large genomes and cells such as *Bombina* and *Arenophryne* or bolitoglossine salamanders have simpler brains than those with smaller genomes, if we disregard miniaturized taxa (Roth *et al.*, 1994). (Miniaturization is a process that independently leads to secondary simplification; Roth *et al.*, 1990.) The same holds for lungfishes; here *Neoceratodus* has a much smaller genome size and a more complex brain anatomy than lepidosirenids (cf. Roth *et al.*, 1993).

An increase in genome and cell size leads to profound retardation of brain development, but not all developmental processes are retarded to the same degree. As a rule, processes appearing late in ontogeny are more affected than those appearing early. Accordingly, the cerebellum – a structure that develops very late – is deeply affected by retardation. In frogs, the cerebellum is small and simple, in nonbolitoglossine salamanders it is even simpler, and the simplest is found in bolitoglossines. The same holds true for the ontogenetically late cell migration processes in the spinal cord, brainstem, torus semicircularis, tectum, and thalamus as well as the formation of anatomically distinct nuclei all over the brain, which are increasingly retarded and even truncated in parallel to the increase in genome and cell size. However, the degree to which the morphology of the amphibian telencephalon is primitive or secondarily simplified remains undecided. This topic is discussed further below.

A phylogenetic comparison of the amphibian CNS with that of other vertebrates is hindered by several facts. It is generally assumed that modern amphibians are closest to the ancestors of all tetrapod vertebrates, but the sister group of tetrapods and that of amphibians is not precisely known. Most presumably, these are extinct members of sarcopterygians, and within this group forms that are most closely related to the extant dipnoans. However, the brains of the majority of lungfish species, i.e., the African *Protopterus* and the South American *Lepidosiren*, are most probably secondarily simplified (Northcutt, 1987).

One of the most interesting aspects is that secondary simplification of sense organs and brain regions at a morphological level has not, or at least not obviously, affected their functions. This is most apparent in the case of bolitoglossine salamanders, which, on the one hand, exhibit the simplest sense organs and brains at a gross morphological level, and on the other hand the most refined prey-catching apparatus and associated neuronal control system (Roth and Wake, 2000).

11.4.2 Comparative Aspects

Differences between amphibians and lungfishes on the one hand and amniotes on the other are minor with respect to the spinal cord, medulla oblongata, midbrain, and the preoptic-hypothalamic diencephalic region (see Evolution of the Nervous System in Fishes). Major differences between amphibians and amniotes are found with respect to (1) the thalamopallial system, (2) the visual system, (3) pallial regions, (4) striatopallidum, and (5) the amygdaloid complex.

11.4.2.1 The thalamopallial system In a series of seminal papers, Butler (1994a, 1994b, 1995) argued that the dorsal thalamus of all jawed vertebrates consists of two divisions, i.e., a collothalamic and a lemnothalamic one. The 'collothalamic' division is characterized by a pathway that originates predominantly from the midbrain (tectum mesencephali, colliculi superiores, and colliculi inferiores in mammals) and projects to dorsal thalamic nuclei such as the nucleus rotundus of reptiles and birds and the posterior dorsal thalamic and intralaminar nuclei of mammals, which in turn send projections to the striatum via the lateral forebrain bundle. The 'lemnothalamic' division is characterized by a

pathway that includes predominantly sensory (lemniscal) afferents and projects to dorsal thalamic nuclei such as the mammalian LGN and the lateral geniculate nucleus of reptiles, which in turn project to the medial and dorsal pallium/cortex via the medial forebrain bundle. One characteristic of the lemnothalamus is a direct sensory (mostly retinal) input to the dorsal thalamic nuclei. Butler (1995) argues that both divisions of the dorsal thalamus were elaborated to some degree during amniote evolution. While diapsid and anapsid amniotes mainly developed the collothalamus (with some further specialization of the lemnothalamus in birds), the evolution of the mammalian brain was characterized by an enormous evolution of the lemnothalamus.

Along this concept, Puelles and collaborators (Puelles *et al.*, 2000), based on a series of experiments in the developing chick, as well as Puelles (2001), developed the idea that the dorsal thalamus of tetrapods is organized in three tiers, i.e., a dorsal tier characterized by the lemnothalamic visual LGN (with direct retinal input), an intermediate tier characterized by collothalamic nuclei such as the nucleus rotundus of birds and reptiles and the intra- and paralaminar nuclei in mammals, and a ventral tier containing the auditory medial geniculate nucleus of mammals and the nucleus ovoidalis complex of birds. The dorsal tier receives direct retinal input and projects to the medial and dorsal pallium/cortex, the intermediate tier receives predominantly tectal/superior collicular input and projects to the striatum and ventral pallium (and its derivatives), and the ventral tier receives input from the torus semicircularis/inferior colliculus and projects to specific auditory regions inside the anterior dorsal ventricular ridge (aDVR) in reptiles and birds and to the auditory cortex in mammals.

The dorsal thalamus of amphibians contains a central nucleus, which in some respects resembles the collothalamus *sensu* Butler, the ventral tier and intermediate tier *sensu* Puelles, and an anterior nucleus resembling the lemnothalamus and the dorsal tier. However, there are major inconsistencies regarding its lemnothalamic nature. The anterior dorsal thalamic nucleus of amphibians projects in a lemnothalamic, or first tier, fashion to the medial and dorsal pallium, but it receives only indirect retinal input via ventral thalamus, tectum, and central dorsal thalamic nucleus. It also sends collaterals to the medial amygdala and lateral septum, which is atypical for a lemnothalamic pathway. It is, therefore, safe to conclude that in amphibians a lemnothalamus in a strict sense does not exist.

The central dorsal nucleus of amphibians projects to the caudal ventral pallium (sparsely) and striatum (massively); also, it receives a projection from the torus semicircularis. Therefore, this nucleus can be regarded as a combined collothalamic nucleus of the intermediate and ventral tier in the sense of Puelles and is probably homologous to the nucleus rotundus of reptiles and birds.

In turtles (Hall and Ebner, 1970; Hall *et al.*, 1977; Zhu *et al.*, 2005) and lizards (Desfilis *et al.*, 2002), three dorsal thalamic nuclei exist that project to pallial–cortical structures and constitute the thalamocortical system of these taxa:

1. the dorsomedial anterior nucleus, which projects to the small-celled medial cortex;
2. the dorsolateral anterior nucleus, which projects to the large-celled dorsal (ventral) medial cortex; and
3. the LGN, which is the main retinorecipient nucleus and projects to the dorsal cortex via the medial forebrain bundle and to the pallial thickening via the lateral forebrain bundle (Zhu *et al.*, 2005).

The dorsomedial and dorsolateral anterior nuclei are multimodal, including visual afferents, but apparently without direct retinal input (Pritz, 1995). The three nuclei mentioned surround the large nucleus rotundus, which does not receive direct retinal but rather visual afferents from the tectum and projects to the anterolateral portion of the dorsal ventricular ridge, but not to the cortex.

It is still controversial whether the cortical areas mentioned project back to the dorsal thalamic nuclei from which they receive afferents (for a discussion see Zhu *et al.*, 2005), as is the case in mammals. The small-celled medial and the large-celled dorsomedial cortices are considered to be homologous to the mammalian hippocampus (possibly Ammon's horn region and dentate gyrus or subiculum), whereas the dorsal cortex is considered homologous to the mammalian isocortex. Auditory information from the torus semicircularis is relayed to the nucleus reuniens, and this nucleus projects to the ventral aDVR, but not to the cortex.

While the projection of the LGN to the dorsal cortex most probably represents a lemnothalamic pathway and the projection of the nucleus rotundus to the anterior dorsal ventricular ridge certainly a collothalamic pathway *sensu* Butler (1994b), it is unclear what kind of pathway originates from the dorsomedial and dorsolateral nucleus (Zhu *et al.*, 2005). Given that in reptiles these two nuclei apparently do not receive direct retinal input, they can only be considered to carry multimodal and limbic information and are not lemnothalamic in a strict

sense, but closely resemble the anterior dorsal thalamic nucleus of amphibians.

11.4.2.2 Pallium The pallium of amphibians is unlaminated, despite extensive cell migration in the medial and to a lesser degree in the dorsal and lateral pallium. In dipnoans, the pallium is relatively small and occupies the dorsolateral telencephalon. Laterally, it is divided from the striatum by the sulcus limitans pallii. In *Neoceratodus* and *Protopterus*, there is a thick periventricular layer and a thin layer of migrated cells. In *Neoceratodus*, a dorsal hippocampal pallium, an intermediate general pallium, and a piriform ventral pallium are distinguished, but there are no clear subdivisions (Nieuwenhuys, 1998). Unfortunately, no modern tracer studies exist on the afferents and efferents of pallial neurons in dipnoans. The reptilian pallium consists of two parts, the cerebral cortex and the dorsal ventricular ridge, unique to reptiles and birds. The cortex is divided into a medial, dorsomedial, dorsal, and lateral cortex plus a pallial thickening. The lateral cortex is the main olfactory cortex (ten Donkelaar, 1998). The medial and dorsomedial cortex receive olfactory information via the lateral cortex plus multisensory and limbic information via the dorsomedial and dorsolateral thalamic nuclei.

The situation found in reptiles and amphibians regarding pallial regions is similar: the small-celled medial and the large-celled mediodorsal cortices of reptiles are largely homologous to the small-celled ventral and the large-celled dorsal portion of the medial pallium of amphibians, and the dorsal cortex of reptiles is largely comparable to the dorsal pallium of amphibians. These regions receive multimodal sensory and limbic afferents from the anterior dorsal thalamus, which in reptiles comprise the dorsomedial and dorsolateral nuclei. The only major difference consists in the existence of a lemnothalamic sensory relay nucleus in the dorsal thalamus of reptiles, the LGN, which receives direct visual input and projects in parallel to the dorsal cortex and to the pallial thickening; such a lemnothalamic nucleus does not exist in amphibians. However, a strict homologization of the LGN of reptiles and the LGN of mammals is problematic, because in the medial, dorsomedial, and dorsal cortex of reptiles, as in the medial and dorsal pallium of amphibians, no precise topographic, unimodal sensory maps have been found to date (see overview in ten Donkelaar, 1998).

The dorsal ventricular ridge (DVR, Johnston, 1923) is a structure uniquely found in reptiles and birds. It is divided into an anterior and a posterior part (aDVR, pDVR) separated by the anterior commissure (ten Donkelaar, 1998). The aDVR is divided into three longitudinal zones as main targets of ascending sensory pathways. Visual information reaches the lateral part, somatosensory information terminates in the central part of the aDVR, and auditory information in the medial part of the aDVR. The pDVR receives nontopographically organized multisensory limbic afferents from the dorsal thalamus. The nucleus sphericus receives the main olfactory and vomeronasal afferents and projects to the ventromedial hypothalamus and to the AOB.

At present, it is debated to which structure of the mammalian telencephalon the aDVR should be considered homologous. Bruce and Neary (1995) regarded the aDVR as homologous to the mammalian basolateral amygdala, whereas Striedter (1997) homologized it with the mammalian endopiriform nucleus/claustrum, and Puelles *et al.* (2000) argued that the ventral pallium gives rise to the endopiriform nucleus and lateral amygdala nucleus in mammals and to the sensory-recipient part of the aDVR in reptiles and birds. More recently, Molnár and Butler (2002) argued that a strict homologization between these structures in the mammalian and sauropsid brain is impossible, but that a field homology can be postulated between the aDVR of sauropsids and the claustrum-endopiriform nucleus plus the basolateral amygdala of mammals, both developing from the collothalamic lateral–ventral pallium.

Amphibians, like mammals, lack a dorsal ventricular ridge. A strict homology of the anterior ventral pallium of amphibians with the aDVR is unlikely, because the ventral pallium receives no substantial visual, auditory, and somatosensory input from the dorsal thalamus, but its dominant input comes from the AOB. Also, it lacks connections with the dorsal pallium. However, one could envision an evolutionary process during the transition from amphibian to reptilian ancestors, in which sensory afferents from the dorsal thalamus extend further rostrally into the anterior ventral pallium replacing the olfactory and vomeronasal input.

In summary, the amphibian pallium is likely to represent a situation prior the divergent evolution of the mammalian cortex and the reptilian–avian cortex aDVR. However, the precise functions of the amphibian pallial regions are unclear. First, inside the medial, dorsal, and lateral pallium of frogs and salamanders, only multimodal responses can be recorded (F. Laberge, unpublished data). Second, the large dorsal and lateral pallium have no extra-telencephalic projections, and the only extra-pallial projection reaches the septum. The extra-telencephalic projections of the medial pallium, which is assumed to be homologous to the mammalian hippocampus,

mostly reach the ventral thalamus and the dorsal and ventral hypothalamus, whereas projections to the dorsal thalamus and the brainstem are weak. The major output regions of the amphibian telencephalon are the nucleus accumbens, septal region, the centromedial amygdala, and the caudal striatopallidum. Whereas nucleus accumbens, septum, and centromedial amygdala have reciprocal connections with pallial regions, the striatopallidum does not. It remains to be investigated how in amphibians the pallium influences the striatopallidal motor output. Only the rostral and ventral pallium exhibit some projections to the striatopallidal complex.

An unsolved question is whether the amphibian pallial regions are primitive or secondarily simplified (cf. Northcutt and Kicliter, 1980). This question can be answered only by a phylogenetic analysis. Such an analysis is hindered by the fact that the dipnoans have pallial regions that exhibit a simple morphology and closely resemble those of amphibians, but themselves are secondarily simplified. The pallium of *Latimeria*, representing the sister group of dipnoans, is assumed not to be secondarily simplified, but likewise gives a primitive appearance (cf. Nieuwenhuys, 1998). This would suggest that the pallium of amphibians is primitive and not secondarily simplified or pedomorphic. However, even in the medial and dorsal pallium of dipnoans, we find an incipient lamination, which is likewise found in turtles and lizards, but completely absent in amphibians. Therefore, it is safe to assume that some lamination in the medial and dorsal pallium is a plesiomorphic feature of sarcopterygians and all tetrapods and was lost in amphibians.

11.4.2.3 The visual system In amphibians, like in all anamniote vertebrates as well as in lizards and turtles and partly in birds, the tectum is the major brain center for visual perception and visuomotor functions. In the amphibian tectum, localization and recognition of objects and depth perception take place, and separate pathways descend to premotor and motor centers in the brainstem and cervical spinal cord involved in the guidance of visual behavior. Ascending pathways run bilaterally to the dorsal and ventral thalamus. Unlike other jawed vertebrates, the amphibian tectum has no saccadic system, because eye movements do not exist in adult amphibians, but this probably is due to a secondary loss, because eye movements are present during ontogeny of amphibians with an aquatic or semiaquatic lifestyle. Also, directionally selective neurons are absent in the amphibian tectum, but exist in all amniotes.

On the basis of recent tract tracing and intracellular and extracellular recording experiments in frogs and salamanders presented above, it appears that the amphibian visual system is organized in essentially the same way as that of amniotes in the sense that object recognition is based on population coding and occurs in a parallel-distributed fashion simultaneously and subsequently at several to many visual centers. Interaction and modulation between these centers occurs to a larger extent, because they are interconnected by several feedback loops, and top-down influences are most likely (Roth *et al.*, 1998; Schuelert and Dicke, 2005). This pattern of interaction is paralleled by a complex chemoarchitecture.

In amphibians as well as in all other vertebrates studied, three separate retinotectal subsystems for object recognition exist, which process information about (1) size and shape, (2) velocity and movement pattern, and (3) changes in ambient illumination (such as that caused by large moving objects). These kinds of information are processed at the level of different types of RGCs and tectal neurons, as described above, in close interaction with neurons in other visual centers such as the nucleus isthmi or the thalamus. Accordingly, different types of tectal neurons receiving different retinal input give rise to separate ascending pathways to thalamic and eventually telencephalic associative and limbic centers and to separate descending pathways to different premotor and motor centers in the medulla oblongata and rostral spinal cord, where they meet other descending pathways from diencephalic and telencephalic centers such as the central amygdala, septum, and striatopallidum.

At least three major streams of information meet at premotor and motor levels in order to elicit the various steps of visually guided behavior:

1. Information about certain properties of the object perceived concerning size, contrast, color, shape, velocity, movement pattern, etc.
2. Information about the precise location of that object. Pathways (1) and (2) need to interact in order to fully identify visual objects, including their absolute size.
3. Information about the level of motivation, most probably coming from limbic telencephalic regions (amygdala, nucleus accumbens/ventral striatopallidum) and the hypothalamus. How these latter influences are mediated to the main visual center is under investigation.

These tectal pathways found in the amphibian visual system strongly resemble those found in amniote vertebrates. However, there are remarkable differences between amphibians and mammals. One

of them is the absence of a visual relay nucleus in the dorsal thalamus in amphibians that receives direct retinal input and projects monosynaptically to the cortex, and another is the presence or absence of primary and topographically organized visual areas in the cortex (the striate cortex). Birds have evolved, apparently independently, a similar system, i.e., a pathway from the retina to the nucleus geniculatus lateralis pars dorsalis, which in turn projects to the visual Wulst. The situation in turtles and reptiles is somewhat intermediate, because there is the LGN, which receives direct retinal input and projects both to the dorsal cortex and the pallial thickening. However, in neither area are retinotopically arranged visual areas found (for details see ten Donkelaar, 1998; Dubbeldam, 1998; Voogd *et al.*, 1998; see Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?).

11.4.2.4 Amygdaloid complex According to a recent concept developed by Laberge and Roth (cf. Laberge *et al.*, 2006), the amphibian amygdaloid complex is composed of an autonomic-visceral component equivalent to the mammalian extended central amygdala including the BNST and to the striatoamygdaloid transition (SAT) area of reptiles (Russchen and Jonker, 1988; Bruce and Neary, 1995; ten Donkelaar, 1998). There is a vomeronasal amygdala of pallial origin in the ventral caudolateral telencephalon including the caudal SPTA and homologous to the mammalian posteromedial cortical amygdala and to the nucleus sphericus and medial amygdala of reptiles. Possibly, in amphibians there is a ventromedially situated subpallial vomeronasal amygdala comparable to the mammalian medial amygdala (F. Laberge, unpublished observation). Also, there is an olfactory amygdala in the caudal lateral pallium homologous to the anterior and posterolateral cortical amygdala of mammals and the external and ventral anterior amygdala in reptiles (Lanuza and Halpern, 1998).

An unsolved problem is which part of the amphibian telencephalon is homologous or at least equivalent to the mammalian basolateral amygdala. Bruce and Neary (1995) as well as Marín *et al.* (1998) and Moreno and González (2003, 2006) assume that this is the case for the ventral pallium. Based on studies of gene expression pattern and topological position, it has been suggested that in vertebrates the ventral pallium is homologous (at least in the sense of a field homology) to the ventral part of the anterior dorsal ventricular ridge in birds and part of the claustrum and lateral amygdala in mammals (Brox *et al.*, 2002; Molnár and Butler, 2002). However, there are problems with considering the ventral pallium of amphibians homologous with the mammalian basolateral amygdala. The latter is characterized by strong reciprocal connections with the hippocampal formation and sensory, associative, and limbic cortical areas as well as receiving collothalamic input from the posterior dorsal thalamus (Alheid *et al.*, 1995; Pitkänen, 2000). In amphibians, there are no primary visual, auditory, and somatosensory pallial regions; furthermore, the anterior ventral pallium receives no or only very weak sensory input from the anterior dorsal thalamic nucleus and only sparse input from the central dorsal thalamic nucleus. Also, this part does not project back to the medial pallium considered homologous to the hippocampal formation and has no connections with the dorsal pallium. Therefore, it cannot exert multisensory integration in close interaction with cortical/pallial and hippocampal regions. The fact that it receives strong olfactory and vomeronasal input and that its efferents join the stria terminalis on its way to the hypothalamus is atypical of the mammalian basolateral amygdala. However, this does not exclude the possibility that during the evolution of the amniote brain this area eventually developed into a mammalian basolateral amygdala.

A region that at least partially fulfills these connectional criteria is the ventromedial portion of the ventral caudal telencephalon of anurans including the ventral-most portion of the lateral septum, traditionally called medial amygdala. This region receives multimodal sensory and limbic input from the anterior dorsal thalamus and projects heavily to the septum; the septum in turn projects to the medial and dorsal pallium. This medial amygdala also contains neurons that project directly to the medial pallium, from where it receives substantial input. On the other hand, it is entirely of subpallial and not of pallial origin, as is the case for the basolateral amygdala of mammals. Therefore, it appears that the medial amygdala of amphibians is not homologous but homoplastic to the basolateral amygdala, i.e., it is of different origin, while serving similar functions (Laberge *et al.*, 2006).

Thus, the evolution of a portion of the amygdala of pallial origin with strong reciprocal connections to sensory, associative, and limbic pallial–cortical areas appears to be a major step in the evolution of the amniote telencephalon, enabling the formation of new and more complex types of emotional learning. The amygdaloid complex found in amphibians with a vomeronasal, olfactory, and a mixed autonomic-visceral and associative amygdala certainly represents the ancestral tetrapod and perhaps vertebrate condition.

In this context, new findings demonstrate that in mammals not only the basolateral amygdala (as was assumed for a long time; cf. LeDoux, 2000), but also the central nucleus are the site of emotional conditioning, albeit in a simpler fashion (Everitt *et al.*, 2003; Paré *et al.*, 2004). This would be consistent with the view that the presence of a mixed autonomic-visceral-associative amygdala enables amphibians to develop simple forms of affects and emotions, while the formation of more complex emotions would be based on the evolution of a basolateral amygdaloid complex (Everitt *et al.*, 2003).

11.4.2.5 Striatopallidum The amphibian dorsal striatopallidal complex is divided into a rostral portion corresponding to the dorsal striatum of mammals, a caudal portion corresponding to the dorsal pallidum of mammals, and an intermediate portion with properties shared by both structures. Enkephalin-ir and substance P-ir neurons are mainly found in the rostral and intermediate part of the dorsal striatopallidal complex, while Leu-enkephalin-ir and serotonin-ir fibers are most abundant in the intermediate and caudal parts. Furthermore, there is a distinct dopaminergic input from the posterior tubercle, which is believed to be homologous to the substantia nigra pars compacta of mammals (Marín *et al.*, 1995). All this characterizes the amniote including mammalian dorsal striatopallidum (Mori *et al.*, 1985; Graybiel, 1990; Reiner *et al.*, 1998).

Major differences between the amphibian and mammalian dorsal striatopallidum consist in the following:

1. A low number of GABAergic neurons in the amphibian striatum (H. Endepols, unpublished data), while in mammals nearly all striatal output cells are GABAergic.
2. A lack of segregation into histochemically different patches and matrix typical of the mammalian striatum; however, in amphibians histochemically different layers can be observed.
3. The complete or nearly complete absence of cholinergic neurons, which in mammals are concentrated in the so-called matrix.
4. Only weak input from the rostral and ventral pallium in amphibians, while there is a strong cortical input to the striatum in mammals.
5. No or only a weak projection of the caudal striatopallidal complex to the dorsal thalamus.

As a consequence, the main motor output of the amphibian striatopallidum is the projection of the pallidum to brainstem and spinal cord motor regions (which, of course, is also present in mammals), rather than the corticospinal tract in mammals. Furthermore, a modulation of sensory and executive functions does not take place in the pallium/cortex, because a projection of the pallidum back to the cortex via ventral thalamic nuclei is lacking. Such a modulation appears to occur either via a projection of the pallidum to the ventral thalamus and from there to the tectum or to the tegmentum (equivalent of the substantia nigra pars reticulata) and from there to the tectum. The tectum, then, projects to the premotor and motor regions in the brainstem and rostral spinal cord.

The striatopallidum of reptiles appears to represent an intermediate evolutionary stage between amphibians and mammals (cf. Reiner *et al.*, 1998; Marín *et al.*, 1998). First, the reptilian striatum contains both GABAergic output neurons and cholinergic interneurons, both of which are either small in number or absent in amphibians. However, the cholinergic neurons are not arranged in a distributed island typical of the mammalian striatum. Second, the striatum of reptiles, like that of amphibians and unlike that of mammals, receives very little input from the cortex. Third, it is presently unclear whether reptiles possess thalamic motor nuclei comparable to the ventral anterior and ventrolateral relay nuclei of mammals, which receive projections from the dorsal pallidum and project to cortical regions with connections with the striatum. Thus, it appears that neither amphibians nor reptiles posses a re-entrant circuitry (dorsal loop) between pallium/cortex, striatopallidum, and thalamus.

11.5 Summary and Conclusions

In zoology and evolutionary biology, amphibians have always played a problematic role. Although modern amphibians, the *Lissamphibia*, are highly derived vertebrates with very little resemblance to the paleozoic ancestors of tetrapods, they usually are considered primitive vertebrates. This misunderstanding results at least in part from many features of sense organs and brains of amphibians appearing to be much simpler than those of nearly all other vertebrates. As discussed above, many of these features have undergone secondary simplification, especially affecting processes of morphological differentiation, formation of laminae, and anatomically distinct nuclei in the brain. Other features, especially those concerning the thalamus and telencephalon, appear to be primitive. Thus, sense organs and brains of amphibians represent a mixture of primitive and secondarily simplified traits.

In recent years, a large amount of new data on the morphology of the brains of frogs and salamanders using modern neuroanatomical methods has

accumulated. These studies demonstrated that the brains of frogs and salamanders possess nearly all the properties characteristic of the brains of amniotes. One main conclusion that can be drawn from these new insights is that in many aspects the brains of turtles and lizards are closer to that of amphibians than to mammals and birds. Major differences between amphibians on the one hand and mammals and birds on the other are the following:

1. Visual object recognition and visual guidance of behavior are mostly exerted by the retinotectopretectal system; a unimodal visual thalamotelencephalic system characteristic of mammals and birds is absent in amphibians and poorly developed in reptiles. Thalamic and telencephalic centers appear to exert a modulatory role.
2. The amphibian medial pallium is partly homologous to the mammalian hippocampal formation, but a dentate gyrus appears to be missing; the precise homologization of the medial and dorsomedial cortex of reptiles to the mammalian hippocampus is likewise unclear.
3. The amphibian dorsal pallium possesses no unimodal, topographically organized areas as found in the mammalian isocortex and the avian hyper-, meso-, and nidopallium (cf. Reiner *et al.*, 2004), and is most probably homologous to the limbic-associative cortex of mammals. The situation found in turtles and lizards is unclear, but the presence of unimodal and topographically organized areas has not been demonstrated in the dorsal cortex.
4. The amphibian striatopallidum receives input from the rostral and ventral pallium and projects to ventral thalamic nuclei, which however do not project to pallial–cortical areas connected with the dorsal striatum (the ventrolateral and ventral anterior thalamic nuclei of mammals). Such a dorsal loop appears to be missing in reptiles as well.
5. Pallial premotor and motor areas are likewise missing in amphibians as well as in lizards and turtles. The main motor output of the telencephalon in amphibians, lizards, and turtles is the projection of the dorsal pallidum to the pretectum and to the mesencephalic tegmentum and from there to the tectum mesencephali.
6. In the anterior striatopallidum of amphibians corresponding to the mammalian dorsal striatum, GABAergic projection neurons and cholinergic interneurons, characteristic of the mammalian dorsal striatum, are largely or completely missing. The striatum of turtles and lizards, in contrast, appears to possess such types of neurons, although not in a quantity comparable to the mammalian situation.
7. The amphibian amygdaloid complex consists of a vomeronasal amygdala of subpallial and pallial origin homologous to the mammalian posteromedial cortical amygdala, a pallial olfactory amygdala homologous to the anterior and posterolateral cortical amygdala of mammals and the external and ventral anterior amygdala in reptile amygdala, and an extended central amygdala homologous to the mammalian BNST, medial amygdala, and central amygdala. An amygdala of pallial origin homologous to the mammalian basolateral amygdala appears to be missing in amphibians, but probably has – at least in part – a functional equivalent in the mediocentral amygdala.

In summary, the differences mentioned between amphibians on the one hand and mammals and birds on the other concern mostly the connection between thalamus and pallium/cortex, the intra-telencephalic connections of the pallium/cortex, predominantly to the striatopallidum, and the further differentiation, enlargement, and specialization of pallial/cortical structures. This further differentiation of the thalamocortical system apparently has occurred independently in mammals and birds originating from different reptilian ancestors.

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12 Evolution of the Nervous System in Reptiles

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Glossary

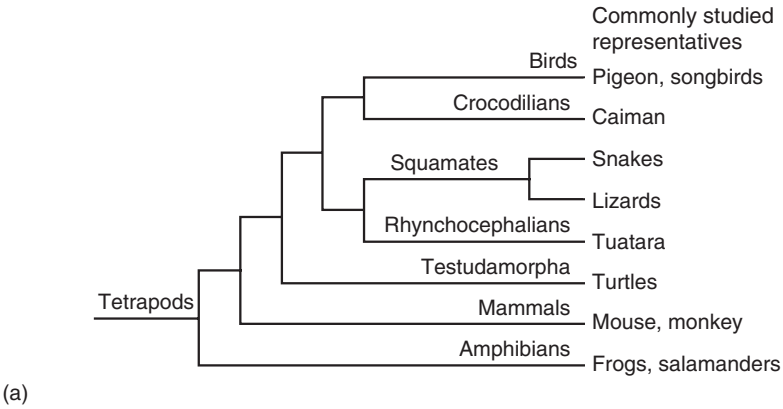
homologue	Traits that are derived from a common ancestral region and formed by similar developmental processes.
pallium	The dorsal part of the telencephalon that arises from the rostral, dorsal neural folds, including hippocampus, cortex, and part of the amygdala.
telencephalon	The rostral expansion of the brain including pallium and subpallium.
tetrapods	Vertebrates with four feet, generally including amphibians, reptiles, birds, and mammals.

12.1 Introduction

Living reptiles are traditionally classified as either anapsids or diapsids based largely on the morphology of a single key character, the temporal fenestrae (e.g., Williston, 1917). These fenestrae are presumed to provide better jaw muscle attachment in the diapsid rather than the anapsid condition. Anapsids lack temporal fenestrae. Diapsids have two fenestrae on each side and evolved from ancestors that had none. Snakes, lizards, crocodiles, and dinosaurs are diapsids. Testudamorpha (turtles and tortoises), as well as many Paleozoic reptiles, are anapsids. The absence of fenestrae is considered a primitive state and the presence of fenestrae is considered a derived state. In traditional interpretations of the phylogeny of extant reptiles, Testudamorpha are considered basal to other reptiles (lizards, snakes, tuatara, crocodiles, and birds) (Figure 1a). Testudamorpha are typically classified as living representatives of the more extinct anapsid reptiles, and placed with the early fossil reptiles. This has led many researchers, including comparative neurobiologists, to consider turtles as the reptile of choice for evolutionary studies.

A growing body of evidence from molecular and osteological analyses suggests that Testudamorpha should be reclassified as specialized diapsids. Cladistic analyses of 168 osteological characters in 14 living and fossil reptilian taxa led Rieppel and De Braga (1996) to conclude that Testudamorpha are nested within the clade Diapsida as the sister group to birds and crocodiles (Figure 1b).

The earliest molecular approach (Fitch and Margoliash, 1967), based on analyses of the cytochrome *c* gene amino acid sequences in vertebrates and invertebrates, resulted in a phylogenetic scheme that was largely similar to the traditional scheme with one notable exception – turtles were more closely associated with birds than with the other living reptiles. Fitch and Margoliash noted this departure from conventional interpretations, but at the time considered it to be an anomaly.



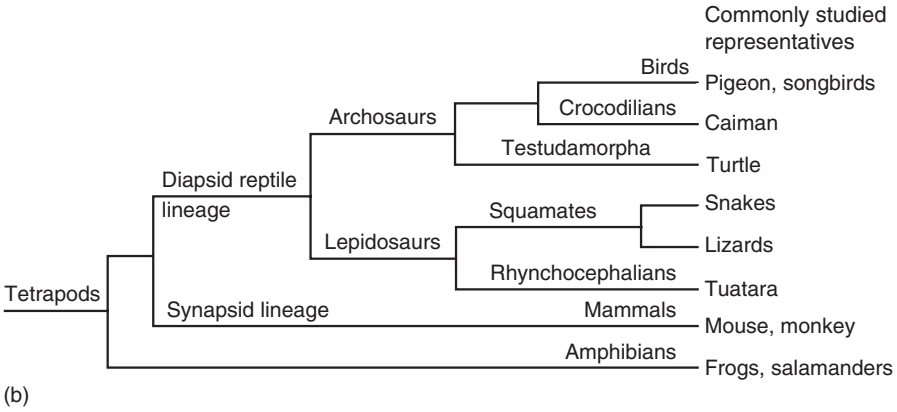


Figure 1 Relationships among the major groups of living tetrapods. a, The traditional phylogeny places Testudamorpha at a basal position relative to other reptiles. b, The consensus phylogenetic tree based on molecular and morphological analyses places Testudamorpha as a sister group to birds and crocodiles. Modified from Rieppel, O. 1999. Turtle origins. *Science* 283, 945 946 and Platz, J. E. and Conlon, J. M. 1997. . . . and turn back again. *Nature* 389, 246.

Platz and Conlon (1997) analyzed the molecular changes of pancreatic polypeptide, utilizing seven amphibians (representing all three orders) as the outgroup to examine living reptile phylogeny (snake, turtle, and alligator, and three birds). Their results were consistent with the earlier analyses of cytochrome *c*. Thus, turtles nest within the diapsids above snakes and are the sister group to birds and crocodiles. More recently, analyses of nuclear genes and mitochondrial genomes (Hedges and Poling, 1999; Janke *et al*., 2001) have provided further support for this tree.

In summary, five independent data sets, one morphological and four molecular, all support testudamorphs as diapsids. Because rhynchocephalians are presently endangered, lizards are the best alternative model to compare with other lepidosaurs, and to identify traits present in the common ancestors of birds and mammals. The evolutionary history of snakes remains controversial and requires further study, although the prevailing view is that they are derived from lizards. For studies of avian evolution, crocodilians (alligators, caiman, and crocodiles) are the most closely related living group. Testudamorpha represent a basal member of the living archosaurian diapsids, and therefore provide insights into crocodilian and avian evolution.

12.2 How Do Differences in Brain Organization Evolve?

The term 'homology' was coined by Owen (1848) to recognize organs of common tissue origin, regardless of form and function in different species. Applied to the brain, 'homology' typically refers to neural units that are formed by similar developmental processes.

The brains of extant animals are studied to identify presumed homologies. This involves examination and demarcation of neural regions that were derived from a common ancestral region (Campbell and Hodos, 1970; Northcutt, 1984). To do this we

must recognize and eliminate nonhomologous regions, in particular those that have arisen by parallel or convergent evolution; meaning those similarities that are not derived from common ancestry. If we can identify homologies, then mapping neuronal traits onto a well-established tree will allow one to differentiate between shared, derived, or primitive states, and those that arose by convergence.

Comparative neuroanatomists, like all scientists, are limited by the tools available to them as well as by assumptions made as to how evolution 'should work'. In the early part of the twentieth century, neuroanatomists made comparisons based on analyses of size, gross morphology, and cytoarchitecture, and followed the principle that brain regions evolved from 'simple' to 'complex' (e.g., Edinger, 1908; Ariëns Kappers *et al*., 1936). They relied on Nissl, myelin, and Golgi stains, and brain dissections, which provided information about neuronal morphology, topology, and some axonal pathways. Though some of their conclusions have been disproved, many are still valid today and others are still being debated. Continued searches for homologies are essential to resolving these issues.

There are well-accepted approaches for studying homology: topology, connections, neurochemical expression patterns, genetic expression patterns, cell morphology, and neurophysiological characteristics. Each approach has strengths and weaknesses. Therefore, evidence for or against a particular homology should be based on multiple approaches, and use multiple techniques. Again, if one has a good molecular-based phylogeny, one can 'map' neural states on to it.

12.2.1 Topology

Using topology as a criteria assumes that the relative position of a given brain region is determined by interactions with adjacent regions. This is useful because the topological fate of a neuronal group and its connections is determined by multiple genes that are expressed in specific spatial and temporal patterns. A weakness of this approach is that neuronal groups may migrate away from their ventricular site of origin. Therefore, topological analyses alone may be misleading. A classic example of a mistaken topology-based homology is Edinger's (1908) comparison of the avian nidopallium (previously named neostriatum; Reiner *et al*., 2004) and reptilian dorsal ventricular ridge (DVR) to part of the mammalian striatum, because they all form a large ridge in the lateral ventricle. Karten (1969) subsequently used histochemical analyses to show that these are pallial, and not striatal, structures.

12.2.2 Connections

The formation of neuronal pathways is regulated by multiple families of genes at both the sites of origin and termination. Identification of similar connections is thus another indication of homology. However, the weakness of this approach is that the same region (e.g., thalamus) may give rise to more than one projection to a target (e.g., telencephalon), or may invade new regions. Thus, a single connection may not be a reliable indicator of homology. The acquisition of novel connections reflects changes in genetic regulation, implying evolutionary change. If, for example, this change took place in a bird, but was not seen in crocodilians or turtles (basal group of the three; Figure 1b), one could tentatively assign the changed state in birds to a derived condition.

12.2.3 Neurochemical Expression Patterns

Comparisons of expression patterns have proven very useful for identifying homologies. Neuronal traits regulated solely by local genetic environment are best for identifying retained evolutionary features. Genes or peptides that are expressed during early developmental stages and maintained into maturity are especially useful for interspecies comparisons. For example, the expression of factors that regulate dopamine, noradrenaline, and serotonin are highly conserved in nuclei among different vertebrate classes (Smeets and Reiner, 1994), and are useful for identifying homologous nuclei and pathways. Care must be taken because some expression patterns can be sculpted by peripheral or environmental influences. For example, expression of calcium-sequestering peptides can be altered by the presence or absence of neuronal input (Britto *et al*., 1994; Diaz de Barboza *et al*., 2003); thus, their presence or absence may not correlate with neuronal ancestry. Finally, if the same antibody is used in two distant taxa, it is uncertain whether it is binding to the homologous peptide.

12.2.4 Gene Expression

Genetic analyses reveal expression patterns at earlier developmental stages than most neurochemical markers. The ontogenetic history of a neural group can be followed from ventricular origin, through proliferation, specification, and migration. A comparison of such embryological fate maps allows the identification of conserved neural domains. For example, analyses of gene-expression patterns

revealed the presence of a ventral pallial domain in tetrapods (Brox *et al*., 2004). However, limitations are that genes may be up- and/or downregulated during development. For example, heterochrony (expression of genes at different stages of development) may result in a positive expression in animal A but not in animal B at one stage, and the opposite expression a few days later. Thus, the stage at which two species are compared must be carefully selected.

12.2.5 Morphology

Neuronal size, shape, and packing density are variable, and are usually not regarded as strong indicators of homology. For example, the laminated optic tectum of reptiles and the nucleated superior colliculus of mammals are homologous although structurally different.

12.2.6 Physiological Characteristics

A neuron's function is determined by a complex interaction of local and nonlocal genetic factors within the brain, as well as influences from peripheral organs and the environment. Thus, comparative analyses of cellular response characteristics are more likely to reveal diverse evolutionary adaptations than retained homologous features. For example, comparisons of cellular responses at each level of the auditory pathway reveal that amphibians, reptiles, birds, and mammals have all evolved unique adaptations for processing auditory information (Grothe *et al*., 2004).

12.2.7 Summary

In summary, every neural region contains both evolutionarily conserved (primitive) and derived states. While recognizing the difficulty in identifying homologous features, using the total evidence from the approaches discussed above should allow us to reach a consensus with regard to homology. Mapping changed character states in the brain to a cladistically derived phylogenetic tree should reveal shared evolved patterns (synapomorphies) and at the same time identify instances of convergent evolution (a form of homoplasy), as well as derived traits.

12.3 How Can We Recognize Brain Areas That Have Evolved from a Common Ancestor?

Each brain region can be recognized by a unique set of traits, including gene-expression patterns, embryology, neurochemistry, and connections. Our null hypothesis is that a region does not change in the evolution from one species to another. Furthermore, we assume that homologous structures develop from topologically equivalent precursors (Braford, 1995). By searching for homologies, one should identify the neural regions that have retained most of their characteristics, and in the process recognize those that have changed.

The greater the number of traits two structures in divergent species have in common, the greater the likelihood that they are homologous. Thus, making a comparison based on a single pathway (e.g., the retino-tecto-thalamo-pallial projection), or a single gene is a weaker argument than a comparison based on multiple connections or characteristics. Controversies over homologues often occur because different investigators rely on or ignore different traits to reach their conclusions.

Evolution happens: brains clearly differ among the vertebrate classes, and the components have evolved to varying degrees. For example, evolutionary geneticists can identify a gene as 90% homologous with a similar gene from another species. Comparative neurology cannot achieve such quantification, yet we know that the homologues of some nuclei are easier to identify than others. As in gene evolution, we expect some neuronal traits to be more variable, while others are more constrained. Our current hypotheses about homologies will continue to be tested, and hopefully confirmed, as more and more traits are identified.

12.4 Sensory Pathways

The first mammalian studies of auditory, visual, and somatosensory pathways to the telencephalon recognized the main components of the sensory pathways, and found that sensory information was relayed through the midbrain and thalamus to the primary sensory areas of the cortex. The sensory pathways of amphibians, reptiles, and birds were compared to these pathways. With the introduction of new techniques, we have since learned that the components and connections of these sensory systems are more numerous and complicated, requiring re-evaluation of proposed homologues.

12.4.1 Auditory System

12.4.1.1 Reptiles In reptiles, peripheral sound is conveyed through a tympanic middle ear to the basilar papilla. The basilar papilla projects centrally to two subdivisions of the cochlear nucleus, nucleus magnocellularis and nucleus angularis (Carr and Code, 2000). A third brainstem target, nucleus laminaris, also receives direct peripheral auditory input in lizards and crocodiles (DeFina and Webster,

1974; Barbas-Henry and Lohman, 1988). Nucleus laminaris also has features comparable to the superior olive: it receives input from the cochlear nuclei and projects to the midbrain torus semicircularis. It is usually poorly developed in turtles and lizards, reflecting its role in all low-frequency processing (Miller, 1975; Miller and Kasahara, 1979; Barbas-Henry and Lohman, 1988). In crocodiles and birds it is a larger, distinctly monolayer structure (Rubel and Parks, 1975). Further studies of the reptilian nucleus laminaris are needed to elucidate its evolutionary status. The next level of the vertebrate auditory system is the lateral lemniscus. Within the rostrolateral hindbrain of reptiles there is a region called the lateral lemniscus, but little is known of its connections or other characteristics.

The reptilian auditory midbrain is located in the medial torus semicircularis. It has similar structure, connections, and embryonic origin among tetrapods, and is thus considered a homologue (Wilczynski, 1988; McCormick, 1999). A tonotopic organization is present in crocodiles (Manley, 1971). In reptiles, as in other vertebrates, there is a core area that is the main target of ascending projections from the brainstem, and a belt or laminar area that appears to receive auditory input from the core or from non-brainstem auditory areas. In lizards and crocodiles the auditory core of the torus projects to the thalamus (Pritz, 1974a; Foster and Hall, 1978). The laminar area projects to thalamus and spinal cord, and parts of it are correlated with vocalizations and social communication behaviors (Kennedy, 1975; Distel, 1978; Butler and Bruce, 1981; Hoogland, 1982).

The auditory midbrain projects to two thalamic areas in reptiles: nucleus medialis (known as nucleus reuniens in turtles and crocodiles), and the dorsolateralis anterior (Pritz, 1974a; Foster and Hall, 1978; Hoogland, 1982). In addition, auditory information from the superior olive projects to an area immediately lateral to nucleus rotundus (Hoogland, 1982). Thus, there may be as many as three distinct auditory thalamic areas, although nucleus medialis/reuniens is the main one. Further studies are needed to resolve this issue.

Nucleus medialis/reuniens projects to the striatum and the medial part of the DVR, and the nucleus dorsolateralis anterior is reciprocally connected with the cortex (Pritz, 1974b; Foster and Hall, 1978; Lohman and van Woerden-Verkley, 1978; ten Donkelaar and de Boer-Van Huizen, 1981; Bruce and Butler, 1984a, 1984b).

12.4.1.2 Amphibian to reptile transition Amphibians have two sensory organs that detect airborne sound, the amphibian papilla and basilar papilla, but only the basilar papilla appears to be homologous to the acoustic-sensing organs of other tetrapods (Fritzsch and Neary, 1998; Fritzsch *et al*., 2002). The amphibian cochlear nucleus, the dorsolateral nucleus, has features unique to amphibians, and its homology with the amniote cochlear nuclei remains unclear (McCormick, 1999). However, cells within the dorsolateral nucleus are morphologically and physiologically similar to those in the cochlear nuclei of other tetrapods. The brainstem auditory nuclei are extensively interconnected (see Fritzsch and Neary, 1998), so only the main connections will be described here (Figure 2a). The amphibian superior olive receives projections from the dorsolateral nucleus, and projects to the torus semicircularis in the midbrain.

The auditory part of the torus semicircularis has similar characteristics among tetrapods, although differences in local circuitry and sound processing are apparent. In amphibians it projects strongly to the central thalamic nucleus and more weakly to the lateral and anterior thalamic nuclei (Hall and Feng, 1987; Neary, 1990). Auditory information from the central and lateral thalamic nuclei is conveyed primarily to the striatum and sparsely to the ventral part of the lateral pallium; that from the anterior thalamic nucleus is conveyed primarily to the medial pallium (Mudry and Capranica, 1980; Wilczynski and Northcutt, 1983a; Hall and Feng, 1987; Neary, 1990; Allison and Wilczynski, 1991; Feng and Lin, 1991).

The basic pattern of connections can be recognized in amphibians and reptiles. Similar thalamic zones can be recognized, although there is increased size and specificity in the reptilian thalamus. The greatest change in the auditory pathway occurred in the telencephalon. Assuming that the common tetrapod ancestor had a forebrain similar to that of extant amphibians, then the ventral part of the lateral pallium in amphibians underwent extensive elaboration to give rise to the reptilian DVR with its distinct sensory regions. The amphibian medial pallium also underwent considerable change, including the development of separate hippocampal and cortical regions. See Sections 12.5.2 and 12.5.3 for further discussion of the evolution of the thalamus and pallium.

12.4.1.3 Reptile to bird transition During the transition from ancestral reptiles to crocodiles and birds, the cochlear nucleus laminaris appears to have enlarged and become distinctly laminated (Rubel and Parks, 1975). The auditory midbrain, torus semicircularis, appears to be generally homologous between reptiles and birds (Figure 2c). Its main

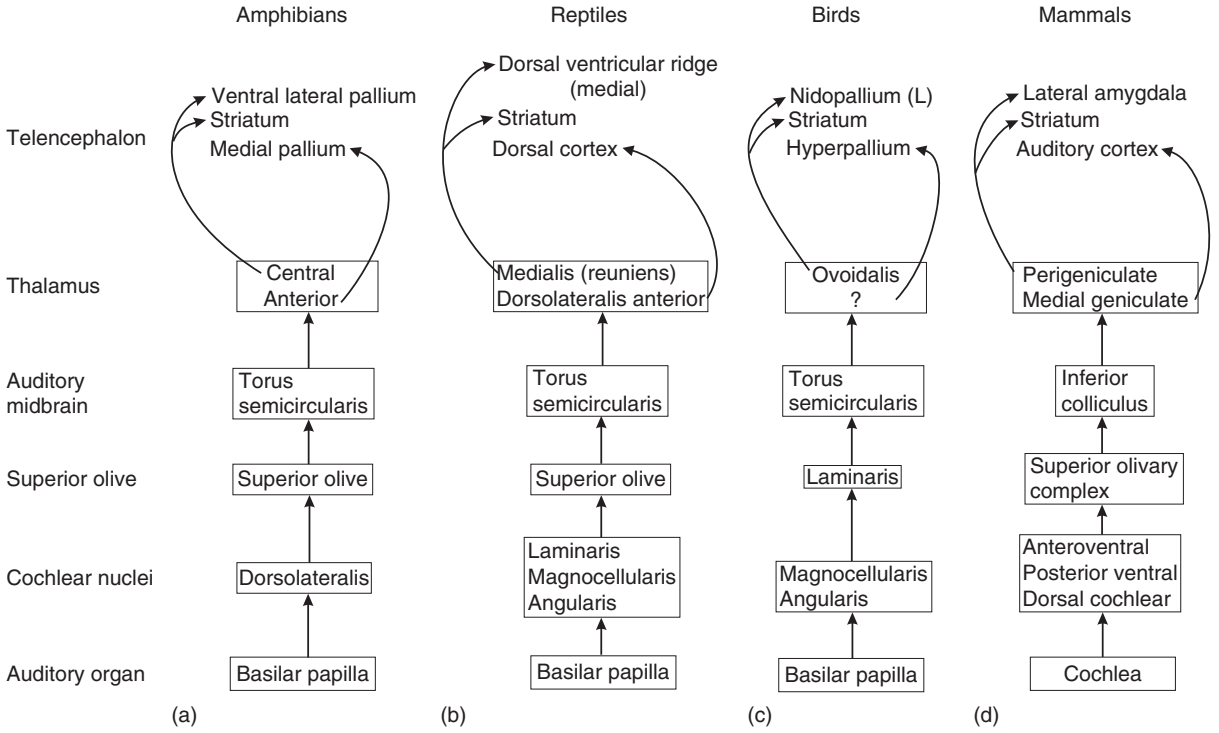


Figure 2 Comparison of the major ascending auditory pathways in tetrapods.

projection in birds is to the auditory thalamic nucleus, ovoidalis (Arthur, 2005). Nucleus ovoidalis projects to the striatum and to Field L in the DVR (Wild *et al.*, 1993). A toral projection to a thalamic nucleus that projects to the hyperpallium has not been described, although Adamo and King (1967) recorded acoustic responses in the medial cortex. The auditory region of the DVR is more elaborate in birds than reptiles, and appears to be correlated with the evolution of vocal behaviors. Many of the connections from the auditory midbrain to circuits involving vocalization appear to be present among tetrapods, although the descending connections like those from the avian arcopallium (formerly archistriatum) to the midbrain have not been reported in reptiles.

12.4.1.4 Reptile to mammal transition The evolution of the mammalian auditory system is correlated with the appearance of more complex cell types and connections (Grothe *et al.*, 2004). The mammalian cochlear nucleus contains intrinsic connections and nonprimary inputs that have no known homologue in nonmammals, and which may be associated with the evolution of a high frequency hearing range, and of mobile pinnae that increased sound-localization cues (Grothe *et al.*, 2004). The correspondence of the three subdivisions of the mammalian cochlear nucleus with those of the reptilian nucleus remains uncertain. Nonetheless, the same basic pattern of connectivity seen in other tetrapods is also present in mammals (Figure 2d). The auditory midbrain, inferior colliculus, projects to two thalamic groups: the medial geniculate and a perigeniculate group. The medial geniculate projects to auditory areas in the temporal cortex, whereas the perigeniculate group, including the medial division of the medial geniculate nucleus, the posterior intralaminar nucleus, and the suprageniculate nucleus, projects to the lateral amygdalar nucleus (Doron and LeDoux, 1999, 2000). The mammalian thalamic nuclei and telencephalic regions devoted to audition and vocalization appear to have undergone considerable expansion and parcellation during the transition to mammals. They show considerable variation from the nonmammalian condition, and identification of their homologues is very controversial and will be dealt with separately (Sections 12.5.2 and 12.5.3).

12.4.1.5 Summary The general features and synaptic levels of the auditory pathways are present in all tetrapods, suggesting a conserved Bauplan. However, within each of these levels there is considerable anatomic and physiologic diversity among the vertebrate taxa. Another trend is the increase in size and complexity of the auditory thalamic and cortical regions, particularly in the reptile to mammal transition. Hypotheses about the homologues of thalamic

and pallial regions in amphibian reptiles, and birds are in general agreement, but comparisons with the mammalian auditory regions have proved more difficult and more controversial (see Shared and Convergent Features of the Auditory System of Vertebrates).

12.4.2 Visual System

The targets of primary retinal projections will first be presented, followed by the visual pathways to the telencephalon.

12.4.2.1 Reptiles *Primary retinal projections.* The primary visual system has been studied in many reptiles, allowing the comparison of a general pattern of retinal projections. The retinal ganglion cells project bilaterally with a contralateral dominance in most lizards, but are predominantly contralateral in crocodiles, and entirely contralateral in *Chameleo* and *Uromastix* lizards (Bennis *et al.*, 1994; Derobert *et al.*, 1999). Retinal fibers terminate in six general targets within the diencephalon and midbrain of all tetrapods studied with modern experimental techniques (lizards: Northcutt and Butler, 1974; Cruce and Cruce, 1975, 1978; Bruce and Butler, 1984b; Reperant *et al.*, 1978; de la Calle *et al.*, 1986; Kenigfest, *et al.*, 1997; Casini, *et al.*, 1993; Bennis, *et al.*, 1994; snakes: Repérant, and Rio, 1976; Schroeder, 1981; Dacey and Ulinski, 1986; turtles: Hall *et al.*, 1977; Bass and Northcutt, 1981a, 1981b; Kunzle and Schnyder, 1983; Sjöström and Ulinski, 1985; Ulinski and Nautiyal, 1988; Hergueta *et al.*, 1992, 1995; crocodiles: Derobert *et al.*, 1999). These studies are summarized in the following paragraphs. A variety of nomenclatures have been used for these retinal-recipient nuclei, so we here follow that of Repérant *et al.*, 1992 except as noted.

1. *Hypothalamus.* Retinal fibers terminate in the suprachiasmatic nucleus. The retinohypothalamic projection helps to synchronize endogenous rhythms with seasonal changes in the diurnal cycle (Underwood and Groos, 1982; Pickard, 1982). In chameleons and crocodiles there is an additional retinal projection to periventricular hypothalamic area, nucleus opticus periventricularis hypothalami posterior (Bennis *et al.*, 1994; Derobert *et al.*, 1999).
2. *Ventral thalamus.* The ventral thalamus is distinguished from the dorsal thalamus by its lack of projections to the telencephalon. Visual projections terminate in nucleus geniculatus lateralis pars ventralis, in a medially adjacent group, nucleus ventrolateralis, and in a dorsal group called either nucleus ovoidalis or geniculatus lateralis pars dorsalis (GLd). The dorsal surface of the retino-recipient ventral thalamic area is capped by a sheet of neuropeptide Y (NPY)-like immunoreactive cells, above which is dorsal thalamus (Medina *et al.*, 1992).
3. *Dorsal thalamus.* Dorsomedial to the ventral thalamic area is a small cell group that receives retinal projections and projects to the telencephalon. In turtles, this nucleus is called the lateral geniculate nucleus (Hall and Ebner, 1970a, 1970b). In lizards, it is called intercalatus (Bruce and Butler, 1984a) or the lateral part of the dorsolateralis anterior (Bennis *et al.*, 1994). It appears to be smaller in lizards than in turtles.
4. *Pretectal nuclei.* There are four visual pretectal nuclei. Nucleus lentiformis mesencephalicus is largest in snakes and some lizards, and poorly developed in most turtles (Reperant *et al.*, 1992). Nucleus griseus tectalis is better developed in lizards than in snakes. Nucleus geniculatus pretectalis and nucleus posterodorsalis are similar features amongst reptiles.
5. *Optic tectum.* The retina terminates in the superficial optic tectum in a retinotopic organization.
6. *Mesencephalic tegmentum.* Nucleus opticus tegmenti is present in all reptiles, but is particularly large in chameleons.

Visual pathways to the telencephalon. Two visual pathways to the telencephalon are usually recognized in the reptilian visual system (see Repérant, *et al.*, 1992, for a historical review). A primary pathway ascends from the retina to the thalamus to the telencephalic cortex. A secondary pathway ascends from the retina to the optic tectum (superior colliculus), to the thalamus, and then to a visual area within the DVR. The primary visual pathway is sometimes referred to as the lemnothalamic pathway, and the pathway through the tectum may be called the collothalamic pathway (Butler and Hodos, 1996). There is, however, a third distinct route through which visual information reaches the telencephalon, raising the question of which pathways are homologous among tetrapods (Figure 3b).

A retino-thalamo-telencephalic projection has been described in turtles (Hall and Ebner, 1970a, 1970b; Hall *et al.*, 1977) and lizards (Bruce and Butler, 1984a; Kenigfest *et al.*, 1997). In turtles it projects to the anterolateral parts of the dorsal cortex (Hall and Ebner, 1970a; Desan, 1988; Zhu *et al.*, 2005). In lizards it projects to or near a rostral telencephalic nucleus, the pallial thickening (Bruce and Butler, 1984a). Further studies are needed to identify the specific telencephalic target in lizards and crocodiles.

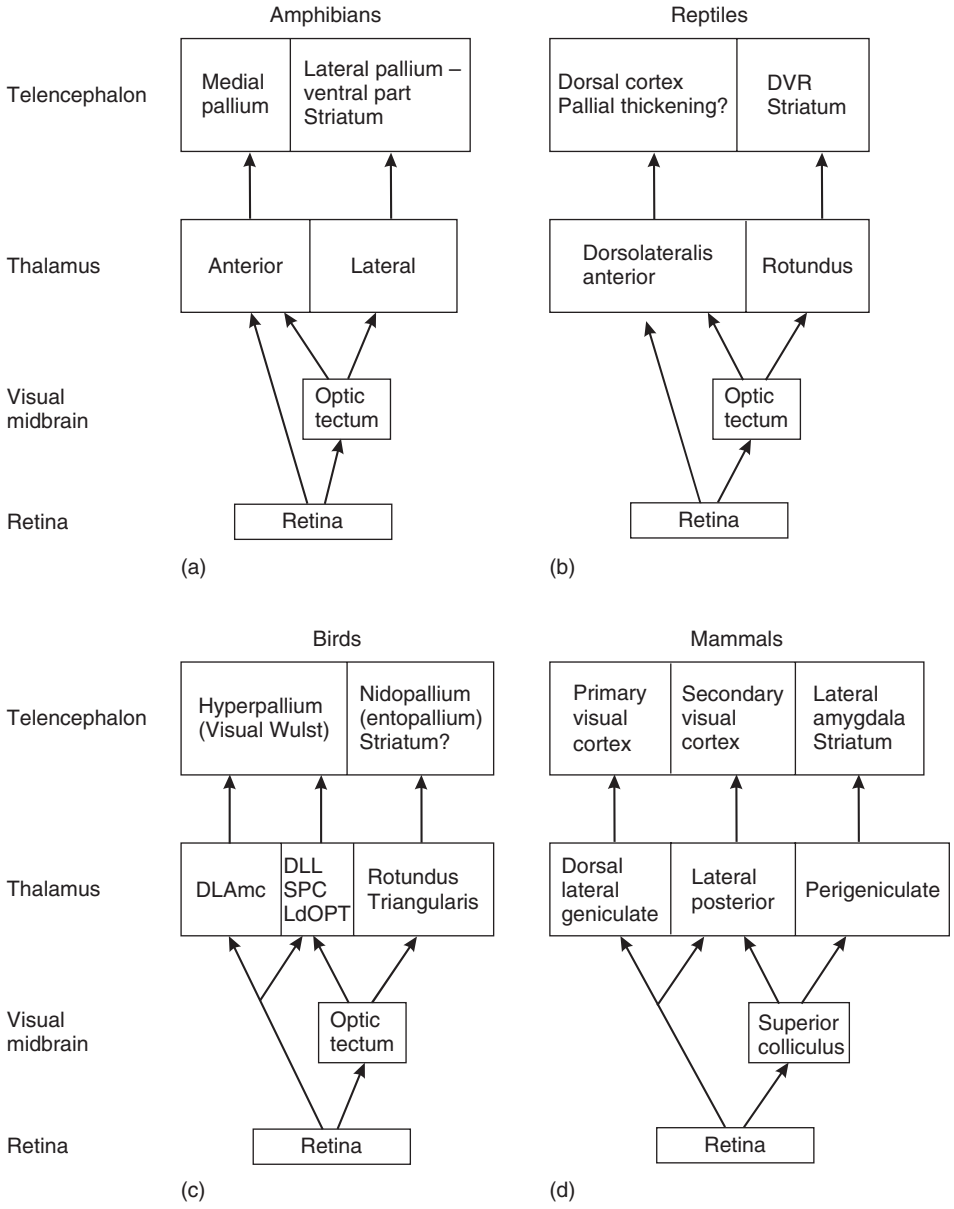


Figure 3 Comparison of the major ascending visual pathways to the telencephalon in tetrapods. Refer to text for abbreviations.

Retino-tecto-thalamo-telencephalic projections have been the focus of a number of studies (lizards: Butler and Northcutt, 1971; Bruce and Butler, 1984b; Guirado *et al*., 2000; turtles: Hall and Ebner, 1970a, 1970b; Balaban and Ulinski, 1981; Rainey and Ulinski, 1982; Foster and Hall, 1975; Hoogland, 1982; Desfilis *et al*., 2002; Belekhova *et al*., 2003; crocodiles: Braford, 1972; Pritz, 1975). Neurons in the optic tectum that extend dendrites into the visual-recipient layers project to rotundus and, at least in lizards, to the dorsolateralis anterior. Nucleus rotundus then projects to the striatum and DVR, and the dorsolateralis anterior projects to the dorsal cortex. Thus, visual information reaching the optic tectum is conveyed to two different thalamic regions, and then each projects to a separate pallial region, either the dorsal cortex or the DVR. Visual responses have been recorded from the rostrolateral dorsal cortex, the lateral part of the medial cortex (Andry and Northcutt, 1976), and from the visual DVR (Peterson and Rowe, 1976; Manger *et al*., 2002). These two regions develop from embryologically distinct pallial domains, dorsomedial and ventromedial, respectively (Fernandez *et al*., 1998).

12.4.2.2 Amphibian to reptile transition The basic pattern of retinal projections seen in reptiles is also present in amphibians, indicating that it was

present in the common ancestor (Figure 3a). Visual projections terminate in the hypothalamus, ventral thalamus, dorsal thalamus, pretectum, mesencephalic tectum, and mesencephalic tegmentum (Scalia and Gregory, 1970; Scalia, 1976; Roth *et al*., 1998; Wye-Dvorak *et al*., 1992).

A retino-thalamo-telencephalic pathway is present in amphibians, as in reptiles. Retinal axons terminate on the dendrites of neurons in the anterior nucleus, within a cell-poor terminal region called the neuropil of Bellonci (Scalia and Gregory, 1970). The anterior thalamic nucleus then projects to the medial, dorsal, and lateral pallia (Neary, 1990; Northcutt and Ronan, 1992).

Two retino-tecto-thalamo-telencephalic pathways are present and comparable to those in reptiles. The optic tectum projects to the lateral and anterior thalamic nuclei. The anterior thalamic nucleus projects to the pallial cortices, especially the medial pallium, whereas the lateral thalamic nucleus projects heavily to the striatum and sparsely to the ventral part of the lateral pallium (Wilczynski and Northcutt, 1977, 1983a; Neary, 1990; Montgomery and Fite, 1991). These two pallial targets develop from embryologically distinct domains, dorsomedial and ventrolateral pallia, respectively (Brox *et al*., 2004).

12.4.2.3 Reptile to bird transition The avian visual system has been the subject of numerous studies, and a great deal more is known about it than the reptilian visual system, so here we focus on comparable studies (Figure 3c). As in other tetrapods the avian retina projects to targets in the hypothalamus, ventral thalamus, dorsal thalamus, pretectum, midbrain tectum, and midbrain tegmentum (Gamlin and Cohen, 1988; Norgren and Silver, 1989a, 1989b). Within the dorsal thalamus the retinorecipient nuclei that project to the hyperpallium are the dorsolateralis anterior thalami, pars lateralis (DLL), dorsolateralis anterior thalami, pars magnocellularis (DLAmc), lateralis dorsalis nuclei optici principalis thalami (LdOPT), and the suprarotundus (SpRt) (Güntürkün *et al*., 1993). This group is sometimes called the nucleus geniculatus lateralis, pars dorsalis. These nuclei project bilaterally to the hyperpallium (visual Wulst), except the SpRt, which projects ipsilaterally.

Thalamic nuclei that receive visual input via the optic tectum include rotundus, triangularis, superficialis parvocellularis (SPC), part of the DLL, and the LdOPT (Karten and Revzin, 1966; Sugita *et al*., 1996). One group of nuclei (SPC, DLL, and LdOPT) projects to the visual Wulst in the hyperpallium (Güntürkün *et al*., 1993). The SPC projects to additional telencephalic targets including the somatosensory Wulst and the area parahippocampalis. The other nuclei (rotundus and triangularis) project to the entopallium, a visual region within the nidopallium (Hellmann and Güntürkün, 2001). A projection from rotundus to the striatum apparently has not been documented, although rotundus axons clearly pass through the striatum enroute to the entopallium. Thus, visual information from the optic tectum projects through two separate thalamic regions and then to the hyperpallium and the nidopallium. The nidopallium, like the reptilian DVR is embryologically derived from the ventrolateral pallium; the hyperpallium, like the dorsal cortex is derived from the mediodorsal pallium (Fernandez *et al*., 1998; Puelles, 2000; Brox *et al*., 2004)

The avian visual system follows the same basic plan seen in reptiles, although the retino- and tectorecipient thalamic nuclei and the visual hyperpallium appear to have enlarged and segregated further during the reptile to bird transition. This enhanced ability to process visual cues may be correlated with the evolution of flight.

12.4.2.4 Reptile to mammal transition As in other tetrapods, ganglion cells in the mammalian retina project to targets within the hypothalamus, ventral thalamus, dorsal thalamus, pretectum, midbrain tectum, and midbrain tegmentum. These are, respectively, the suprachiasmatic nucleus, ventral lateral geniculate, dorsal lateral geniculate, several pretectal nuclei, superior colliculus, and medial terminal nucleus (Figure 3d; Sefton and Dreher, 1985).

The flow of information from the retina to the telencephalon is often regarded as a 'dual visual system', but in fact visual information reaches the telencephalon through at least three distinct pathways. One pathway is from retina to thalamus (dorsal lateral geniculate) to primary visual cortex. The second and third pathways are relayed through the superior colliculus to the thalamus. However, the superior colliculus projects to multiple thalamic groups, including perigeniculate, midline, and intralaminar nuclei, in addition to the well-known lateral posterior/pulvinar nuclei (Holstege and Collewijn, 1982; Linke, 1999; Linke *et al*., 1999). Thus, the second pathway is from the retina to the superior colliculus (midbrain tectum) to the lateral posterior (or pulvinar in primates) thalamic nuclei to secondary visual cortical areas (Diamond, 1976). The third pathway is from the retina to the superior colliculus to the visual perigeniculate thalamus (particularly the suprageniculate nucleus), which projects to the striatum and lateral amygdala (Linke *et al*., 1999, 2000; Doron and LeDoux, 1999). Embryological studies

show that the lateral amygdala is derived from the ventrolateral pallium, whereas the visual cortex arises from the dorsomedial pallium (Fernandez *et al*., 1998; Puelles *et al*., 2000; Brox *et al*., 2004). The midline and intralaminar nuclei have widespread, nonspecific projections to cortical and striatal regions. Which of these colliculo-thalamotelencephalic targets are comparable among the vertebrate classes is currently under considerable debate. However, connectional and embryological data indicate that the visual areas of the reptilian DVR and mammalian lateral amygdala are comparable, and those of the reptilian dorsal cortex and mammalian visual cortices are comparable (see Section 12.5.3).

12.4.2.5 Summary In all tetrapods the retina projects to the hypothalamus, ventral thalamus, dorsal thalamus, pretectum, mesencephalic tectum, and mesencephalic tegmentum. Thus, this pattern was present in a common ancestor. Furthermore, in all tetrapods retinal information is conveyed to the telencephalon by at least three distinct pathways: (1) a thalamocortical pathway that runs from the retina directly to a cell group in the thalamus, and then to a dorsomedial pallial region; (2) a tecto-thalamo-cortical pathway that travels from the retina to the midbrain tectum to a cell group in thalamus and then to a dorsomedial pallial region. This dorsomedial pallial region corresponds to visual parts of the medial pallium in amphibians, dorsal cortex in reptiles, hyperpallium in birds, and isocortex in mammals; and (3) a tecto-thalamo-amygdalar pathway that runs from the retina to the optic tectum to a different cell group in the thalamus, and then to both the striatum and a nucleated region within the ventrolateral pallium. This ventrolateral pallium corresponds to a visual area of the ventral lateral pallium in amphibians, DVR in reptiles, nidopallium in birds, and lateral amygdala in mammals.

Another noteworthy trend is the increase in size and complexity of the visual thalamic and cortical regions, which occurred during each transition, but was especially remarkable in the reptile to mammal transition. Identification of homologues between amphibians, reptiles, and birds has been relatively straightforward, but comparisons with the mammalian visual system have proven more difficult to make and more controversial.

12.4.3 Somatosensory System

12.4.3.1 Reptiles Somatosensory information about the body reaches the thalamus from the spinal cord and dorsal column nuclei (Figure 4b). Information about the head reaches the thalamus from the trigeminal nuclei. In addition, the spinothalamic, dorsal column and trigeminal regions project to a somatosensory midbrain area, which then projects to the thalamus (snakes: Ebbesson, 1969; lizards: Ebbesson, 1967; Bruce and Butler, 1984b; Ebbesson, 1978; turtles: Ebbesson, 1969; Siemen and Kunzle, 1994; Kunzle and Schnyder, 1983; Kunzle and Woodson, 1982; crocodiles: Pritz and Northcutt, 1980; Ebbesson and Goodman, 1981; Pritz and Stritzel, 1994).

Spinal and dorsal column somatosensory information appears to terminate in three thalamic regions in reptiles: (1) a posterior thalamic group called medialis posterior and posterocentralis nuclei in lizards, called the medialis complex in crocodilians, and called the lateral part of nucleus reuniens in turtles; (2) the dorsolateralis anterior nucleus; and (3) the ventral thalamic nuclei (Pritz and Northcutt, 1980; Ebbesson and Goodman, 1981; Bruce and Butler, 1984b; Belekhova *et al*., 1985; Siemen and Kunzle, 1994). Trigeminal nuclei have a similar projection pattern, terminating in the dorsolateralis anterior nucleus, in a region near medialis posterior, and in the ventral thalamus (Hoogland, 1982; Desfilis *et al*., 2002).

These three somatosensory-recipient thalamic areas have different ascending projections. The posterior thalamic group projects to posterior regions of the striatum and to the caudal part of the anterior DVR; the caudal DVR may project back to the somatosensory thalamus. Nucleus dorsolateralis anterior has reciprocal projections to the dorsal, medial, and lateral cortices, although the main cortical somatosensory target is believed to be the dorsal cortex. The ventral thalamic nuclei lack telencephalic connections (Bruce and Butler, 1984a, 1984b; Pritz and Northcutt, 1980; Balaban and Ulinski, 1981; Gonzalez *et al*., 1990; Pritz and Stritzel, 1994; Lohman and van Woerden-Verkley, 1978; Voneida and Sligar, 1979).

12.4.3.2 Amphibian to reptile transition Two spinal somatosensory pathways to the telencephalon are present in amphibians, and thus are presumed to be present in the common tetrapod ancestor (Figure 4a). In amphibia the spinal cord projects directly to the thalamus, or indirectly via the torus semicircularis in the midbrain. There are three spinal and midbrain thalamic targets: a massive projection to the ventral nuclei, a moderate projection to the central nucleus, and a sparse projection to the anterior nucleus (Munoz *et al*., 1997). A homologue of the dorsal column nuclei appears to project only to the ventral thalamic nuclei (Munoz

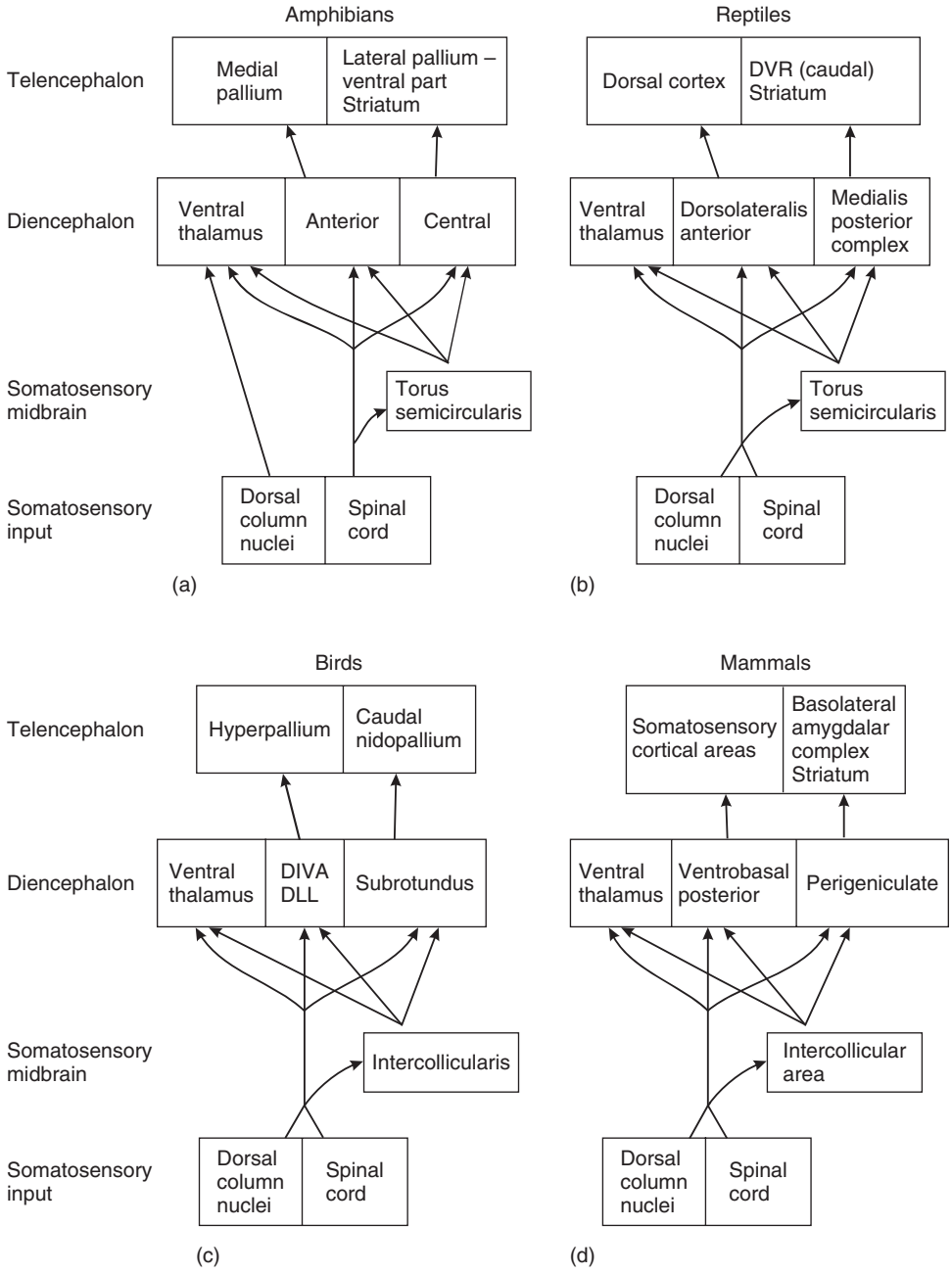


Figure 4 Comparison of the major ascending somatosensory pathways to the telencephalon in tetrapods. Refer to text for abbreviations.

et al., 1996) and thus more extensive brainstem-thalamic projections appear to have evolved in reptiles.

Three somatosensory-recipient thalamic groups in amphibians appear to correspond to those in reptiles: (1) the central thalamic nucleus, which projects heavily to the striatum and sparsely to the ventral part of the lateral pallium; (2) the anterior thalamic nucleus, which projects heavily to the medial pallium and sparsely to the dorsal and lateral pallia. Evoked potential studies suggest that the medial pallium is the target of polysensory ascending sensory information from the anterior thalamus (Karamian *et al*., 1966; Northcutt, 1970; Vesselkin *et al*., 1971; Mudry and Capranica, 1980); and (3) a ventral thalamic group lacks telencephalic projections (Neary, 1990). Thus, the central and anterior nuclei appear to be comparable to the reptilian posterior somatosensory group (medialis posterior and posterocentralis nuclei), and to the dorsolateralis anterior, respectively. Projections from the dorsal column nuclei to the dorsal thalamus appear to be

absent in amphibians, but present in reptiles, suggesting that the axons may have invaded new territory in a reptilian ancestor, or that the projection was present in the common tetrapod ancestor, but was lost in extant amphibians.

12.4.3.3 Reptile to bird transition The dorsal column nuclei, spinal cord, and trigeminal nuclei project to the midbrain (nucleus intercollicularis; Karten, 1963; Arends *et al*., 1984; Necker, 1989; Wild, 1997). They also have extensive thalamic projections, including: (1) three ventral thalamic nuclei, intercalatus, ventrolateral, and reticular nuclei; (2) nuclei that project to the hyperpallium, dorsointermedius ventralis anterior (DIVA), and DLL; and (3) a nucleus that projects to the caudal nidopallium, subrotundus. In addition, sensory information from the body and face reaches several intralaminar-like nuclei in birds (dorsolateralis posterior, and dorsolateralis anterior, pars medialis), but a comparable projection has not been reported in reptiles (Figure 4c; Karten, 1963; Schneider and Necker, 1989; Delius and Bennetto, 1972; Arends *et al*., 1984; Wild, 1989, 1997; Korzeniewska and Güntürkün, 1990; Veenman *et al*., 1997; Kroner and Güntürkün, 1999). There is a projection from the principle trigeminal nucleus directly to the nucleus basalis in the telencephalon, which is devoted to the bill and beak cavity sensation, and appears to be unique to birds (Cohen and Karten, 1974; Dubbeldam *et al*., 1981).

Thus, the basic pattern of most somatosensory connections was conserved during the evolution from reptiles to birds, although the thalamic targets were greatly elaborated in birds. A direct trigeminal projection to the telencephalon appears to be a unique avian feature. It may have evolved by the invasion of primary trigeminal fibers into the nearby parabrachial nucleus, which projects to the telencephalon in reptiles and mammals.

12.4.3.4 Reptile to mammal transition In mammals, spinothalamic, dorsal columnar, and trigeminal nucleus projections terminate in the mesencephalon (intercollicular area) and in four thalamic areas including: ventral thalamus (zona incerta); the ventrobasal and posterior thalamic nuclei; a perigeniculate area at the ventromedial edge of the medial geniculate; and the intralaminar nuclei, particularly the central lateral nucleus (Giesler *et al*., 1981; 1988; Cliffer *et al*., 1991; Willis and Coggeshall, 1991; LeDoux *et al*., 1987).

These four thalamic nuclei can be classified based on their additional connections: (1) a ventral thalamic nucleus that lacks projections to the telencephalon; (2) nuclei that project to the somatosensory cortex: the ventrobasal and posterior nuclei; (3) a limbic thalamic area that projects to the striatum and a ventrolateral pallial derivative, the basolateral amygdaloid complex, (LeDoux *et al*., 1987; Turner and Herkenham, 1991; Bordi and LeDoux, 1994; Price, 1995; Linke *et al*., 2000); and (4) intralaminar nuclei which project to the striatum and frontal motor cortex (Figure 4d).

12.4.3.5 Summary Somatosensory information from the spinal cord reaches the forebrain through similar pathways in all tetrapods, and thus appears to be a phylogenetically ancient feature. Somatosensory information from dorsal column nuclei appears to reach the telencephalon only in amniotes, suggesting that modifications of this pathway may have occurred in the common amniote ancestor. Three somatosensory recipient thalamic nuclei are common to all tetrapods including: (1) ventral thalamic nuclei; (2) nuclei that project to a cortical target; and (3) nuclei that project to a striatal and ventrolateral pallial target. An intralaminar-like somatosensory thalamic region has been identified in birds and mammals, and further studies are needed in amphibian and reptiles to determine its evolutionary origins (see The Evolution of the Dorsal Thalamus in Mammals).

This scheme suggests that the thalamic nuclei that project to the amphibian ventral lateral pallium, reptilian DVR, avian nidopallium, and mammalian pallial amygdala are homologous. This comparison is considered further in Section 12.5.3 (see The Evolution of the Somatosensory System, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?).

12.4.4 Olfactory System

In most tetrapods olfaction is detected in two peripheral organs, the main olfactory organ, which is sensitive to lighter, airborne molecules and the vomeronasal organ, which is sensitive to heavy odor molecules (Johnson and Leon, 2000). The vomeronasal system is unique to tetrapods and is involved in both foraging and reproductive behaviors, and in some pheromonally mediated behaviors (Eisthen, 1992; Halpern and Martinez-Marcos, 2003). The sensory neurons in the main olfactory organ and vomeronasal organ project to the main olfactory and accessory olfactory bulbs, respectively. This section compares the projections of the main and accessory olfactory bulbs.

12.4.4.1 Reptiles A vomeronasal organ is present in rhynchocephalians (tuatara), lizards, snakes, and turtles, but is absent in crocodilians and birds (Figure 5b). The central projections have been studied in four species of lizards, two snakes, a turtle, and caiman (Heimer, 1969; Scalia *et al.*, 1969; Halpern, 1976; Ulinski and Peterson, 1981; Reiner and Karten, 1985; Martinez-Garcia *et al.*, 1991; Lohman and Smeets, 1993; Lanuza and Halpern, 1998). The later studies that used more sensitive techniques provide greater details, and are the basis for the following summary.

The main olfactory bulb projections travel in a superficial sheet over the telencephalon to targets that include the olfactory tubercle, lateral cortex, rostral septum, nucleus of the diagonal band, and several cortical amygdalar nuclei. Olfactory fibers continue caudally in the stria medullaris, cross in the habenular commissure, and then innervate similar targets in the contralateral hemisphere.

The accessory olfactory bulb projects caudally, terminating in the medial amygdala and its medial extension (the bed nucleus of stria terminalis), in the nucleus sphericus in both lizards and snakes, and in

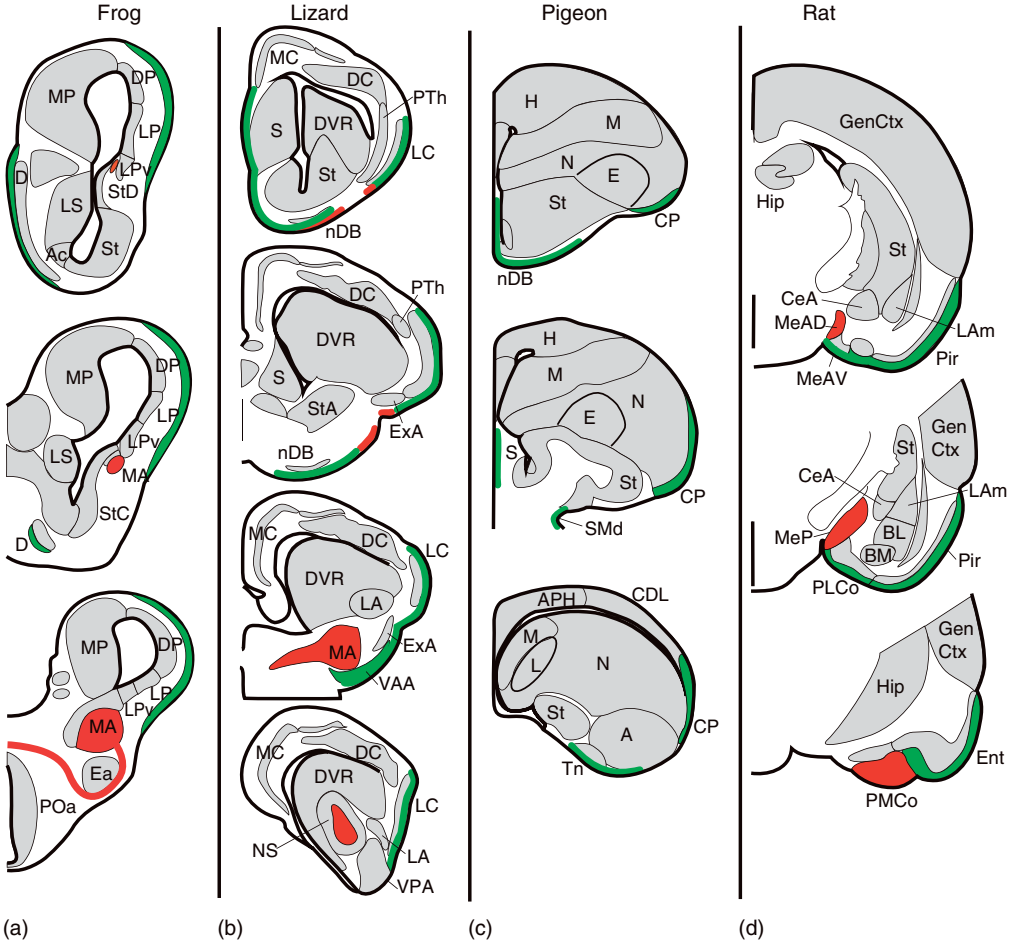


Figure 5 Comparison of the pathways from main olfactory bulb (green) and accessory olfactory bulb (red) in (a), amphibians; (b), reptiles; (c), birds; and (d), mammals. Sections are through the right hemisphere, with rostral sections at the top. Abbreviations: Frog Ac, accumbens; D, nucleus of the diagonal band; DP, dorsal pallium; Ea, anterior entopeduncular nucleus; LP, lateral pallium; LPv, ventral part of the lateral pallium; LS, lateral septal nucleus; MA, medial amygdala; MP, medial pallium; POa, anterior preoptie area; St, striatum; StC, caudal striatum; StD, dorsal striatum. Lizard DC, dorsal cortex; DVR, dorsal ventricular ridge; ExA, external amygdalar nucleus; LA, lateral amygdala; LC, lateral cortex; MA, medial amygdala; MC, medial cortex; nDB, nucleus of the diagonal band; NS, nucleus sphericus; PTh, pallial thickening; S, septal nuclei; St, striatum; StA, striatoamygdalar area; VAA, ventral anterior amygdala; VPA, ventral posterior amygdala. Pigeon A, arcopallium; APH, area parahippocampalis; CDL, dorsal lateral cortex; CP, cortex piriformis; E, entopallium; H, hyperpallium; L, Field L; M, mesopallium; nDB, nucleus of the diagonal band; N, nidopallium; S, septal nuclei; St, striatum; SMd, stria medullaris tract, dorsal part; Tn, nucleus Taeniae. Rat BL, basolateral amygdala; BM, basomedal amygdala; CeA, central amygdala; Ent, entorhinal cortex; GenCtx, general (nonolfactory, nonhippocampal) cortex; Hip, hippocampus; LAm, lateral amygdala; MeAD, anterodorsal division of medial amygdala; MeAV, anteroventral division of medial amygdala; MeP, medial pallium; Pir, piriform cortex; PLCo, posterior lateral cortical amygdala; PMCo, posterior medial cortical amygdala; St, striatum. Modified from Bruce, L. L. and Neary, T. J. 1995c. The limbic system of tetrapods: A comparative analysis of cortical and amygdalar populations. *Brain Behav. Evol.* 46, 224 234.

the ventral posterior amygdala in at least one lizard (Martinez-Garcia *et al*., 1991). Nucleus sphericus is present in most squamate reptiles, and is correlated with the importance of chemosensory function in their ecology (Halpern and Martinez-Marcos, 2003). Although a vomeronasal system is present in Testudamorpha, it is uncertain whether or not Reiner and Karten included it in their olfactory bulb injections (Reiner and Karten, 1985).

12.4.4.2 Amphibian to reptile transition Olfactory pathways have been studied in tiger salamanders and three species of ranid frogs (Northcutt and Royce, 1975; Kokoros and Northcutt, 1977; Kemali and Guglielmotti, 1987; Scalia *et al*., 1991; Moreno *et al*., 2005). The targets of the main olfactory bulb are very similar to those in reptiles, and include the postolfactory eminence, lateral and dorsal pallia, rostral septum, nucleus of the diagonal band, and ventral part of the lateral pallium (LPv; sometimes named the lateral amygdala); and a rostral part of the medial amygdala. The lateral and dorsal pallia are comparable to the reptilian lateral cortex, and the LPv and rostral medial amygdala may be comparable in part to the reptilian olfactory cortical amygdala (Bruce and Neary, 1995c; Moreno *et al*., 2005). A fascicle of fibers decussates in the habenular commissure, comparable to that in reptiles (Figure 5a).

A vomeronasal system is present in most aquatic and terrestrial amphibians, but is absent in some aquatic salamanders (Eisthen, 2000). The accessory olfactory bulb projects to the medial amygdaloid nucleus (formerly called the lateral amygdala) and to part of the caudal LPv. Accessory fibers cross in both the anterior commissure and the habenular commissure, unlike the ipsilateral reptilian condition. Thus, most accessory olfactory targets receive bilateral input. The amphibian medial amygdala thus appears comparable to the reptilian medial amygdala, and part of the caudal LPv appears comparable to the reptilian accessory recipient cortical nucleus. Furthermore, the reptilian nucleus sphericus appears to be a specialization of the accessory olfactory amygdala, reflecting the importance of vomeronasal information in the ecology of squamate reptiles.

12.4.4.3 Reptile to bird transition Birds, like crocodilians, have a main olfactory system but lack a vomeronasal system (Figure 5c). Olfactory bulb projections have been reported in pigeons and ducks (Reiner and Karten, 1985; Ebinger *et al*., 1992). The targets are remarkably similar to those seen in reptiles. Targets of the olfactory bulb include the olfactory tubercle, cortex pyriformis, rostral septum, nucleus of the diagonal band, and cortical amygdalar nuclei in the caudal telencephalon, including nucleus Taeniae. Axons cross in the habenular commissure. Unlike reptiles, the olfactory bulb does not project to most of the caudal telencephalic pole (the arcopallium).

12.4.4.4 Reptile to mammal transition In mammals, the main and accessory olfactory bulb projections are entirely ipsilateral (Figure 5d). Main olfactory bulb targets include the olfactory tubercle, olfactory cortex (pyriform and entorhinal), nucleus of the diagonal band, several cortical amygdalar areas (anterior amygdala, anterior cortical, and posterolateral cortical amygdalar nuclei), and the ventral anterior part of the medial amygdala (de Olmos *et al*., 1978; Switzer *et al*., 1985). Accessory olfactory bulb projections include most of the medial amygdala, the medial bed nucleus of the stria terminalis, and the posteromedial cortical amygdalar nucleus. Thus, the main and accessory olfactory targets of mammals are very similar to those of reptiles, except that mammals lack contralateral main olfactory projections.

12.4.4.5 Summary The main olfactory bulb targets are similar in all tetrapods, suggesting they were present in a common ancestor. A contralateral projection through the habenular commissure is present in amphibians, reptiles, and birds, but not mammals, suggesting it was lost in a mammalian ancestor.

Likewise, the accessory olfactory bulb targets are similar among most tetrapods, suggesting they were established in a common ancestor. However, contralateral projections are present only in amphibians, suggesting they were lost in ancestral reptiles. In squamate reptiles, the vomeronasal targets include nucleus sphericus, which is probably derived from the vomeronasal amygdala (Bruce and Neary, 1995c; Lanuza and Halpern, 1998). The absence of the vomeronasal system in crocodilians and birds, cetaceans, and in some primates suggests that it was lost independently in several taxa (see Vertebrate Olfactory Subsystems and their Evolution).

12.5 Brain Regions

12.5.1 Hindbrain: Cranial Motor Nuclei

There are a number of extensive reports and reviews on the development and evolution of the tetrapod hindbrain (Barbas-Henry and Lohman, 1984, 1988; Roth *et al*., 1988; Szekely and Matesz, 1988; Bruce *et al*., 1997; Fritzsch, 1998; Gilland and Baker, 2005). These are summarized in the following description of cranial nerve motor nuclei (Figure 6).

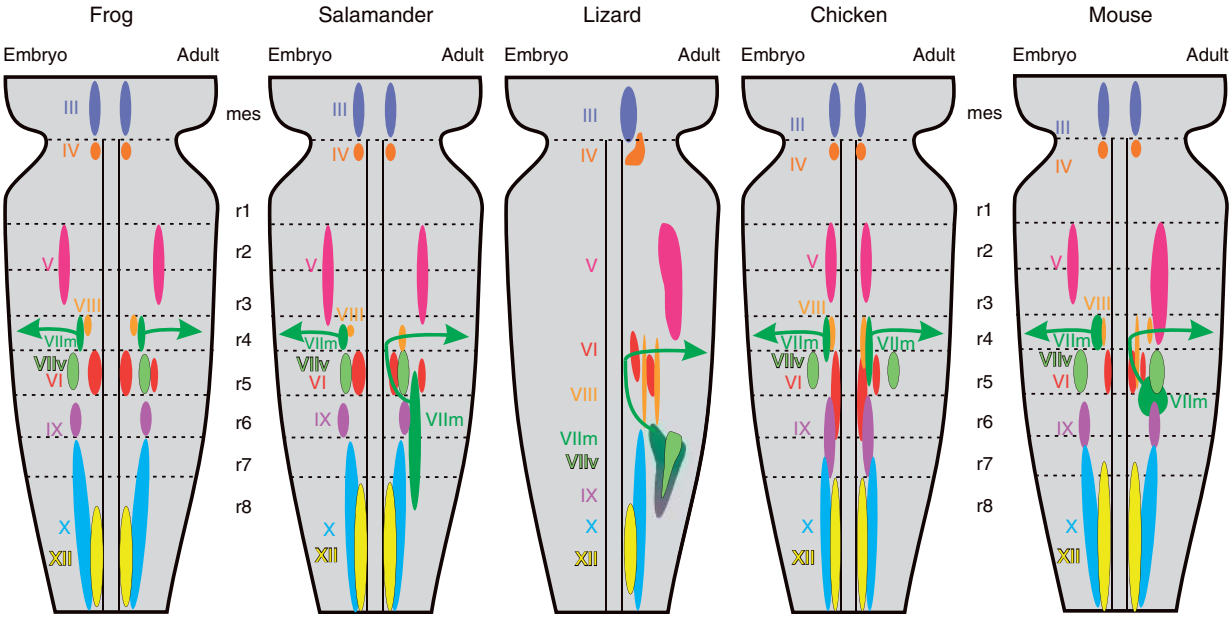


Figure 6 Comparison of the embryonic (left side) rhombomeric origin (r1 r8) and adult (right side) locations of cranial nerve motor nuclei in frog, salamander, lizard (adult only), chicken, and rat. Green arrows show the trajectory of VIIm axons. Note that the facial genu is formed by the caudal migration of VIIm neurons. Modified from Gilland, E. and Baker, R. 2005. Evolutionary patterns of cranial nerve efferent nuclei in vertebrates. *Brain Behav. Evol.* 66, 234 254 and Fritzsch, B. 1998b. Ontogenetic and evolutionary evidence for the motoneuron nature of vestibular and cochlear efferents. In: The Efferent Auditory System: Basic Science and Clinical Applications (*ed.* C. I. Berlin), pp. 31 60. Singular.

Hindbrain motor neurons can generally be divided into three major groups: somatic (cranial nerves III, IV, VI, and XII), branchiomotor (V, VII, IX, X, and XI), and visceral (VII, IX, and X). Somatic and branchiomotor neurons innervate striated muscle, and visceromotor neurons innervate parasympathetic ganglia. The octavolateral efferents innervate the auditory and vestibular periphery of tetrapods, as well as mechanosensory hair cells in the lateral line of amphibians, and are closely related to the branchiomotor neurons of VII.

The cranial nerve nuclei of all vertebrates are born near the midline floorplate and are derived from similar rostrocaudal segments, or rhombomeres, and from similar dorsoventral partitions within the hindbrain. A variety of genes regulate rhombomeric (e.g., Hox and Fgf gene families) and dorsoventral organization (e.g., Shh). Variations in gene expression are correlated with variations in the origin of the cranial nerve nuclei, and are beyond the scope of this review. The hindbrain is divided into eight rhombomeres (r1–r8), although in mammals r8 is indistinct and usually included in r7. The development of hindbrain motor neurons has not been adequately studied in reptiles, but they are believed to follow the avian pattern.

12.5.1.1 Oculomotor nuclei (III) In all tetrapods the oculomotor motor neurons are born in the somatic column in the caudal mesencephalon, and remain near their origin. Their axons project mainly ipsilaterally, with contributions from a few cells in the contralateral caudal pole.

12.5.1.2 Trochlear nuclei (IV) Trochlear motor neurons of tetrapods derive from the contralateral somatic column in the rostral part of r1, and remain there. Their axons take a unique dorsal trajectory, decussating at the dorsal midline and then exiting the brain. A few ipsilateral trochlear neurons are present in mice.

12.5.1.3 Trigeminal nuclei (V) In all tetrapods the trigeminal motor nuclei are derived from r2 and the rostral two-thirds of r3. They remain close to their origin. Motor neurons derived from r2 appear to innervate muscles homologous to jaw adductors; those from r3 innervate the jaw openers (Song and Boord, 1993).

12.5.1.4 Abducens nuclei (VI) The origin of the abducens nucleus is variable, arising from r5 alone (frogs, salamanders, and mammals) or from both r5and r6 (chickens). In all tetrapods the abducens gives rise to the principal abducens, which remains adjacent to the medial longitudinal fascicle and innervates the lateral rectus muscle of the eye, and to the accessory abducens, which migrates laterally and innervates the ocular retractor muscles.

12.5.1.5 Facial motor nuclei (VIIm) In salamanders the facial motor nuclei are born in the caudal half of r4; in frogs, chickens, and mammals they originate from the full extent of r4. The facial motor nucleus migrates caudally but variably in salamanders, chickens, mice, but not in frogs. In salamanders it migrates to r5 and further caudally so that it overlaps the entire glossopharyngeal and parts of the vagus and hypoglossal nuclei. In chickens it extends from r4 to r5, and in mice from r5 to r6. In adult lizards the facial motor nucleus is located caudal to the abducens, suggesting that this is also a migrated population. Adult lizards have a flexure in their caudal brainstem, making relationships to other tetrapods imprecise. In animals with a migrated facial population, an internal facial genu is formed by the axons that remain in the migratory route. The facial motor nucleus innervates muscles derived from the second branchial arch.

12.5.1.6 Octavolateral nuclei (VIII) The octavolateral nucleus derives from the same ventricular area of r4 as the facial motor nucleus. In all tetrapods the octavolateral nuclei remain in r4, but in lizards and mice some of the neurons migrate laterally and away from the ventricle and form two clusters. In chickens a contralateral octavolateral population forms by neuronal migration across the midline; in mice it forms by the growth of axons to the contralateral side. Lizards also have a contralateral projection, although its origin is unknown. The octavolateral efferents innervate hair cells in the auditory and vestibular epithelia. In amphibians a group of octavolateral efferents arising from r4 (with the facial motor) and r6 (with the glossopharyngeal) innervate mechanoreceptors of the lateral line.

12.5.1.7 Facial visceral nuclei (VIIv) Also known as the superior salvitory nucleus, the facial visceral nucleus arises from r5 in all tetrapods, and remains there in adults. Although in reptiles the facial motor, facial visceral, glossopharyngeal, and vagal nuclei appear to overlap in dorsal view because of the flexure in this region, they are largely distinct nuclei (Figure 6). The facial visceral nucleus innervates postganglionic neurons to the Haderian gland.

12.5.1.8 Glossopharyngeal nuclei (IX) The glossopharyngeal nuclei include both a branchiomotor and a visceral group. In frogs and mammals the glossopharyngeal nuclei originate from r6, but in chickens they originate from both r6 and r7. The neurons remain in their rhombomere of origin. In adult salamanders and lizards the glossopharyngeal nuclei are intermingled with the facial motor neurons. The glossopharyngeal nucleus innervates muscles derived from the third branchial arch.

12.5.1.9 Vagal nuclei (X) The vagal nuclei of frogs and mice originate from r7 and r8, whereas those of chickens originate from r8. In adults the vagal nuclei may extend into the spinal cord, but it is not clear if this is due to neuronal migration.

12.5.1.10 Spinal accessory nuclei (XI) In tetrapods the spinal accessory nuclei originate in the rostral spinal cord. Its rostral extent often blends with the caudal extent of the vagal nuclei.

12.5.1.11 Hypoglossal nuclei (XII) Hypoglossal nuclei are present in all tetrapods with tongues. The rostral pole originates from progressively more rostral levels in frogs (caudal border of r8; called the first spinal nerve nucleus), chickens (caudal border of r7; called the supraspinal nucleus), and mice (middle of r7). The hypoglossal nucleus innervates the tongue musculature.

12.5.1.12 Summary The comparative development of the tetrapod hindbrain motor nuclei is fairly sparse, and requires analysis of more taxa, particularly from reptiles. The rostral poles of the motor nuclei have similar rhombomeric limits in all tetrapods, and thus appears to be a highly conserved feature. In contrast, the caudal pole may vary by as much as one rhombomere, particularly in chickens, and thus appears to be under fewer genetic constraints. Motor nuclei V, VIIm, and VIII migrate caudally from their ventricular origins in some taxa. In frogs there is no caudal migration, in salamanders only the facial motor nucleus migrates, in chickens the facial motor and octavolateralis nuclei migrate, and in mice the facial motor, octavolateralis, and trigeminal nuclei migrate caudally. Thus, caudal migration appears to be most prominent in mammals and least prominent in amphibians. The genu of the facial nerve forms as a result of the caudal neuronal migration, and is prominent in salamanders, lizards, and mammals. Thus, the facial genu may have been present in the common ancestor of amphibians, mammals, and reptiles, but lost in the anuran lineage.

12.5.2 Dorsal Thalamus

The reptilian diencephalon consists of the epithalamus, dorsal thalamus, ventral thalamus, and the hypothalamic regions. The organization of the dorsal thalamus is the best-studied region, yet some of its mammalian homologues are controversial. Thus,

the functional connections will be described and compared in this section.

12.5.2.1 Reptiles The reptilian dorsal thalamus consists of nuclei that project to and often receive projections from the telencephalon. It can be subdivided into at least four groups on the basis of connectional and functional characteristics (Table 1).

1. *Nuclei that receive specific sensory projections and project to the cortex*. In squamates this group includes the dorsolateralis anterior (DLA) and a retino-recipient nucleus, called either lateral geniculate, intercalatus, or the lateral part of the dorsolateralis anterior (Hall and Ebner, 1970a, 1970b; Cruce and Cruce, 1975; Hall *et al*., 1977; Hoogland, 1982; Bruce and Butler, 1984a, 1984b; Bennis *et al*,. 1994; Desfilis *et al*., 2002; Guirado and Davila, 2002). The DLA receives spinal, septal, and hypothalamic projections and a small projection from the auditory/vocal area of torus semicircularis in the midbrain. In some lizards the DLA is further subdivided into the pars magnocellularis (DLAm), which projects bilaterally to the lateral and medial cortices, and the pars parvocellularis (DLAp), which projects ipsilaterally to the dorsal cortex and pallial thickening. In Testudamorpha these subdivisions correspond to the dorsomedial anterior and the dorsolateral anterior, respectively (Desan, 1988; Zhu *et al*., 2005).
2. *Nuclei that receive specific sensory brainstem projections and project to specific regions of the striatum and DVR*. These include rotundus, medialis (reuniens in turtles and crocodilians), and medialis posterior (caudalis in turtles; medialis complex in crocodilians), which convey visual, auditory, and somatosensory information, respectively (see Section 12.4).
3. *A nonspecific midline area that receives widespread input and projects to cortex, DVR, central amygdala, accumbens and striatum* (Hoogland, 1982; Gonzalez *et al*., 1990; Desfilis *et al*., 2002; Heredia *et al*., 2002). In lizards the dorsomedial nucleus projects to the accumbens, striatum, and receives projections from the septum, preoptic area, ventromedial hypothalamus, ventral thalamus, and locus coeruleus.
4. *Calcitonin gene-related peptide (CGRP)-positive neurons that project to the pallium (cortex and DVR)*. In lizards these cells lie at the posterior ventral pole of the thalamus (Martinez-Garcia *et al*., 2002a).

Table 1 Comparisons of dorsal thalamic nuclei in tetrapods

	Reptile				*Mammal (rodent)*	
Amphibian	*Lizard*	*Crocodilian*	*Turtle*	*Birds*	*Revised*	*Traditional*
	DM		DM	DLM, DMA, DMP, SHL, DIP, DLP	Paraventricular, MD, CL, CM, PC, PF, IMD	Paraventricular, MD, CL, CM, PC, PF, IMD
Anterior	DLAm and DLAp		Dorsomedial anterior and Dorsolateral anterior	Superficialis parvocellularis, DLA	Anterior, reuniens, VPM, VPL, MGN, LP	Anterior, reuniens
Anterior	Intercalatus (lateral DLA)		Dorsal lateral geniculate	Dorsal optic complex	Dorsal lateral geniculate	Dorsal lateral geniculate
Lateral	Rotundus	Rotundus	Rotundus	Rotundus	Perigeniculate: Supragenículate	Lateral posterior (pulvinar)
Central	Medialis	Reuniens	Reuniens	Ovoidalis	Perigeniculate: MGm	Medial geniculate
Central	Medialis Posterior and posterocentral	Medialis complex	Caudalis	Subrotundus	Perigeniculate: Posterior intralaminar	VPM, VPL
				Ventral intermediate area	VA, VL	VA, VL

CM, central medial thalamic nucleus; CL, centrolateral thalamic nucleus; DIP, nucleus dorsointermedius posterior thalami; DM, nucleus dorsomedialis; DLA, dorsolateral anterior thalamic nucleus; DLAm, dorsolateral anterior thalamic nucleus, magnocellular part; DLAp, dorsolateral anterior thalamic nucleus, parvocellular part; DLM, nucleus dorsolateralis anterior thalami, pars medialis; DMA, nucleus dorsomedialis anterior thalami; DMP, nucleus dorsomedialis posterior thalami; DLP, nucleus dorsolateralis posterior thalami; IMD, intermediodorsal nucleus; LP, lateral posterior thalamic nucleus; MD, mediodorsal nucleus; MGN, medial geniculate nucleus; PC, paracentral thalamic nucleus; PF, parafascicular thalamic nucleus; SHL, lateral subhabenular nucleus; VA, ventral anterior thalamic nucleus; VL, ventral lateral thalamic nucleus; VPM, ventral posterior medial thalamic nucleus; VPL, ventral posterior lateral thalamic nucleus.

12.5.2.2 Amphibian to reptile transition The amphibian dorsal thalamus consists of the anterior, central, and lateral thalamic nuclei (Neary and Northcutt, 1983).

1. *Nuclei that receive specific sensory projections and project to the cortex* (Mudry and Capranica, 1980; Wilczynski and Northcutt, 1983a; Neary, 1984, 1988, 1990; Allison and Wilczynski, 1991; Montgomery and Fite, 1991; Northcutt and Ronan, 1992). The anterior thalamic nucleus receives visual inputs from the retina, somatosensory inputs from the obex region, spinal cord, and torus semicircularis, and auditory inputs from the lateral torus semicircularis and pretectal grey. It projects heavily to the medial and dorsal pallia and the septum, and more weakly to most parts of the telencephalon and the ventral hypothalamus.
2. *Nuclei that receive specific sensory brainstem projections and project to specific regions of the striatum and DVR*. Both the central and lateral thalamic nuclei receive widespread sensory brainstem projections and project to specific regions within the striatum and ventral part of the lateral pallium (Wilczynski and Northcutt, 1983a, 1983b; Hall and Feng, 1987; Neary, 1990, 1995; Allison and Wilczynski, 1991; Feng and Lin, 1991). The central nucleus receives input from the optic tectum and somatosensory part of the torus semicircularis. The lateral thalamic nucleus receives input from the auditory part of the torus semicircularis. Both the lateral and central nuclei project to the striatum and the ventral part of the lateral pallium.
3. *A nonspecific midline area that receives widespread input and projects to the telencephalon.* Neurons with these characteristics have not been described in amphibians.
4. *CGRP-positive neurons that project to the pallium.* These neurons are scattered within the ventromedial and posterior part of the central thalamic nuclei (Petko and Santa, 1992).
5. Thus, at least three of the four thalamic groups present in reptiles are also recognized in amphibians, suggesting that they were present in the common tetrapod ancestor. A nonspecific midline thalamic area has not been identified in amphibians, and further studies are needed to determine if it exists. Furthermore, the reptilian thalamus appears to have increased in size and become further segregated during the amphibian to reptile transition.

12.5.2.3 Reptile to bird transition The following thalamic groups are present in birds:

1. *Nuclei that receive specific sensory projections and project to the cortex*. Visual information from both the retina and optic tectum reaches the dorsolateralis pars lateralis (DLL), SPC, and LdOPT, whereas only the retina projects to the DLAmc. Somatosensory information from the torus semicircularis reaches the DIVA and DLL. All these thalamic nuclei project to visual and somatosensory Wulst areas in the hyperpallium (hyperstriatum).
2. *Nuclei that receive specific sensory brainstem projections and project to specific regions of the striatum and DVR (see Section 12.5.3)*. Auditory, visual, and somatosensory information reaches nucleus ovoidalis, nucleus rotundus, and nucleus subrotundus, respectively. Each of these thalamic nuclei then projects to a unique target in the nidopallium (previously called the neostriatum).
3. *A nonspecific midline area that receives widespread input and projects to cortex, nidopallium, accumbens, and striatum*. The dorsomedialis posterior, dorsomedialis anterior, subhabenular nucleus, dorsointermedius posterior nucleus, and dorsolateralis posterior meet these connectional criteria, and have also been compared to mammalian homologues based on neurochemical and mRNA expression patterns (Veenman *et al*., 1997; Bruce *et al*., 2002; Atoji and Wild, 2005).
4. *CGRP-positive neurons that project to the pallium*. A scattered population of CGRP-expressing neurons that projects to the hyperpallium and nidopallium is mainly located in the shell surrounding nucleus ovoidalis and in nucleus dorsolateralis posterior (Brauth and Reiner, 1991; Lanuza *et al*., 2000).
5. *Thalamic nuclei that receive motor input from cerebellar nuclei, substantia nigra, and globus pallidus, and project to the pallium*. In pigeons the ventral intermediate area has these characteristics (Medina *et al*., 1997).

Thus four of the five thalamic groups (1, 2, 3, and 4) described in birds can also be identified in reptiles (Table 2). However, a motor thalamic nucleus (5) has been identified in birds, but not reptiles. Further studies are needed to determine if it has a reptilian homologue. Also noteworthy is the increased size of many of the avian thalamic nuclei, compared to those in reptiles.

Table 2 Comparison of current models of the evolution of the avian hyperpallium, mesopallium, and sensory nidopallium

	Hyperpallium dorsal cortex	*Mesopallium pallial thickening*	*Sensory nidopallium anterior DVR*	*Caudal nidopallium posterior DVR*
Bruce and Neary (1995c), and Bruce (this article)	Isocortex	Claustrum and endopiriform	Lateral amygdala (sensory part)	Basolateral amygdala
Striedter (1997)	Isocortex	Claustrum	Endopiriform	Pallial amygdala
Puelles *et al.* (2000) and Martinez-Garcia *et al.* (2002b)	Isocortex claustrum	Isocortex claustrum	Endopiriform	Basal and lateral amygdalar nuclei
Butler and Molnar (2002)	Isocortex	Not mentioned	Isocortex, claustrum, endopiriform, basal and lateral amygdalar nuclei	Isocortex, claustrum, endopiriform, basal and lateral amygdalar nuclei
Karten (1997) and Reiner (2000)	Isocortex	Isocortex	Isocortex	Isocortex

These four avian territories (top row) are compared to their suggested mammalian homologue according to the proposals of various authors (left column).

12.5.2.4 Reptile to mammal transition The following thalamic groups are present in mammals:

1. *Nuclei that receive specific sensory projections and project to the cortex*. Auditory information from the inferior colliculus reaches the medial geniculate nucleus. Visual information from the retina reaches the dorsal lateral geniculate, and from the superior colliculus reaches the lateral posterior nucleus in rodents (LP; called pulvinar in primates). Somatosensory information from the spinal cord, dorsal column nuclei, trigeminal nuclei, and intercollicular area reaches the ventrobasal and posterior nuclei. All of these thalamic nuclei project to specific auditory, visual, and somatosensory areas in the isocortex.
2. *Nuclei that receive specific sensory brainstem projections and project to specific regions of the striatum and amygdala (see Section 12.5.3)*. Auditory, visual, and somatosensory information reaches the perigeniculate region, sometimes called the posterior intralaminar region. Each of these thalamic nuclei then projects to a different region of the lateral amygdala (Doron, and LeDoux, 1999; LeDoux *et al.*, 1987, 1990; Linke, 1999; Linke *et al.*, 1999, 2000; Linke and Schwegler, 2000).
3. *A nonspecific midline area that receives widespread input and projects to cortex, amygdala, accumbens, and striatum*. The centromedial thalamic nucleus, intermediodorsal, paraventricular, and parafascicular nuclei meet these connectional criteria and also have similar neurochemical expression patterns (Veenman *et al.*, 1997; Bruce *et al.*, 2002).
4. *CGRP-positive neurons that project to the pallium*. CGRP-expressing neurons project to diffuse targets in the cortex, amygdala, and striatum. They are scattered in the peripeduncular nucleus, posterior intralaminar nucleus, subparafascicular nucleus pars lateralis, and subparafascicular nucleus (Brauth and Rener, 1991; Yasui *et al.*, 1991).
5. *Thalamic nuclei that receive motor input from cerebellar nuclei, substantia nigra, and globus pallidus, and project to the pallium*. The ventral lateral and ventral anterior thalamic nuclei meet these criteria (Price, 1995).

The reptile to mammal transition is marked by a considerable increase in size and subdivisions of the thalamic nuclei that project to the cortex. At least three of these thalamic populations are comparable in reptiles and mammals (1, 3, and 4). A motor thalamic nucleus (5) has not yet been identified in reptiles, although one is present in birds. Whether the reptilian thalamic nuclei that project to the DVR should be compared with the mammalian thalamic nuclei that project to the cortex (traditional view) or with those that project to the lateral amygdala (revised view), is an ongoing controversy. These two thalamic populations appear to have few discriminating neurochemical expression patterns, which would resolve the issue. The group they are compared to is currently determined by the homology of their telencephalic target, a controversy addressed in Section 12.5.3. Most evidence suggests that the reptilian thalamo-DVR population is comparable to the mammalian thalamo-amygdalar population.

12.5.2.5 Summary The transition from amphibians to reptiles, and from reptiles to birds and mammals is marked by an increased hypertrophy and segregation of the thalamic groups with projections to the cortex. Homologues of the reptilian thalamic nuclei that project to the DVR have been identified in amphibians and birds, but the comparable group in mammals is controversial, with some

comparing these nuclei to the sensory nuclei that project to specific cortical areas (traditional view), and others to sensory nuclei that project to the lateral amygdala (revised view). Two additional thalamic groups, sensory nuclei that project to the cortex, and a CGRP-positive group, are present in amphibians, reptiles, birds, and mammals, suggesting that they were present in the common ancestor of tetrapods. A nonspecific midline group with widespread pallial projections is present in reptiles, birds, and mammals, but has not been identified in amphibians, and a motor thalamic group has been identified in birds and mammals, but not in reptiles or amphibians. Further study is needed to determine if these are present, before its evolutionary appearance can be considered.

12.5.3 Telencephalon

12.5.3.1 Reptiles The reptilian telencephalon contains two major divisions, the pallium and the subpallium. This section focuses on the pallium (telencephalic roof), including the pallial amygdalar nuclei. The pallium includes the medial, dorsal, and lateral cortices, the pallial thickening, DVR, and several amygdaloid nuclei (Figures 7b and 8b). The subpallium includes the striatum, central amygdaloid nucleus, and most septal nuclei. The medial amygdaloid nucleus contains both pallial and subpallial characteristics (Northcutt, 1978; Puelles *et al.*, 2000; Brox, *et al.*, 2004).

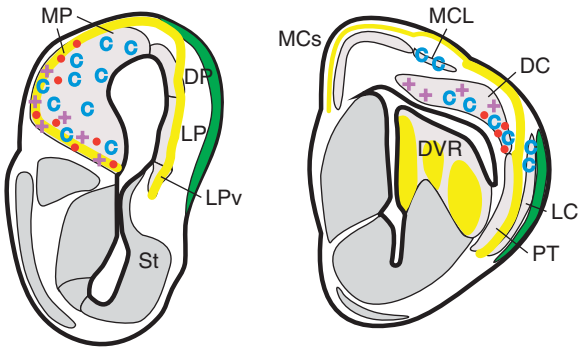


Figure 7 Comparison of pallial connections in frog (a), and lizard (b). Schematic shows the locations of neurons that project to the contralateral pallium (c), to the thalamus (+), and to the hypothalamus (red dots) in sections through the right hemisphere. Terminal areas from neurons in the olfactory bulb (green), and thalamic nuclei (yellow) (anterior nucleus in frogs; dorsolateral and dorsal lateral geniculate in reptiles) and caudal thalamic nuclei (lateral and central in frogs; medialis, medialis posterior, and rotundus in lizards: orange) are also shown. Rostral-most sections are at the top. Note that the connections of the ventral part of the frog medial pallium are most similar to those of the lizard DC. Abbreviations: frog DP, dorsal pallium; LP, lateral pallium; LPv, ventral part of the lateral pallium; MP, medial pallium; St, striatum. Lizard DC, dorsal cortex; DVR, dorsal ventricular ridge; LC, lateral cortex; MCL, large-celled part of medial cortex; MCs, small-celled part of medial cortex; PT, pallial thickening.

Cortical regions. The lateral cortex is the primary target of the main olfactory bulb, and reciprocates this connection (Lohman and Mentink, 1972; Martinez-Garcia *et al.*, 1991; Lohman and Smeets, 1993; Lanuza and Halpern, 1998). The medial cortex is generally regarded to be a hippocampal region based on connectional and neurochemical staining patterns (e.g., Ariëns Kappers *et al.*, 1936; Northcutt, 1969; Belekhova and Kenigfest, 1983; Bruce and Butler, 1984b; Olucha, 1988; Perez-Clausell, 1988; Smeets *et al.*, 1989, Butler, 1994), and corresponds to the medial cortex of Testudamorpha (Desan, 1988; Zhu, 2005). The dorsal cortex appears to be a general cortical area (e.g., nonhippocampal, nonolfactory cortex). It is reciprocally connected with sensory thalamic nuclei and with the contralateral hemisphere (Northcutt, 1969, 1981; Hall and Ebner, 1970a; Bruce and Butler, 1984a; Desan, 1988; Hoogland and Vermeulen-Vanderzee, 1989; Reiner, 1991; Butler, 1994; Bruce and Neary, 1995c).

Pallial thickening. In turtles part of the pallial thickening lies deep to the olfactory-recipient lateral cortex, but the rest (the primary visual thalamic target) extends superficially and becomes continuous with the dorsal cortex (Desan, 1988; Zhu, 2005). The deep part of the pallial thickening lies over the sensory-recipient DVR, and has mainly intrinsic telencephalic connections. In lizards the pallial thickening receives a projection from the dorsolateral anterior nucleus and from the retinothalamic nucleus (intercalatus or dorsal lateral geniculate) (Bruce and Butler, 1984a; Kenigfest *et al.*, 1997; Desfilis *et al.*, 2002). Further studies are needed to determine if these projections are segregated into two regions of the pallial thickening, as in turtles. The pallial thickening appears to be mainly a lateral pallial derivative (Fernandez *et al.*, 1998).

Dorsal ventricular ridge (DVR). The anterior DVR contains three sensory-recipient regions (auditory, visual, and somatosensory). The posterior DVR contains a caudomedial region that projects to the ventromedial hypothalamus, and a caudodorsal region that projects to the lateral hypothalamus (Bruce and Neary, 1995c). Based on Testudamorpha and avian data, the anterior DVR appears to be a ventral pallial derivative and the posterior DVR appears to include both ventral and lateral pallial territories (Fernandez *et al.*, 1998; Puelles *et al.*, 2000; Martinez-Garcia *et al.*, 2002b).

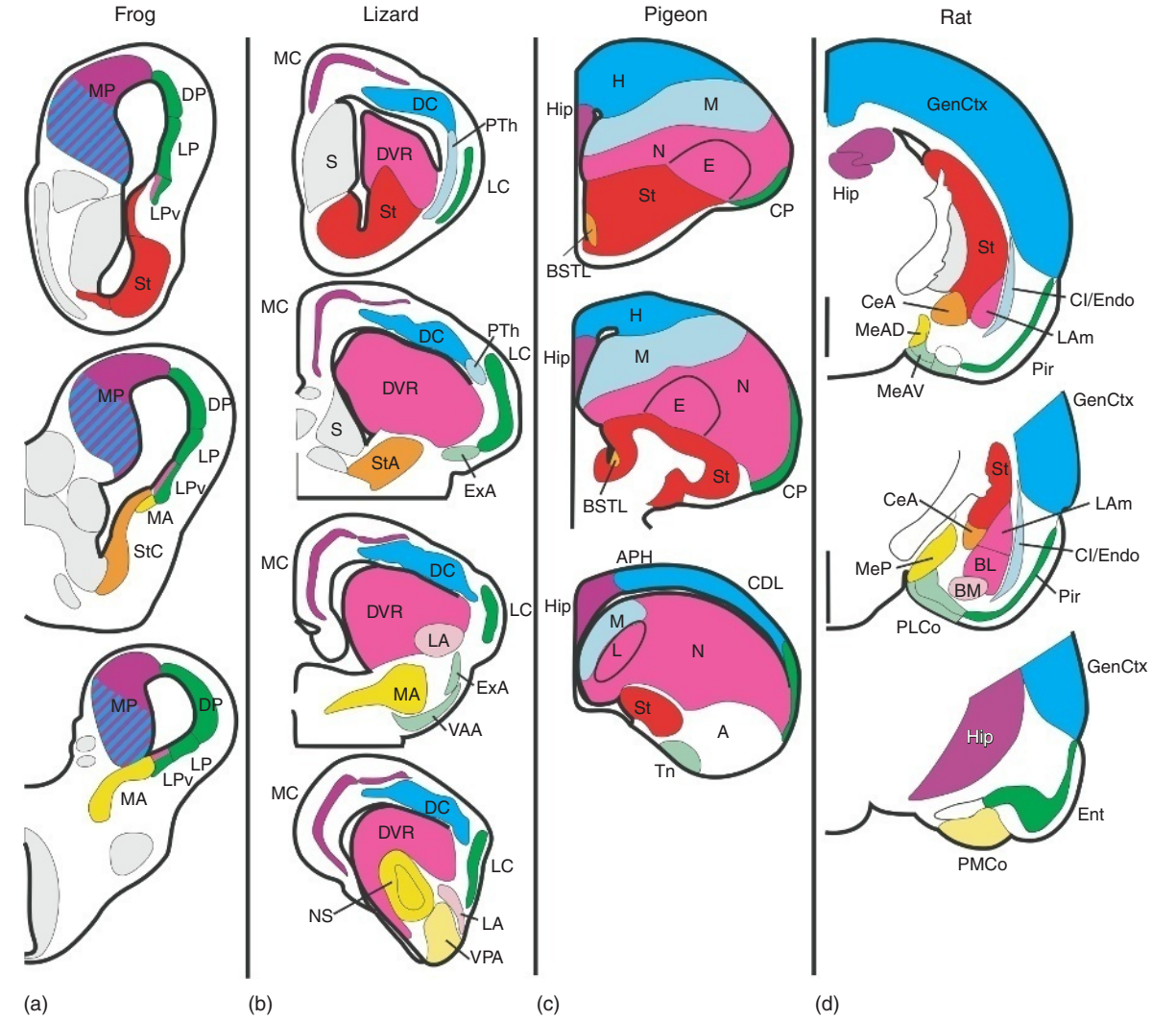


Figure 8 Summary of homologous telencephalic areas in a, amphibians; b, reptiles; c, birds; and d, mammals. Homologous areas are identified by the same color. Abbreviations: frog DP, dorsal pallium; LP, lateral pallium; LPv, ventral part of the lateral pallium; MA, medial amygdala; MP, medial pallium; St, striatum; StC, caudal striatum. Lizard DC, dorsal cortex; DVR, dorsal ventricular ridge; ExA, external amygdala; LA, lateral amygdalar nucleus; LC, lateral cortex; MA, medial amygdala; MC, medial cortex; NS, nucleus sphericus; PTh, pallial thickening; S, septal nuclei; St, striatum; StA, striatoamygdalar area; VAA, ventral anterior amygdala; VPA, ventral posterior amygdala. Pigeon A, arcopallium; APH, area parahippocampalis; BSTL, lateral bed nucleus of stria terminalis; CDL, dorsolateral cortex; CP, cortex piriformis; E, entopallium; H, hyperpallium; Hip, hippocampus; L, Field L; M, mesopallium; N, nidopallium; St, striatum; Tn, nucleus Taeniae. Rat BL, basolateral amygdala; BM, basomedal amygdala; CeA, central amygdala; cl/Endo, claustrum and endopiriform; Ent, entorhinal cortex; GenCtx, general (nonolfactory, nonhippocampal) cortex; Hip, hippocampus; LAm, lateral amygdala; MeAD, anterodorsal division of medial amygdala; MeAV, anteroventral division of medial amygdala; MeP, medial pallium; Pir, piriform cortex; PLCo, posterior lateral cortical amygdala; PMCo, posterior medial cortical amygdala; St, striatum. Adapted from Bruce, L. L. and Neary, T. J. 1995c. The limbic system of tetrapods: A comparative analysis of cortical and amygdalar populations. *Brain Behav. Evol.* 46, 224 234, with permission from Karger, Basel.

Amygdalar groups. The medial amygdaloid nucleus and nucleus sphericus receive input from the accessory olfactory bulb. The medial amygdaloid nucleus projects to the ventromedial and lateral hypothalamus (Bruce and Neary, 1995a, 1995b). The lateral amygdaloid nucleus receives sensory information from the anterior DVR and projects to the core of the ventromedial hypothalamus (Voneida and Sligar, 1979; Bruce and Neary, 1995a). The dorsolateral amygdaloid nucleus (DLA) receives sensory input from nucleus medialis posterior in the thalamus and from the anterior DVR. It receives dopaminergic input from the ventral tegmental area and cholinergic input from the basal forebrain. Its main output is to the striatum and accumbens (Lanuza *et al*., 1998). The ventral anterior amygdaloid nucleus lies superficial to the medial amygdala. It receives olfactory input and projects to the

ventromedial hypothalamus. The ventral posterior amygdaloid nucleus lies superficial to nucleus sphericus, receives vomeronasal input, and projects to the lateral hypothalamic area. Further studies are needed to determine if these groups are derived from the ventral or lateral pallium.

The central amygdaloid nucleus has a subpallial origin, and is the only subpallial region with long descending projections to both the hypothalamus and brainstem (Russchen and Jonker, 1988; Bruce and Neary, 1995b).

12.5.3.2 Amphibian to reptile transition *Lateral, dorsal, and medial pallia*. The pallium of amphibians is divided into lateral, dorsal, and medial pallial fields (Northcutt, 1981), which were traditionally compared to the lateral, dorsal, and medial cortices of reptiles. However, a re-analysis of the amphibian pallial connections led Bruce and Neary (1995c) to conclude that both the dorsal and lateral pallia are comparable to those of the reptilian lateral cortex, whereas the medial pallium is comparable to both the reptilian medial and dorsal cortices (Figures 7a, 7b, and 8a). The connections of the reptilian lateral cortex and the amphibian lateral and dorsal pallia are similar: they receive a substantial projection from the main olfactory bulb, moderate input from the rostral thalamus, lack commissural connections, and do not project outside the hemisphere (Northcutt and Royce, 1975; Neary, 1990; Scalia *et al*., 1991). The amphibian medial pallium is topographically and connectionally similar to the reptilian medial cortex, but it also has connections in common with the reptilian dorsal cortex. These include projections to the olfactory bulb, hypothalamus, thalamus, and midbrain, contralateral projections, and the lack of direct olfactory input (Bruce and Butler, 1984a; Hoogland and Vermeulen-Vanderzee, 1989; Neary, 1990; Northcutt and Ronan, 1992).

Ventral part of the lateral pallium (LPv). The LPv is an embryologically and connectionally unique subdivision of the lateral pallium that is comparable to the DVR and overlying olfactory cortex in reptiles (Bruce and Neary, 1995c; Brox *et al*., 2004; Moreno and Gonzalez, 2004). Both the anterior LPv and the anterior DVR are ventral pallial derivatives, whereas the posterior LPv and part of the posterior DVR are lateral pallial derivatives (Fernandez *et al*., 1998; Martinez-Garcia *et al*., 2002b; Brox *et al*., 2004). The caudal LPv is the only pallial region that receives input from the main olfactory bulb and the hypothalamus, and projects to the medial hypothalamus.

Medial amygdala. Traditionally known as the pars lateralis of the amygdala (Northcutt and Kicliter, 1980), the medial amygdala receives input from the accessory olfactory bulb and projects to the hypothalamus (Northcutt and Royce, 1975; Wilczynski and Allison, 1989; Scalia *et al*., 1991; Neary, 1995), and thus appears to be homologous to the reptilian medial amygdaloid nucleus, nucleus sphericus, and the ventral posterior amygdalar nucleus (Bruce and Neary, 1995c).

12.5.3.3 Reptile to bird transition *Cortical areas*. There is general agreement about the homologues between the reptilian and avian cortical regions (Figures 7c and 8c). The squamate medial cortex is comparable to the avian hippocampus (dentate and Ammon's horn). The reptilian dorsal cortex is comparable to the avian parahippocampal area, dorsolateral cortex, and hyperpallium. The reptilian lateral cortex is comparable to the avian cortex piriformis (e.g., Ariëns Kappers *et al*., 1936; Bruce and Neary, 1995c; Striedter, 1997; Reiner, 2000).

Nidopallium and arcopallium. The reptilian dorsal ventricular ridge has been compared to the avian nidopallium, mesopallium, and arcopallium (Striedter, 1997), or to only the nidopallium (Bruce and Neary, 1995c). These homologues are evaluated in detail below. Several connections of the arcopallium have not been identified in reptiles (e.g., afferents to the optic tectum), and further studies are needed to identify its homologue with certainty.

Mesopallium. Although the mesopallium (formerly named hyperstriatum ventrale) and nidopallium together form a ventricular ridge like the reptilian DVR, the connections of the mesopallium compare best to those of the reptilian pallial thickening, rather than the DVR. The mesopallium is reciprocally connected with the nidopallium, hyperpallium and striatum, and receives input from the posterior amygdala, substantia nigra, and locus coeruleus (Bradley *et al*., 1985; Alpar and Tombol, 1998; Metzger *et al*., 1998; Csillag *et al*., 1994). Comparable connections are associated with the pallial thickening of lizards (Bruce and Butler, 1984a).

Amygdalar groups. In birds the posterior amygdala and olfactory recipient nucleus Taeniae are the only regions of the avian brain consistently agreed to be amygdalar. Thus, a great deal more work is needed to identify avian amygdalar groups. A homologue of the posterior amygdala has not been identified, but nucleus Taeniae is homologous in part with olfactory-recipient amygdalar groups of reptiles.

12.5.3.4 Reptile to mammal transition *Cortical areas*. The lateral, dorsal, and medial cortices are generally compared to the piriform, general (i.e., nonolfactory, nonhippocampal), and hippocampal

cortices of mammals (Figures 7d, 8b, and 8d). There is little controversy over these comparisons as they occupy similar positions in the hemisphere and have similar connections (Ariëns Kappers *et al*., 1936; Northcutt, 1969; Bruce and Neary, 1995c). In addition to the massive increase in the size of the mammalian cortex relative to the reptilian dorsal cortex, the major difference is that the mammalian general cortex develops with an 'inside-out' migration pattern (Goffinet *et al*, 1986).

Claustrum, endopiriform, and the basolateral amygdalar complex. The homologues of these regions are highly controversial and will be addressed in detail below.

Basomedial amygdala. The reptilian lateral amygdalar nucleus and basomedial amygdalar nucleus of mammals have similar connections, including a projection to the ventromedial hypothalamus, and appear to be homologues (Bruce and Neary, 1995c; Martinez-Garcia *et al*., 2002b).

Vomeronasal and olfactory amygdaloid nuclei. Amygdaloid nuclei receiving vomeronasal and olfactory input have been identified in reptiles and mammals, and appear to be homologuous, in part (see Section 12.4.4).

Central amygdala. Both the mammalian central amygdala and reptilian striatoamygdalar area have long descending projections to the brainstem and appear to be homologues (Russchen and Jonker, 1988; Bruce and Neary, 1995c; Martinez-Garcia *et al*., 2002b).

12.5.3.5 Homologues of the DVR and pallial thickening The identification of mammalian homologues of the reptilian DVR and pallial thickening, and the avian nidopallium and mesopallium has been a long-standing controversy (Table 2). The avian nidopallium (neostriatum) and reptilian DVR are often compared to parts of the mammalian neocortex (Karten, 1969, 1997; Reiner, 2000). However, a number of laboratories have recognized similarities between the avian nidopallium, the reptilian DVR, and the mammalian basolateral amygdalar group. Several of these have focused on the comparison between the sensory nidopallium and its mammalian homologue. Striedter (1997) compared the mesopallium and nidopallium to the claustrum and endopiriform areas, respectively. Puelles *et al*. (2000) and Martinez-Garcia *et al*. (2002b) suggested that the nidopallium/DVR may contain cells comparable to the endopiriform area and the basolateral amygdalar complex. Butler and Molnar (2002) compared the nidopallium/DVR to the mammalian basolateral amygdalar complex plus temporal isocortex and the claustrum-endopiriform area. Bruce and Neary (1995c) compared only the basolateral amygdalar group to the nidopallium, and specifically compared the rostral sensory nidopallium to the mammalian lateral amygdala (also a sensory thalamic target).

To determine which evolutionary model fits the current data best, the expression patterns of various early genetic markers, receptors, and ligands in reptiles, birds, and mammals will be summarized, as well as developmental and connectional data (Table 3).

Emx-1. In turtles Emx-1 expression appears in the cortices and pallial thickening, but little or none is expressed in the anterior (sensory) DVR; in mammals it is expressed in the claustrum and cortex, but not in the lateral amygdala (sensory amygdala) or the endopiriform nucleus; in birds Emx-1 is expressed in the hyperpallium (Wulst) and mesopallium, but not in the sensory nidopallium (Fernandez *et al*., 1998; Puelles *et al*., 2000). Thus, the mammalian cortex and claustrum are in the same Emx-1

Table 3 Comparison of various traits

	Mammalian structures				*Avian structures*		
	Iso	*Cl*	*E*	*LA*	*H*	*M*	*Ns*
Emx-1 gene	+++	+++			+++	+++	
a-adrenergic receptor	++	+++	+++	+	++	+++	+
Opiate delta receptor	+++	++++	++++	+++	+/++	++	+/++
Opiate kappa receptor	++	++++	++++	+++	+++	++++	++
VIP	+++	+++	+++	++	+/++	+++	+
NT receptor	+	+	+++	+/++	+	+/+++	+
Development	Dorsal	Lateral	Lateral	Ventral	Dorsal	Lateral	Ventral & lateral
Sensory thalamic afferents	Yes	No	No	Yes	Yes	No	Yes
Sensory thalamic efferents	Yes	No	No	No	Yes	No	No

Cl, claustrum; E, endopiriform nucleus; H, hyperpallium; Iso, isocortex; LA, lateral amygdala; M, mesopallium; Ns, sensory nidopallium; NT, neurotensin; VIP, vasoactive intestinal protein.
\+ density of expression from low (+) to very dense (++++).

positive pallial domain as the avian hyperpallium and mesopallium, and the reptilian cortex and pallial thickening. The mammalian endopiriform nucleus and lateral amygdalar nucleus, the avian sensory nidopallium, and most, if not all, of the reptilian anterior DVR belong to an Emx-1 negative region.

Alpha 2 adrenergic receptor. In quails the hyperpallium, cortex dorsolateralis, and mesopallium contain high levels of expression, in contrast to the adjacent nidopallium, which expresses low to moderate levels (Ball *et al*., 1989; Ball, 1994; Fernandez-Lopez *et al*., 1990). In rats the alpha 2A adrenoreceptor subtype is expressed only in cortical layers 1–4 and in the claustrum and endopiriform nuclei, but not within the amygdala, and the alpha 2C adrenoreceptor is expressed in the striatum but not the pallium (Uhlen *et al*, 1997). These data indicate that the alpha 2 adrenoreceptor differentiates the mammalian cortico-claustro-endopiriform areas and the avian cortico-hyperpallial-mesopallial areas from the mammalian lateral amygdala and avian neostriatum.

Opiate receptors. In turtles the delta opiate receptor is expressed more densely in the pallial thickening than surrounding areas (figures 2 and 4 of Xia and Haddad, 2001). In pigeons the delta and kappa opiate receptors are more abundant in the hyperstriatum ventrale relative to the neostriatum, and this is especially true for the kappa opiate receptor (Reiner *et al*., 1989). In rats the endopiriform-claustrum exhibits very dense binding with delta and kappa opiate receptors (Mansour *et al*., 1987). The lateral and basolateral amygdalar nuclei exhibit moderate to dense delta and kappa labeling. The density of labeling in the lateral amygdala appears to be considerably less than in the endopiriform-claustrum. Thus, the delta and kappa opiate receptors are expressed throughout the pallium of mammals, birds, and turtles, but are expressed more strongly in the mammalian claustro-endopiriform, avian mesopallium, and turtle pallial thickening than in adjacent areas.

Vasoactive intestinal protein (VIP). In birds VIP-like immunoreactivity and mRNA expression is present in most, but not all, of the hyperpallium and mesopallium, whereas little or none is expressed throughout the nidopallium (Shimizu and Karten, 1990; Hof *et al*., 1991; Kuenzel *et al*., 1997). In rats VIP is expressed in neurons and terminals in the claustrum and endopiriform, but in the lateral amygdala cell bodies but not terminal fibers express VIP (Sims *et al*., 1980; Kowianski *et al*., 2001). Thus, VIP expression is higher in the mammalian claustro-endopiriform area and avian mesopallium than in adjacent cortical and amygdalar/neostriatal areas.

Neurotensin. In turtles neurotensin immunoreactive terminals are particularly dense in the molecular layer of the dorsal cortex and lateral to the pallial thickening (Reiner, 1992). In lizards it is expressed in the lateral (olfactory) cortex, including a region that appears to correspond to the pallial thickening (Bello *et al*., 1994). In mammals they are scattered in the piriform cortex and have a dense accumulation in the claustrum and a small part of the lateral amygdalar nucleus (Jennes *et al*., 1982).

Neurotensin receptors. In pigeons neurotensin receptors are distributed densely in the hyperpallium and mesopallium, although a small region within the lateral mesopallium is conspicuously lightly labeled (Brauth *et al*., 1986). The nidopallium is less intensely labeled except for a group of cells surrounding Field L that are densely labeled. In rats, very high densities of neurotensin receptors occur in the superior rhinal cortex and in the endopiriform cortex, whereas the core of the claustrum has a very low density. Labeling in the lateral amygdala has a lower density of expression than in the adjacent endopiriform area (Young and Kuhar, 1981; Quirion *et al*., 1982).

Development. Developmental studies have concluded that the nidopallium develops from a precursor region that in mammals gives rise to olfactory cortex, the endopiriform, claustrum, and the basolateral amygdaloid complex (Bayer and Altman, 1991; Fernandez *et al*., 1998; Striedter *et al*., 1998; Puelles *et al*., 2000; Reblet *et al*., 2002; Brox *et al*, 2004).

Connections. The connections of the avian hyperpallium and nidopallium are similar to those of the reptilian dorsal cortex and DVR, and of the mammalian isocortex and lateral amygdala, respectively (see Bruce and Neary, 1995c). In mammals the endopiriform projects principally to olfactory cortical areas including amygdalocortical nuclei, and sparsely to all the deep amygdalar nuclei except the accessory basal nucleus to which it projects densely (Behan and Haberly, 1999). The claustrum is extensively interconnected with cortical areas, receives projections from the nucleus centralis thalami, and probably from the locus coeruleus and the lateral hypothalamus (LeVay and Sherk, 1981). The avian mesopallium receives projections from the hyperpallium accessorium, caudal and intermediate parts of the nidopallium. Thalamic projections to mesopallium arise from the nucleus dorsolateralis posterior thalami (Funke, 1989; Gamlin and Cohen, 1986; Metzger *et al*., 1998; Leutgeb *et al*., 1996; Lanuza *et al*., 2000), which is comparable to a

group of mammalian thalamic nuclei that includes the central lateral nucleus (Veenman, 1997; Bruce *et al*., 2002). Efferents from the mesopallium include a projection to the nidopallium (Wild *et al*., 1993). Most studies indicate that the mesopallium projects principally within the telencephalon, including a projection to the dorsal arcopallium (Karten, 1969; Nauta and Karten, 1970; Metzger *et al*., 1998). The connections of the mammalian endopiriform and claustrum, avian mesopallium, and reptilian pallial thickening are similar and thus are consistent with a possible homology. However, none of the connections are unique to claustrum-endopiriform, mesopallium, or pallial thickening, and so cannot be used as strong indicators of a homology.

Together the data indicate that:

1. The best comparison of the mammalian general cortex (including isocortex) is with the avian hyperpallium and dorsolateral cortex and with reptilian dorsal cortex. This is consistent with all models.
2. The best comparison of the mammalian claustrum and endopiriform areas is with the avian mesopallium and reptilian pallial thickening. This is consistent with the models of Bruce and Neary (1995c), Bruce (this article), Striedter (1997), and Puelles *et al*. (2000).
3. The best comparison of the mammalian sensory lateral amygdala is with the avian sensory nidopallium and reptilian DVR. This is consistent with the models of Bruce and Neary (1995), Puelles *et al*. (2000), and Butler and Molnar (2002).
4. The hypotheses that the avian sensory nidopallium is comprised of overlapping territories corresponding to the mammalian claustrum, endopiriform nucleus, and lateral amygdala (Butler and Molnar, 2002), or the mammalian endopiriform nucleus and lateral amygdala (Striedter, 1997; Puelles *et al*., 2000) are weakly supported by this data.
5. The hypothesis that the avian sensory nidopallium is comparable to parts of the mammalian neocortex (Karten, 1997; Reiner, 2000) is also poorly supported by this data.

12.5.3.6 Summary If we assume that the forebrain of the common tetrapod ancestor was similar to that of extant amphibians, then the amphibian–reptile transition was marked by segregation of pallial fields (Figure 8). The medial pallium gave rise to the medial and dorsal cortices of reptiles, the dorsal and lateral pallia gave rise to the pallial thickening and most of the lateral cortex, and the ventral part of the lateral pallium gave rise to the DVR and overlying lateral and amygdalar cortices.

The changes during the transition from the reptilian to avian forebrain were not dramatic, since recognition of homologous regions is fairly straightforward. However, the identification of a homologue of the avian arcopallial region is uncertain. In reptiles the vomeronasal system projects to a topographically similar region as the arcopallium. Thus, the appearance of the arcopallium may result from an adaptation to the loss of the vomeronasal input.

Connectional as well as genetic and neurochemical expression patterns indicate that the reptilian DVR, avian nidopallium, and mammalian basolateral amygdalar complex are homologous. The reptile to mammal transition is marked by a massive hypertrophy of the dorsal (general) cortex, which may be associated with a more caudal location of the amygdaloid groups in mammals, compared to reptiles or birds (see The Origin of Neocortex: Lessons from Comparative Embryology, How Can Fossils Tell us About the Evolution of the Neocortex?, Reconstructing the Organization of the Forebrain of the First Mammals, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, The Evolution of the Amygdala in Vertebrates).

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13 Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?

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Glossary

amniote Group of vertebrates that develop an amniotic membrane around the embryo; includes reptiles, birds, and mammals.

arcopallium A caudal (or posterior) subdivision of the dorsal ventricular ridge in birds.

cortex A laminar brain structure consisting of cells arranged in layers parallel to the ventricular/pial surfaces and generally orthogonal (perpendicular) to radial glial fibers.

developmental regulatory gene A gene encoding a transcription factor (or a cofactor) or a signaling protein that is expressed during development in specific patterns, and is able to control expression of other genes and regulate patterning and morphogenesis of specific body parts.

DVR Dorsal ventricular ridge: a large region of the ventrolateral pallium of the telencephalon of birds and reptiles.

hippocampal formation Derivative of the medial pallium in different vertebrates. In mammals it includes the dentate gyrus, the hippocampus proper (Ammon's fields), and the subiculum. In birds it includes the hippocampus and the area parahippocampalis, whereas in reptiles it includes the medial and dorsomedial cortices.

homologous Having the same relative position (topological position), embryonic origin, and common ancestor; exhibiting biological homology.

homologue The same organ in different animals under every variety of form and function (Owen's definition, in 1843; see History of Ideas on Brain Evolution on this concept).

homology A similarity attributed to common evolutionary origin (see 'homologous').

hyperpallium A dorsal region of the avian telencephalon that develops from the dorsal pallium. It typically forms a bulge on the dorsal surface of the telencephalon. It was often called Wulst.

M1 Primary motor area of the mammalian neocortex.

mesopallium A dorsal subdivision of the DVR in birds.

neocortex Derivative of the dorsal pallium in mammals, that typically shows a six layered organization. It is also known as isocortex.

nidopallium A ventral subdivision of the DVR in birds.

pallial thickening A lateral expansion of the dorsal cortex of reptiles, showing a non cortical organization. It is generally considered a lateral part of the dorsal cortex. However, only part of it may be a dorsal pallial derivative, and more comparative and developmental studies of this structure are needed before reaching any conclusion.

pallium A major dorsocaudal division of the telencephalon in all vertebrates, which in mammals gives rise to the cortical regions, claustrum, and part of the amygdala (including the cortical areas plus the basolateral complex). It is subdivided into four parts, called medial, dorsal, lateral, and ventral pallia.

piriform cortex Olfactory cortex of different vertebrates. It derives from the ventrolateral pallium. In reptiles, it is also known as lateral cortex.

S1 Primary somatosensory area of the mammalian neocortex.

sauropsid Group of vertebrates that includes reptiles and birds.

subpallium A major ventrorostral (or basal) division of the telencephalon in all vertebrates, that in mammals gives rise to most of the septum, the basal ganglia, part of the amygdala (including the intercalated and centromedial nuclei), and other cell groups of the basal telencephalon, such as the cholinergic corticopetal groups. It is subdivided into striatal, pallidal, and anterior entopeduncular parts.

telencephalon Bilateral evaginations of the rostral forebrain. It shows two major divisions in all vertebrates: pallium and subpallium.

tetrapod Group of vertebrates having two pairs of limbs, that includes amphibians, reptiles, birds, and mammals.

thalamus Forebrain structure that derives from the alar plate of the diencephalon (in particular, from prosomeres 2 to 3). It is subdivided into a ventral thalamus (also called prethalamus, which derives from prosomere 3), and a dorsal thalamus (or simply thalamus, which derives from prosomere 2). The dorsal thalamus contains cell groups that typically relay sensory information to the subpallium and pallium in vertebrates. In amniotic vertebrates, the dorsal thalamus contains specific cell groups that relay unimodal sensory and/or motor information to specific areas of the dorsal pallium.

topology Geometric configuration of any given structure (such as the brain) according to internal coordinates, which remain unaltered independent of deformations or differential growth of subdivisions that occur during development. According to this, the topological position of any subdivision within the structure, and its relation to neighbors, remains the same throughout ontogeny. Further, in organisms sharing the same configuration and basic organization plan (for example, vertebrates), the topological position of homologous subdivisions should be the same across species.

V1 Primary visual area of the mammalian neocortex.

Wulst German term previously employed to name the hyperpallium. It literally means bulge, making reference to the swollen or protuberant appearance of this subdivision of the avian telencephalon.

13.1 Introduction

One of the most challenging questions in brain evolution is to ascertain the origin of neocortex and to know whether a comparable cortical (pallial) region is present in extant birds and reptiles, which would mean that a primordium of this structure was already present in stem amniotes. This question has been addressed by researchers since the end of the

nineteenth century and continues to be discussed nowadays (for example, see article by Aboitiz *et al.*, 2003, and commentaries on it). However, many issues related to this question still remain uncertain and controversial. Nevertheless, the combination of developmental, paleontological, and adult anatomical plus functional data, analyzed using a cladistic approach, has proven to be very useful for evolutionary studies; this combined approch has helped to clarify some aspects of cortical evolution and has offered some light on what direction to follow in this research (for example, Northcutt and Kaas, 1995; Striedter, 1997, 2005; Medina and Reiner, 2000; Puelles, 2001; Butler and Molnár, 2002; Aboitiz *et al.*, 2003). Here I will review evidence based on this approach that suggests that: (1) the pallium of birds and reptiles contains a sector that is homologous as a field to the mammalian neocortex (i.e., they evolved from the same primordium present in stem amniotes); (2) this pallial sector contains a primary visual and a primary somatosensory area that might be homologous to V1 and S1, respectively, of mammalian neocortex; and (3) the frontal part of this pallial sector contains a somatomotor control area in birds (apparently overlapped with the somatosensory field) and mammals (M1), but these areas likely evolved independently and are, therefore, nonhomologous (see Evolution of the Nervous System in Reptiles, The Origin of Neocortex: Lessons from Comparative Embryology, Reconstructing the Organization of the Forebrain of the First Mammals, The Evolution of Motor Cortex and Motor Systems).

13.2 Finding the Homologue of Neocortex in the Pallium of Nonmammals

The neocortex is a six-layered structure located in the dorsolateral part of the telencephalon in mammals, above the ventricle, and it covers the central and basal region that is occupied by the basal ganglia and other basal telencephalic cell groups (Figure 1a). The neocortex is also located above the rhinal fissure, which separates it from the piriform cortex and olfactory tract. In contrast to the neocortex, the basal ganglia and other basal telencephalic cell groups show a nuclear (nonlaminar) organization. The difference between laminar versus nuclear organization together with the relative position of the cell masses with respect to the ventricle was once considered a criterion to identify cortical (pallial) and basal (subpallial) regions in the telencephalon of nonmammals, and based on it the telencephalon of birds and reptiles was thought to be made of a very large basal ganglia and a very tiny cortical region (reviewed in Medina and Reiner, 1995; Striedter, 1997; Reiner *et al.*, 1998; Jarvis *et al.*, 2005). This is now known to be wrong, and there is a large amount of evidence showing that the telencephalon of birds and reptiles contains a large pallial region, a major part of which shows a nuclear organization and is located below the lateral ventricle (Figures 1b–1d) (Karten and Hodos, 1970; Reiner, 1991, 1993; Butler, 1994b; Striedter, 1997, 2005; Smith-Fernández *et al.*, 1998; Medina and Reiner, 2000; Puelles *et al.*, 2000; Jarvis *et al.*, 2005). The evidence showing this includes developmental and adult anatomical and functional data, and it has mainly been obtained after the development of modern techniques that allowed detection of gene products (such as enzymes, proteins and, more recently, mRNAs), tracing of axonal pathways, fate mapping, and functional studies of the brain.

The cortical region of mammals is subdivided into medial, dorsal, and lateroventral units during development and in the adult (Figures 1a and 2c), and the neocortex derives from the dorsal subdivision (Holmgren, 1925; Striedter, 1997). The cortical region (pallium) of birds and reptiles is also subdivided into medial, dorsal, and lateroventral units (Figure 1) (Reiner, 1991, 1993; Butler, 1994b; Striedter, 1997; Puelles *et al.*, 2000). The problem comes when trying to compare one-to-one these subdivisions of the avian/reptilian pallium with those of mammals. Some authors employing only adult anatomical and functional data (including connectivity patterns) believe that the dorsal subdivision of the avian/reptilian pallium is homologous to the dorsomedial part of the neocortex, whereas a large part of the lateroventral pallium of birds/reptiles (called dorsal ventricular ridge or DVR; in particular, its ventral part in birds) is homologous to the dorsolateral part of the neocortex or to specific cell groups of it (Karten, 1969, 1997; Reiner, 1993; Butler, 1994b). However, homologous structures must originate from the same embryonic primordium (Striedter, 1997; Puelles and Medina, 2002). In this sense, developmental studies indicate that only the dorsal subdivision of the avian/reptilian pallium can be compared to the neocortex, but not the DVR (in particular, its ventral part, which includes the nidopallium in birds) (Striedter, 1997; Puelles *et al.*, 2000). Rather, both developmental and some adult connectivity data suggest that the avian/reptilian DVR is homologous to the claustrum and pallial amygdala (Bruce and Neary, 1995a, 1995b, 1995c; Striedter, 1997; Guirado *et al.*, 2000; Puelles *et al.*, 2000; Puelles, 2001; Dávila *et al.*, 2002; Martínez-García *et al.*, 2002). Below I

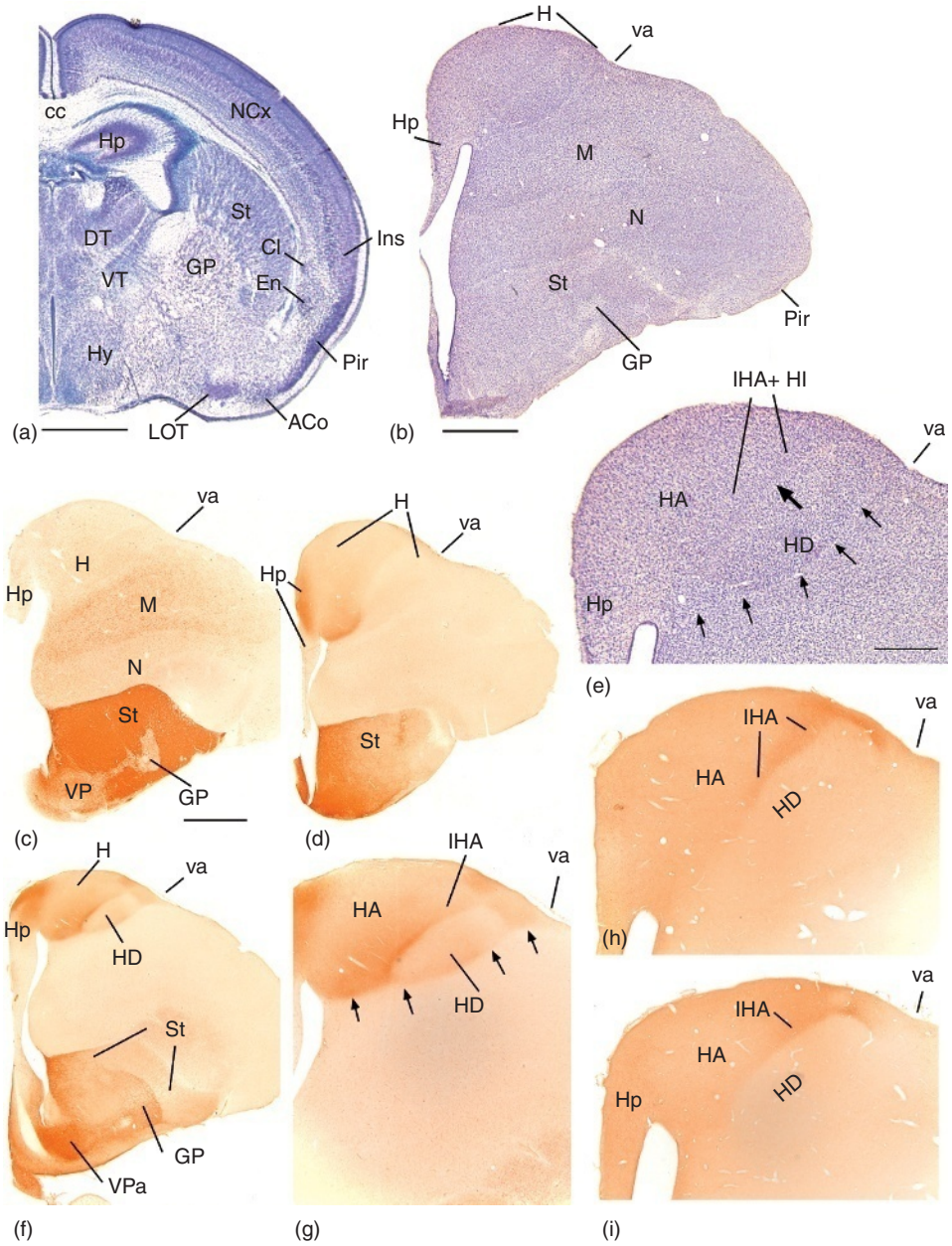

Figure 1 Photomicrographs of frontal sections through the telencephalon of a postnatal mouse (a) or adult pigeon (b i), showing the general cytoarchitecture, as observed in Nissl staining (a), (b), (e), and some subdivisions based on immunostaining for tyrosine hydroxylase (c), substance P (d), (f), (g), of choline acetyltransferase (h), (i). Note the typical lamination in the cerebral cortex of mouse (a), that differs from the nuclear-like organization in the basal ganglia (striatum and pallium). In pigeon (b), as in other birds and reptiles, most of the telencephalon is not laminated. Nevertheless, neurochemical data help to locate the main intratelencephalic boundary in birds and reptiles, separating subpallium and pallium. The subpallium is relatively rich in tyrosine hydroxylase (c) and substance P (d), (f), and includes the basal ganglia (striatum and pallidum). The avian pallium includes four major subdivisions, including the hippocampal formation (medially), the hyperpallium (dorsally), the mesopallium (laterodorsally), and the nidopallium (lateroventrally). At caudal levels, the avian lateroventral pallium includes the arcopallium and part of the amygdala. The avian hyperpallium appears to be the only derivative of the dorsal pallium and is therefore comparable (homologous as a field) to the mammalian neocortex (see Figure 2). The avian hyperpallium (H) has four mediolateral subdivisions, called apical (HA), interstitial nucleus of apical (IHA), intercalated (HI), and densocellular (HD) hyperpallium (e i). These subdivisions are not comparable to neocortical layers, although they show some functional features that resemble them. The lateral extension of the hyperpallium coincides with a cell-free lamina called superior frontal lamina (arrows in (e) and (g)), and generally relates to a superficial groove called vallecula (va), although this is not true at rostral levels. See text for more details. ACo, anterior cortical amygdalar area; cc, corpus callosum; Cl, claustrum; DT, dorsal thalamus; En, endopiriform nucleus; GP, globus pallidus; H, hyperpallium; HA, apical hyperpallium; HD, densocellular hyperpallium; HI, intercalated hyperpallium; Hp, hippocampal formation; Hy, hypothalamus; IHA, interstitial nucleus of the apical hyperpallium; Ins, insular cortex; LOT, nucleus of the lateral olfactory tract; M, mesopallium; N, nidopallium; NCx, neocortex; Pir, piriform cortex; St, striatum; va, vallecula; VPa, ventral pallidum; VT, ventral thalamus. Scale bars: 1cm (a, b); 0.5cm (c, d, f; scale in c); 1cm (e, h, i; scale in e).

review the evidence that supports that the dorsal part of the avian/reptilian cortical region is homologous as a field to the mammalian neocortex, and that both evolved from a similar pallial subdivision present in the telencephalon of stem amniotes.

13.2.1 Developmental Evidence: Histogenetic Origin and Transcription Factors

During development, the telencephalon of vertebrates becomes parcellated into radial histogenetic divisions and subdivisions that are comparable across species (Striedter, 1997; Puelles and Medina, 2002). Each division/subdivision shows a unique molecular profile and produces specific cell groups, most of which stay within the radial domain, except for some selective cell populations that undergo tangential migration across boundaries (Striedter and Beydler, 1997; Striedter *et al.*, 1998; Puelles *et al.*, 2000; Cobos *et al.*, 2001; Marín and Rubenstein, 2001, 2002; Puelles and Medina, 2002). This conclusion is strongly supported by data on developmental regulatory genes (encoding transcription factors or signaling proteins that regulate the expression of other genes), which are expressed in specific and generally comparable spatiotemporal patterns in the telencephalon of different vertebrates during development (Smith-Fernández *et al.*, 1998; Puelles *et al.*, 2000; Brox *et al.*, 2003, 2004; Medina *et al.*, 2005), and play key roles in the regional specification and formation of telencephalic divisions and subdivisions (Marín and Rubenstein, 2002).

13.2.1.1 Pallial subdivisions in mammals and neocortical origin Classical and modern developmental studies, including data on developmental regulatory genes, indicate that the mammalian neocortex derives from the pallium, one of the major divisions of the telencephalon (Figure 2c) (Holmgren, 1925; Källén, 1951b; Puelles *et al.*, 2000). During development, the pallium shows specific expression of numerous transcription factor-expressing genes, including *Pax6*, *Emx1/2*, *Tbr1/2,* and several *LIM-homeobox* (*Lhx*) genes (Simeone *et al.*, 1992; Stoykova and Gruss, 1994; Bulfone *et al.*, 1995, 1999; Rétaux *et al.*, 1999; Puelles *et al.*, 2000; Bulchand *et al.*, 2001, 2003; Medina *et al.*, 2004), which play key roles in pallial specification and parcellation, cell proliferation, and/or cell differentiation (Stoykova *et al.*, 1996, 2000; Zhao *et al.*, 1999; Bulchand *et al.*, 2001; Hevner *et al.*, 2001, 2002; Yun *et al.*, 2001; Bishop *et al.*, 2002, 2003; Muzio *et al.*, 2002; Campbell, 2003). For example, *Pax6, Emx1,* and *Emx2* are involved in pallial specification and parcellation (Bishop *et al.*, 2002, 2003; Muzio *et al.*, 2002). *Emx1* and *Emx2* are also involved in pallial growth (cell proliferation) (Bishop *et al.*, 2003). On the other hand, *Tbr1* appears to be involved in the differentiation of glutamatergic neurons, which are typical in the pallium (Hevner *et al.*, 2001).

What part of the pallium gives rise to the neocortex? Classical developmental studies and studies on the expression and function of developmental regulatory genes indicate that the pallium of mammals contains three main radial subdivisions (Figure 2c): (1) a medial pallium, giving rise to the hippocampal formation; (2) a dorsal pallium, giving rise to the neocortex; and (3) a lateroventral pallium, giving rise to the piriform cortex, claustrum, and pallial amygdala (the lateroventral pallium is sometimes referred to as the piriform lobe, and has the olfactory tract at the surface) (Holmgren, 1925; Striedter, 1997; Puelles *et al.*, 2000; Puelles, 2001). The lateroventral pallium is also subdivided into dorsal and ventral parts (called lateral and ventral pallia, respectively, by Puelles *et al.*, 2000), which show distinct expression of the several developmental regulatory genes, including *Emx1* and *Dbx1*, and give rise to different parts of the claustrum and pallial amygdala (Figure 2c) (Puelles *et al.*, 2000; Yun *et al.*, 2001; Medina *et al.*, 2004). During early development, the lateral pallium expresses strongly *Emx1* but not *Dbx1*, whereas the ventral pallium expresses *Dbx1* in the ventricular zone but only shows *Emx1* expression in the subpial surface (Puelles *et al.*, 2000; Yun *et al.*, 2001; Medina *et al.*, 2004). However, a recent fate-mapping study indicates the existence of numerous *Emx1*-expressing cells in both lateral pallial and ventral pallial parts of the amygdala around and after birth (Gorski *et al.*, 2002), suggesting that there may be a high degree of cellular mixing between these subdivisions (Figure 2c).

The pallial subdivisions are apparently formed by the early action of: (1) signaling proteins (such as Wnt proteins) that diffuse from organizer centers such as the cortical hem (Figure 2c), which, by way of receptors, apparently control the expression of downstream genes (including genes encoding transcription factors) in adjacent pallial areas in a concentration-dependent way (Ragsdale and Grove, 2001); (2) transcription factors that are expressed in opposing gradients in the pallium during early development, involved in regional specification, parcellation, cell proliferation, and/or cell differentiation of the pallium (for example, *Pax6, Emx2, Lhx2*; Donoghue and Rakic, 1999; Bulchand *et al.*, 2001; Bishop *et al.*, 2002); (3) cofactors (activators or repressors) and other regulatory proteins that regulate directly or indirectly the expression of specific transcription factors, that

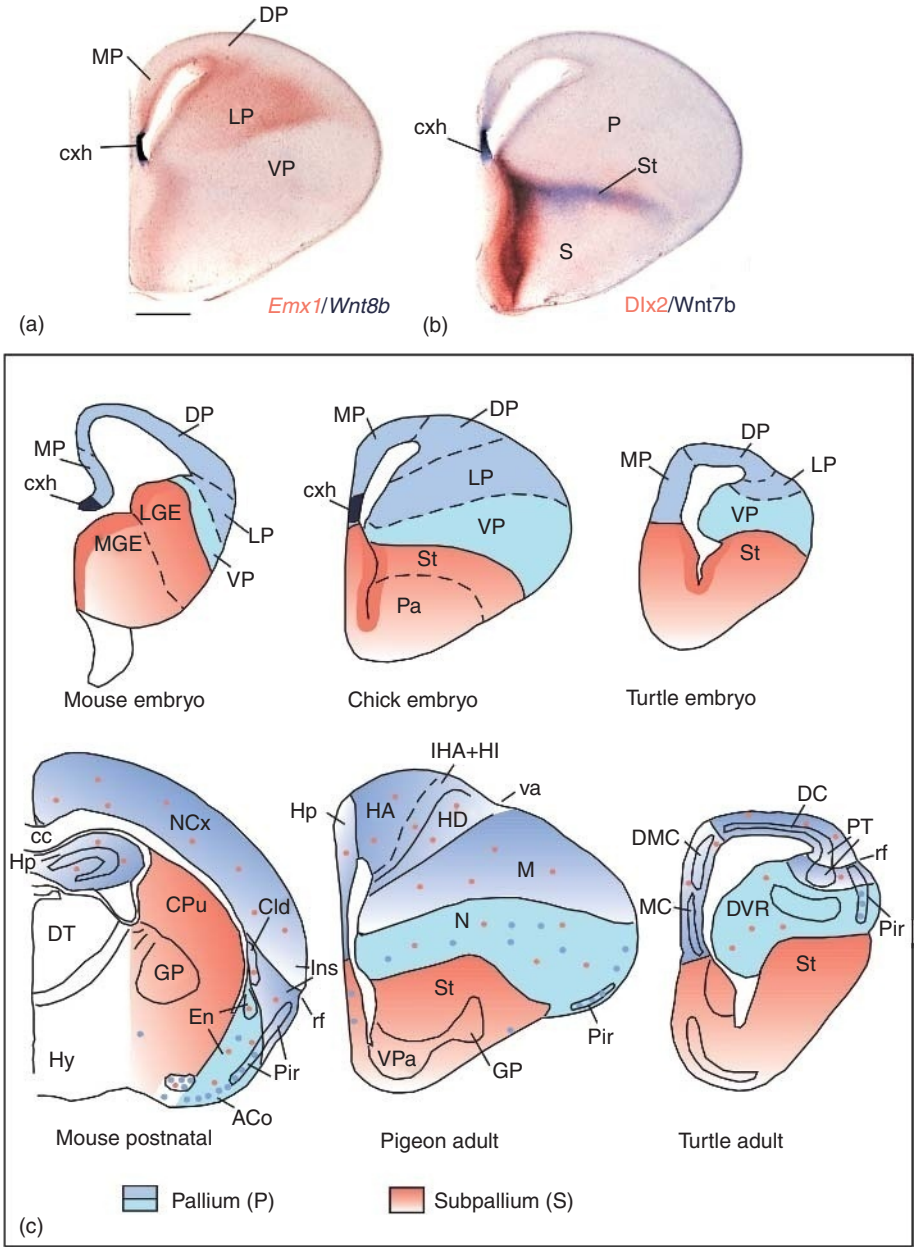

Figure 2 a and b, Photomicrographs of frontal sections through the telencephalon of chick embryos (10 days of incubation), showing expression of the chick genes *Emx1* (red in a), *Wnt8b* (blue in a), *Dlx2* (red in b), or *Wnt7b* (blue in b). The genes *Dlx2* and *Wnt7b* are expressed in the subpallium, either in the ventricular/subventricular zone of the whole subpallium (*Dlx2*) or the ventricular zone and mantle of the striatum (*Wnt7b*). Expression of *Emx1* helps to distinguish a ventral pallial (VP) subdivision, poor in *Emx1* expression and poor in subpallial marker genes. Note the expression of *Wnt* genes in the avian cortical hem, a putative secondary organizer comparable to the cortical hem of mammals. c, Schematics of the telencephalon of a mammal (mouse), a bird (chick or pigeon), and a reptile (turtle), as seen in frontal sections during development or in the adult. The pallial and the major subpallial subdivisions are represented in the different species, based on known expression patterns of developmental regulatory genes observed during development. Four major pallial subdivisions appear to exist in all groups, although the lateral and ventral subdivisions appear to have a large degree of cellular mixing in the adult (which occurs at the level of the ventral pallium). The dorsal pallium gives rise to the neocortex (NCx) in mammals, to the hyperpallium (H) in birds, and to the dorsal cortex (DC) in reptiles. The pallial thickening (PT) is often considered a lateral part of the dorsal cortex. However, available data suggest that only part of it may be a dorsal pallial derivative, and more studies are needed to know where the exact boundary between the dorsal and lateral pallium is, in reptiles. ACo, anterior cortical amygdalar area; cc, corpus callosum; Cld, dorsolateral claustrum; CPu, caudoputamen (dorsal striatum); cxh, cortical hem; DC, reptilian dorsal cortex; DMC, reptilian dorsomedial cortex; DP, dorsal pallium; DT, dorsal thalamus; DVR, dorsal ventricular ridge; En, endopiriform nucleus; GP, globus pallidus; H, hyperpallium; HA, apical hyperpallium; HD, densocellular hyperpallium; HI, intercalated hyperpallium; Hp, hippocampal formation; Hy, hypothalamus; IHA, interstitial nucleus of the apical hyperpallium; Ins, insular cortex; LGE, lateral ganglionic eminence; LP, lateral pallium; M, mesopallium; MC, reptilian medial cortex; MGE, medial ganglionic eminence; MP, medial pallium; N, nidopallium; NCx, neocortex; P, pallium; Pa, pallidum; Pir, piriform cortex; PT, pallial thickening; rf, rhinal fissure; S, subpallium; St, striatum; va, vallecula; VP, ventral pallium; VPa, ventral pallidum. Scale bar: 400μm.

show sharp expression boundaries between subdivisions (for example, the LIM-only protein Lmo3, with a sharp expression boundary between dorsal and lateroventral pallial subdivisions; Bulchand *et al.*, 2003; Vyas *et al.*, 2003). The main pallial subdivisions are differentially affected by mutations targeting some of the above-mentioned developmental regulatory genes, supporting that they represent distinct histogenetic compartments. For example, a mutation in the LIM-homeobox gene *Lhx2* produces a severe malformation of the hippocampal formation and neocortex, but the piriform lobe appears unaffected (Bulchand *et al.*, 2001; Vyas *et al.*, 2003).

13.2.1.2 Pallial subdivisions in nonmammals: the dorsal pallium in birds and reptiles Since developmental regulatory genes generally show highly conserved sequences and expression patterns, they have become very useful tools for identifying comparable brain regions in different vertebrate species and for studies of brain evolution (Puelles *et al.*, 2000; Medina *et al.*, 2005). Classical and modern developmental studies, including radial glial analysis, fate-mapping studies, and expression of developmental regulatory genes, indicate that the telencephalic pallium in reptiles and birds contains three main radial divisions (Figure 2c): (1) a medial pallium, which gives rise to the medial/dorsomedial cortices in reptiles and to the hippocampal formation (hippocampus and parahippocampal area) in birds; (2) a dorsal pallium, which gives rise to the dorsal cortex in reptiles and the hyperpallium or Wulst in birds; and (3) a lateroventral pallium, which gives rise to the lateral or piriform cortex, to a large nuclear structure called the DVR and to some pallial amygdalar nuclei in reptiles and birds (Holmgren, 1925; Källén, 1951a, 1953, 1962; Striedter, 1997; Striedter and Beydler, 1997; Striedter *et al.*, 1998; Puelles *et al.*, 2000; Cobos *et al.*, 2001; Martínez-García *et al.*, 2002). As in mammals, the pallium of reptiles and birds shows specific expression of *Pax6*, *Tbr1/2,* and *Emx1* during development (Smith-Fernández *et al.*, 1998; Bulfone *et al.*, 1999; Puelles *et al.*, 2000; Garda *et al.*, 2002). Similarly to mammals, in birds there is an organizer center at the medial edge of the pallium expressing *Wnt*-family genes (the avian cortical hem; Figures 2a–2c), which may control the formation of the medial pallial subdivision (Garda *et al.*, 2002). This indicates that the specification and parcellation of the avian/reptilian pallium are controlled by many of the same regulatory genes and mechanisms that control pallial development in mammals.

Further, as in mammals, the lateroventral pallium of birds and reptiles is subdivided into a lateral pallium, showing broad and strong expression of *Emx1*, and a ventral pallium, which expresses *Emx1* only in a thin band of the subpial mantle (Figures 2a–2c) (Smith-Fernández *et al.*, 1998; Puelles *et al.*, 2000). These two pallial subdivisions of birds also differ by their distinct expression of *Dachsund* and several *Cadherin* genes during development (Redies *et al.*, 2001; Szele *et al.*, 2002). However, as in mammals, the derivatives of the lateral and ventral pallial subdivisions of birds apparently display a high degree of cellular mixing (Figure 2c), based on radial glial fiber disposition and fate-mapping analysis in chick embryos (Striedter and Beydler, 1997; Striedter *et al.*, 1998; Striedter and Keefer, 2000). The lateral pallium includes the so-called mesopallium and posterior amygdalar nucleus of birds, whereas in reptiles it appears to include a small dorsolateral part of the DVR plus the dorsolateral amygdalar nucleus (Smith-Fernández *et al.*, 1998; Guirado *et al.*, 2000; Puelles *et al.*, 2000; Martínez-García *et al.*, 2002). The ventral pallium includes the so-called nidopallium and arcopallium of birds, whereas in reptiles it appears to include most of the DVR plus the lateral and other amygdalar nuclei (Smith-Fernández *et al.*, 1998; Guirado *et al.*, 2000; Puelles *et al.*, 2000; Martínez-García *et al.*, 2002). The olfactory tract is located at the surface of the ventral pallium (or ventral DVR) in mammals, birds, and reptiles (Striedter, 1997; Guirado *et al.*, 2000; Puelles *et al.*, 2000; Puelles, 2001). Both the relative (topological) position and molecular profile of the pallial subdivisions (including expression of *Pax6, Tbr1,* and *Emx1*) suggest that the dorsal pallial subdivision of reptiles and birds, from which derive the reptilian dorsal cortex and avian hyperpallium, is comparable and possibly homologous as a field to the dorsal pallium of mammals, which gives rise to the neocortex (Striedter, 1997; Puelles *et al.*, 2000). As in mammals, in reptiles the rhinal fissure separates the dorsal cortex from the piriform cortex and olfactory tract (Figure 2c). These data also indicate that the ventral pallial part of the reptilian/avian DVR (which has the olfactory tract and piriform cortex at the surface, and is poor in *Emx1* expression) is not comparable and cannot be homologized to the neocortex, since they derive from different embryonic primordia (Striedter, 1997; Striedter and Beydler, 1997; Smith-Fernández *et al.*, 1998; Striedter *et al.*, 1998; Puelles *et al.*, 2000).

However, one important issue that developmental studies have not yet resolved is where to locate the exact boundary between the dorsal and lateral

pallial subdivisions, since both subdivisions express many of the same developmental regulatory genes (for example, *Emx1*) and the morphological landmarks are not clear in birds and many reptiles. In other words, what is the exact lateral extension of the dorsal pallium in birds and reptiles? In mammals, some developmental regulatory genes are expressed differently in the lateral and dorsal pallium (for example, the LIM-only genes *Lmo2* and *Lmo3*), but, unfortunately, data on the orthologue genes are lacking in nonmammalian vertebrates (Medina *et al.*, 2005). I will return to this issue below.

13.2.2 Adult Anatomical Evidence: Morphological Landmarks, Molecular Markers, and Connections

13.2.2.1 Morphological landmarks and molecular markers: problematic delimitation of the dorsal pallium in birds and reptiles As noted above, the dorsal cortex of reptiles and the hyperpallium of birds appear to derive from the same pallial embryonic subdivision as the neocortex. In adult animals, these structures show cellular and molecular features typical of pallium. For example, they contain a majority of excitatory (glutamatergic) neurons (the principal or projection neurons) and only a relatively small subpopulation of inhibitory (GABAergic) interneurons (Ottersen and Storm-Mathisen, 1984; Reiner, 1993; Veenman and Reiner, 1994, 1996; Swanson and Petrovich, 1998; Fowler *et al.*, 1999; Medina and Reiner, 2000; Broman *et al.*, 2004). In mammals, birds, and reptiles, the principal pallial neurons have excitatory projections to the striatum and brainstem (Ottersen and Storm-Mathisen, 1984; Veenman and Reiner, 1996; Kenigfest *et al.*, 1998; Fowler *et al.*, 1999; Broman *et al.*, 2004). Some of the strongest evidence showing that the principal pallial neurons are glutamatergic has been provided recently by the localization of vesicular glutamate transporters VGLUT1 and VGLUT2, although data on these transporters exist only in mammals (Fujiyama *et al.*, 2001; Herzog *et al.*, 2001; Broman *et al.*, 2004; Fremeau *et al.*, 2004), but are lacking in birds and reptiles. The GABAergic interneurons of the mammalian neocortex and avian hyperpallium, as those of the rest of the mammalian and avian pallium, originate in the subpallium and migrate tangentially to the pallium during development (Figure 2c) (Anderson *et al.*, 1997, 2001; Pleasure *et al.*, 2000; Cobos *et al.*, 2001; Marín and Rubenstein, 2001; Nery *et al.*, 2002; Legaz *et al.*, 2005). This situation appears to be typical in all tetrapods, since it is also described in amphibians (Brox *et al.*, 2003).

In addition to these and other molecular and cellular features typical of the whole pallium, there are no comparative data on molecular markers that clearly distinguish the neocortex/dorsal pallium from other pallial subdivisions in adult animals. In mammals, the neocortex can be distinguished from the adjacent pallial subdivisions because of its typical six-layered structure and the presence of the rhinal fissure on its lateral egde. However, in birds and reptiles there are no clear morphological landmarks for distinguishing the lateral boundary of the dorsal pallium. The absence of dorsal pallial molecular markers has become an additional obstacule for delimiting the lateral extension of the dorsal pallium in adult birds and reptiles. As noted above, more comparative studies on the expression of developmental regulatory genes are also needed to resolve this issue. In reptiles, the dorsal cortex appears to include a rostrolateral extension called pallial thickening (reviewed in Reiner, 1993; Medina and Reiner, 2000). However, the identification of the pallial thickening varies between authors and reptilian species, and it appears that a ventral part of it is located deep to the piriform cortex and, thus, may be part of the lateral pallium (Figure 2c). Analysis of radial glial fiber disposition in that part of the reptilian pallium (Monzón-Mayor *et al.*, 1990) suggests that only the dorsal-most part of the pallial thickening may belong to the dorsal pallium (Figure 2c). This dorsal part of the pallial thickening appears located above the rhinal fissure (visible in only some reptiles), which is consistent with its dorsal pallial nature. As in reptiles, so also in birds, there is some confusion on where to locate the lateral boundary of the hyperpallium or Wulst. According to numerous studies, the hyperpallium includes the so-called apical, interstitial nucleus of apical, intercalated, and densocellular hyperpallium (HA, IHA, HI, and HD, respectively), and its lateral (or lateroventral) boundary coincides with both the superior frontal lamina and a superficial groove called vallecula (Figures 1b–1i and 2c) (Karten *et al.*, 1973; Shimizu and Karten, 1990; reviewed in Medina and Reiner, 2000). However, although this is generally true, the HD exceeds laterally the vallecula at rostral levels (Shimizu and Karten, 1990), and the superior frontal lamina appears to bend laterally when approaching the vallecula (Suárez *et al.*, 2006; see Figures 1e, 1g, and 2c). Further, the HD is sometimes misidentified and either confused with the dorsal part of the mesopallium (a part

of the DVR that belongs to the lateral pallium) or vice versa. This is partly due to the fact that the HD (or part of it) shares with the mesopallium expression of some molecular markers, such as some glutamate receptor subunits (Wada *et al.*, 2004). This has raised the question of whether the HD should or should not be considered part of the hyperpallium. However, the HD also differs from the mesopallium in many other molecular features, such as expression of calcium-binding proteins (Suárez *et al.*, 2006), expression of delta and mu opiate receptors (Reiner *et al.*, 1989), and expression of GluR1 glutamate receptor subunit, neurotensin receptors, or the neuropeptide substance P (Reiner *et al.*, 2004) (Figures 1f and 1g). Further, recent evidence indicates the existence of an additional pallial division (the laminar pallial nucleus) clearly located at the boundary between HD and mesopallium, showing distinct expression of calcium-binding proteins throughout development and in adult chicks (Suárez *et al.*, 2006). Thus, the questions raised on the identity and nature of HD may be partially due to the use of different species. Whereas all four subdivisions of the hyperpallium are clearly distinguished in birds with a large hyperpallium (such as the owl), it appears that the more lateral hyperpallial subdivisions, HI or HD, are difficult to distinguish in either pigeons/chicks or songbirds, respectively.

13.2.2.2 Connections One of the most typical features of the mammalian neocortex is that it contains unimodal sensory and motor areas that receive their input directly from specific nuclei of the dorsal thalamus (Northcutt and Kaas, 1995) (Figure 3). These primary functional areas of mammals show a detailed point-to-point representation of the body and/or world (Krubitzer, 1995). The presence of these primary, unimodal sensory and motor areas makes the neocortex unique and different from adjacent pallial divisions (such as the hippocampal formation, which typically receives multimodal thalamic input, and the piriform cortex, which typically receives olfactory input from the bulb), and this has been used for identifying the dorsal pallium or specific dorsal pallial functional areas in other vertebrates. Nevertheless, the use of connections (or any other single data) alone for identification of homologies is highly risky since they may have changed during the course of evolution (Striedter, 2005). For this reason, when searching for homologies and for evolutionary interpretations, data on connections need to be used in combination with other data, including embryological origin and/or topological position.

The neocortex contains: (1) a primary visual area (V1), receiving input from the retinorecipient dorsal lateral geniculate nucleus; (2) a primary somatosensory area (S1), receiving input from specific nuclei of the ventrobasal (or ventral posterior) thalamic complex (including the ventral posterolateral (VPL) and ventral posteromedial (VPM) nuclei in rodents), which in turn receive somatosensory information from the head and body via the trigeminal sensory and dorsal column nuclei; and (3) a primary motor area (M1), receiving input from specific nuclei of the ventrobasal thalamic complex (including the ventral anterior (VA) and ventral lateral (VL) nuclei in rodents) that receive motor information from the basal ganglia and deep cerebellar nuclei (Figure 3) (Krubitzer, 1995; Groenewegen and Witter, 2004; Sefton *et al.*, 2004; Tracey, 2004; Guy *et al.*, 2005). These primary functional areas are present in most groups of mammals and have a similar relative position within the neocortex, with M1 and S1 being always rostral to V1 (Krubitzer, 1995; Medina and Reiner, 2000; Kaas, 2004). This suggests that these areas (at least V1 and S1, as well as some other sensory areas) were likely present in the origin of the mammalian radiation (Krubitzer, 1995; Slutsky *et al.*, 2000). Further, it appears that the neocortex of early mammals had multiple somatosensory representations of the body, each one corresponding to a distinct area (Krubitzer *et al.*, 1995, 1997; Catania *et al.*, 2000a, 2000b; Kaas, 2004). However, current available data suggest that early mammals did not possess a separate motor cortical area (M1), and this possibly appeared with the origin of placental mammals (Kaas, 2004). In mammals with a small neocortex, the visual and somatomotor areas show a close spatial contiguity (for example, in monotremes or in the hedgehog). In mammals with a large neocortex, such as rodents, carnivores, and primates, V1 becomes secondarily displaced to the most caudal part of the neocortex (occipital lobe) by the development of novel cortical areas involved in higher-order or multimodal information processing. In these mammals, S1 is located in the parietal lobe (in the postcentral gyrus of the primate parietal lobe), whereas M1 is located in the frontal lobe (in the precentral gyrus of the primate frontal lobe). In rodents and primates, the initial parcellation of these functional areas during development is related (among other things) to their expression of specific ephrin ligands and receptors, some of which can be used as early markers of S1 (ephrinA5) or V1 (Ephrin receptor EphA6) (Donoghue and Rakic, 1999; Yun *et al.*, 2003).

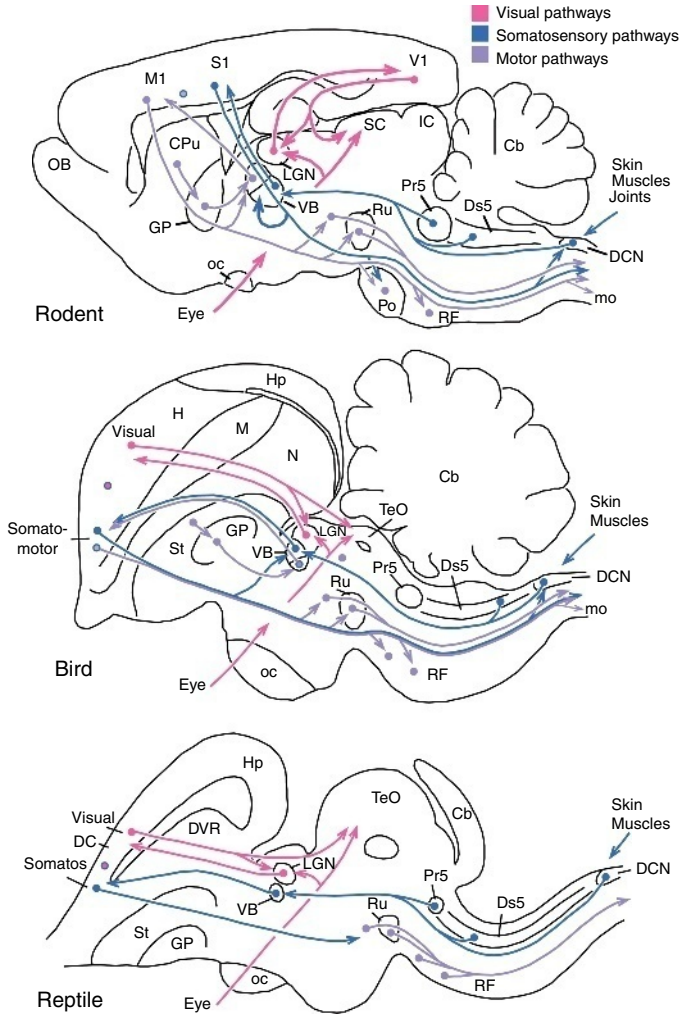

Figure 3 Schematics of lateral views of the brain of a rodent, a bird, and a reptile, showing the major connectivity patterns of the visual, somatosensory, and motor pathways. In mammals, birds, and reptiles, partially comparable visual and somatosensory pathways to the dorsal pallium are present. Visual information reaches a dorsal pallial primary visual area (V1 in mammals) by way of the lateral geniculate nucleus (LGN), located in the lemnothalamus. In turn, the visual cortical (dorsal pallial) area projects back to this thalamic nucleus and to the optic tectum. Somatosensory information reaches a dorsal pallial primary somatosensory area (S1 in mammals) by way of the ventrobasal complex of the lemnothalamus (VB). In birds, the body is mainly represented in the dorsal pallium, whereas in mammals and at least some reptiles, there is representation of both body and head. In birds and mammals, the ventrobasal complex has a motor subdivision that receives basal ganglia and cerebellar input, and projects to the dorsal pallium. In birds, this motor input ends in the somatosensory field (thus becoming a true somatomotor area). In placental mammals, such as rodents, the motor input ends mostly in a separate primary motor area (M1), although a small somatosensory motor overlap occurs at the interface between M1 and S1. See text for details. In mammals, both S1 and M1 project back to the ventrobasal thalamic complex and to the brainstem and spinal cord by way of the pyramidal tract. S1 projections primarily end on precerebellar centers (such as the pontine nuclei) and somatosensory-relay centers (such as the dorsal column nuclei and the dorsal horn of the spinal cord). M1 projections primarily reach premotor precerebellar and reticulospinal centers (such as prerubral and rubral neurons), and also provide some input to motoneuron pools, such as those of the ventral horn of the spinal cord. In birds, the somatomotor dorsal pallial area shows descending projections resembling both S1 and M1 (especially in some avian species). In reptiles, the putative ventrobasal complex does not include any motor subdivision. The somatosensory area of the reptilian dorsal pallium only shows projections to diencephalic and midbrain tegmentum, reaching premotor precerebellar and reticulospinal cell groups, and suggesting that it may control or modulate motor behavior. However, this area lacks most of the connections typical of a true somatomotor area, suggesting that this likely evolved independently in birds and mammals. Cb, cerebellum; CPU, caudoputamen (dorsal striatum); DC, reptilian dorsal cortex; DCN, dorsal column nuclei; Ds5, descending sensory trigeminal nucleus; DVR, dorsal ventricular ridge; GLN, dorsal lateral geniculate nucleus; GP, globus pallidus; H, hyperpallium; Hp, hippocampal formation; IC, inferior colliculus; M, mesopallium; M1, primary motor area of neocortex; mo, motoneuron pools; N, nidopallium; OB, olfactory bulb; oc, optic chiasm; Po, pontine nuclei; Pr5, principal sensory trigeminal nucleus; RF, reticular formation; Ru, nucleus ruber; S1, primary somatosensory area of neocortex; SC, superior colliculus; St, striatum; TeO, optic tectum; V1, primary visual area of neocortex; VB, ventrobasal thalamic complex.

Are these areas present in the dorsal pallium of reptiles and/or birds? Data on ephrin ligands and receptors in the dorsal pallium of birds and reptiles are lacking but, in any case, the search for functional areas in the dorsal pallium of birds and reptiles requires the analysis of the thalamopallial projections in these vertebrate groups and/or electrophysiological recordings in the pallium.

Of interest, the adult hyperpallium of birds and dorsal cortex of reptiles show some patterns of connections with the dorsal thalamus similar to those of the neocortex (Figure 3). In particular, the patterns of connections suggest the existence of a primary visual area and a primary somatosensory area in the reptilian dorsal cortex and avian hyperpallium that are comparable and might be homologous to the primary visual (V1) and primary somatosensory (S1) areas of the mammalian neocortex (reviewed in Medina and Reiner, 2000; see also Wild and Williams, 2000). The somatosensory area found in the avian hyperpallium shows some connections, suggesting that it may represent a true somatomotor area, able to play a role in modulation of somatosensory input as well as in motor control, resembling aspects of mammalian S1+M1 (perhaps like the somatomotor area present in the origin of mammals). However, available data suggest that the motor control features of this avian hyperpallial area evolved independently from those found in M1.

The conclusion of homology of the visual and somatosensory cortical areas is partially based on assumption of homology of the thalamic nuclei of reptiles, birds, and mammals relaying the visual or somatosensory information to the dorsal pallium. But, for this to be true, these nuclei not only need to share similar connections, but also need to originate from the same embryonic primordium (or be located in the same histogenetic field). This will be analyzed in the following section.

13.3 Thalamopallial Projections and Sensory and Motor Areas in the Dorsal Pallium of Mammals, Birds, and Reptiles

13.3.1 Divisions of the Thalamus: Specific Relation of the Lemnothalamus with the Dorsal Pallium

To know whether the thalamic nuclei projecting to V1, S1, or M1 of mammalian neocortex are located in the same histogenetic unit as the avian and reptilian thalamic nuclei projecting to the hyperpallium/dorsal cortex, it is important to analyze the development and adult organization of the thalamus. In this sense, Butler (1994a) proposed the existence of two dorsal thalamic divisions, the lemnothalamus and the collothalamus, which receive sensory input through different systems and have different connections with the pallium. The lemnothalamus includes nuclei receiving sensory input primarily from lemniscal systems and projecting to the medial and/or dorsal pallium (Butler, 1994a, 1994b). The collothalamus includes nuclei receiving a major collicular input and projecting to the lateroventral pallium (Butler, 1994a, 1994b). A similar but more complex subdivision of the thalamus was later proposed by other authors based on differential expression of cadherins or calcium-binding proteins by thalamic subdivisions during development or in the adult (Dávila *et al.*, 2000; Redies *et al.*, 2000). According to these authors, the dorsal thalamus is subdivided into three main histogenetic divisions, called dorsal, intermediate, and ventral tiers, each one showing a specific immunostaining profile and connections with a particular pallial subdivision (Dávila *et al.*, 2000; Redies *et al.*, 2000; Puelles, 2001). The dorsal tier corresponds roughly to the lemnothalamus, whereas the intermediate and ventral tiers roughly correspond to the collothalamus of Ann Butler (Butler, 1994a, 1994b; Dávila *et al.*, 2000; Redies *et al.*, 2000). Thus, the thalamic nuclei projecting to V1, S1, and M1 in mammals are all located in the dorsal tier or lemnothalamus (Puelles, 2001; Butler, 1994a). What about the visual and somatosensory/somatomotor thalamic nuclei projecting to the avian hyperpallium and reptilian dorsal cortex?

13.3.2 A Primary Visual Area in the Dorsal Pallium of Birds and Reptiles and Its Comparison to V1 of Mammals

In mammals, V1 (area 17) receives unimodal visual input from the dorsal lateral geniculate nucleus and projects back to this nucleus and to the superior colliculus (Figure 3) (Krubitzer, 1995; Sefton *et al.*, 2004). A comparable retinorecipient dorsal lateral geniculate nucleus is present in the lemnothalamus of birds and reptiles that projects to the avian hyperpallium and reptilian dorsal cortex (Figure 3) (Karten *et al.*, 1973; Hall *et al.*, 1977; Miceli and Repérant, 1982; Miceli *et al.*, 1990; Mulligan and Ulinski, 1990; Butler, 1994a, 1994b; Kenigfest *et al.*, 1997; Medina and Reiner, 2000; Zhu *et al.*, 2005). In lizards, this nucleus is sometimes called intercalatus (Bruce and Butler, 1984a) and apparently corresponds to the deeper part (cell plate) of the dorsal lateral geniculate nucleus of other authors (Kenigfest *et al.*, 1997; Dávila *et al.*, 2000). In lizards, the geniculate thalamic input only reaches a lateral extension of the dorsal cortex, called pallial thickening (Bruce and Butler, 1984a; Kenigfest

et al., 1997). In birds, the geniculate thalamic input mainly reaches a hyperpallial subdivision called interstitial nucleus of the apical hyperpallium or IHA (Karten *et al.*, 1973; Watanabe *et al.*, 1983). As in mammals, the dorsal pallial area of birds and at least some reptiles (such as turtles) that receives visual input from the geniculate nucleus projects back to this thalamic nucleus and the optic tectum (Figure 3) (Karten *et al.*, 1973; Hall *et al.*, 1977; Miceli and Repérant, 1983, 1985; Reiner and Karten, 1983; Ulinski, 1986; Mulligan and Ulinski, 1990; Butler, 1994a, 1994b; Kenigfest *et al.*, 1998). Therefore, their similar position, histogenetic origin, and connections suggest that the visual lemnothalamic nuclei and related pallial areas of the neocortex, hyperpallium, and dorsal cortex of mammals, birds, and reptiles are homologous, and evolved from similar areas present in their common ancestor.

13.3.3 A Primary Somatosensory Area in the Dorsal Pallium of Birds and Reptiles and Its Comparison to S1 of Mammals

In the mammalian neocortex, S1 receives somatosensory input from the ventrobasal or ventral posterior thalamic complex (in particular, from VPL, receiving body information via the dorsal column nuclei, and from VPM, receiving head information via the principal sensory trigeminal nucleus; restricted parts of VPL/VPM also receive pain and temperature information directly from the spinal cord through the dorsal horn and spinal trigeminal nucleus) (Figure 3). In turn, S1 shows descending projections back to this thalamic complex and to the brainstem and spinal cord (reaching primarily precerebellar and/or somatosensory relay centers) (Weisberg and Rustioni, 1977; McAllister and Wells, 1981; Torigoe *et al.*, 1986; Krubitzer, 1995; Desbois *et al.*, 1999; Manger *et al.*, 2001; Martínez-Lorenzana *et al.*, 2001; Killackey and Sherman, 2003; Craig, 2004; Friedberg *et al.*, 2004; Gauriau and Bernard, 2004; Leergaard *et al.*, 2004; Oda *et al.*, 2004; Tracey, 2004; Waite, 2004; Guy *et al.*, 2005). Similarly to S1, the frontal part of the avian hyperpallium (Wulst) receives somatosensory input from the dorsointermediate ventral anterior thalamic nucleus (DIVA), which is a target of both the dorsal column nuclei (Wild, 1987, 1989, 1997; Funke, 1989a, 1989b; Korzeniewska and Güntürkün, 1990) and the spinal cord (Schneider and Necker, 1989) (Figure 3). The avian DIVA develops in the dorsal tier/lemnothalamus of the dorsal thalamus (Redies *et al.*, 2000) and, thus, appears comparable in position, histogenetic origin, and connections to the mammalian ventrobasal thalamic complex (mainly to VPL). In addition to receiving somatosensory input from DIVA, the frontal part of the Wulst (hyperpallium) projects back to this thalamic nucleus, to the brainstem, and, in some species of birds, to the cervical spinal cord (Figure 3) (Wild, 1992; Wild and Williams, 2000). As with S1, the frontal hyperpallial descending projections predominantly reach precerebellar areas and somatosensory relay areas, such as the thalamic DIVA, the dorsal column nuclei, and the vicinity of medial lamina V in the cervical spinal dorsal horn, which suggests that the frontal hyperpallium may be primarily concerned with the control/modulation of somatosensory input (Wild and Williams, 2000). This suggests that the avian frontal hyperpallium contains a primary somatosensory area that appears comparable to S1 of mammals (Wild, 1992; Medina and Reiner, 2000; Wild and Williams, 2000). However, unlike S1, a sensory trigeminal representation (with head information) has not been found in the hyperpallium (Wild *et al.*, 1985). To know whether these primary somatosensory areas of the avian hyperpallium and mammalian neocortex are homologous we need to analyze if a similar pallial area is present in the dorsal pallium of reptiles.

The frontal part of the reptilian dorsal cortex was previously thought to contain a somatosensory area based on input from a spinorecipient thalamic nucleus (Ebbesson, 1967, 1969, 1978; Hall and Ebner, 1970). Modern tract-tracing data in lizards indicate that the rostral dorsal cortex receives distinct ipsilateral input specifically from a ventral part of the dorsolateral thalamic nucleus (Guirado and Dávila, 2002), which receives somatosensory input from the dorsal column and trigeminal sensory nuclei as well as from the spinal cord (Figure 3) (Hoogland, 1982; Desfilis *et al.*, 1998, 2002). Of interest, the dorsolateral thalamic nucleus of lizards is located in the dorsal tier/lemnothalamus of the dorsal thalamus, and its ventral part DLV, which differs from the rest of the nucleus by its connections and calbindin immunostaining profile – shows a location that resembles that of avian DIVA and ventrobasal complex of mammals (Dávila *et al.*, 2000). The projection from the dorsal column and sensory trigeminal (both descending and principal) nuclei and from the spinal cord to the dorsolateral thalamic nucleus appears to reach specifically its ventrolateral part or DLV (plus the area adjacent to it, called intermediodorsal nucleus; Ebbesson, 1967, 1969, 1978; Hoogland, 1982). Thus, DLV of lizards appears to be a distinct subnucleus of the lemnothalamus that may be primarily involved in somatosensory information processing. Using

modern tract-tracing techniques, similar thalamocortical projections have also been described in crocodiles (Pritz and Stritzel, 1987; these authors also found a specific dorsolateral cell population projecting only ipsilaterally to the dorsal cortex) and in adult and developing turtles (Hall *et al.*, 1977; Cordery and Molnár, 1999). In turtles, the thalamocortical nucleus appears located in a perirotundal position, medially adjacent to the dorsal lateral geniculate nucleus and ventral to the dorsolateral thalamic nucleus (a position that resembles the DLV of lizards), and this perirotundal area is the site of termination of spinal and dorsal column nuclei (but not retinal) projections (Künzle and Schnyder, 1983; Siemen and Künzle, 1994). However, other authors studying turtles have not found any thalamic relay center projecting to the dorsal cortex other than the geniculate nucleus (Zhu *et al.*, 2005). This may be due to the more caudal location of the injections in the dorsal cortex (note that the somatosensory area is located at its rostral pole) or to the employment of *in vitro* tract-tracing techniques in the study done by Zhu *et al.* (2005). Based on the evidence presented above, two different thalamic relay centers conveying either visual or somatosensory information to the dorsal cortex are present in most reptilian groups, and were likely present in their common ancestor. Thus, the frontal part of the dorsal cortex of most reptiles contains a primary somatosensory area that is comparable and may be homologous to those present in the hyperpallium of birds and the neocortex of mammals. Consistent with this, the relative position of the primary somatosensory area in the dorsal pallium is similar in mammals, birds, and reptiles, being always located rostral (in a more frontal position) to the primary visual area.

13.3.4 Do Birds and/or Reptiles Possess a Somatomotor Dorsal Pallial Area Comparable to M1 of Mammals?

In the mammalian neocortex, M1 (area 4) receives motor input from a specific part of the lemnothalamic ventrobasal complex (VA/VL nuclei in rodents), and shows descending projections back to this thalamic complex, and to the brainstem and the spinal cord (Figure 3), where the projections reach precerebellar (including the red and pontine nuclei) and sensory-relay areas (including dorsal column nuclei and dorsal horn of the spinal cord), but also reach premotor reticulospinal cell groups (including the prerubral and rubral neurons) and, in some species (such as rodents and primates), motor neuron pools such as those of the ventral horn in the spinal cord (Weisberg and Rustioni, 1977; Humphrey *et al.*, 1984; Torigoe *et al.*, 1986; Liang *et al.*, 1991; Krubitzer, 1995; Song and Murakami, 1998; Kuchler *et al.*, 2002; Leergaard *et al.*, 2004). In general, the descending projections of M1 are similar to those of S1, and axons from both areas contribute to form the pyramidal tract. However, the descending projections of S1 and M1 are somewhat different. For example, in the brainstem, S1 projects significantly more heavily to the precerebellar pontine nuclei than M1 (Leergaard *et al.*, 2004), whereas M1 is the major source of corticorubral axons (Giuffrida *et al.*, 1991; Burman *et al.*, 2000) (Figure 3). Further, in the spinal cord, S1 axons primarily reach dorsal horn laminae, whereas M1 axons, but not S1 axons, also reach the motoneuron pools in the ventral horn (Figure 3) (Ralston and Ralston, 1985; Martín, 1996). Current available data suggest that early mammals lacked a separate motor cortical area (M1), and that a separate M1 likely evolved with the origin of placental mammals (Kaas, 2004). Thus, it appears that early mammals only had an S1 where somatosensory and motor attributes were overlapped, a situation which resembles that found in marsupials (Kaas, 2004).

Similarly to M1, the frontal part of the avian hyperpallium (Wulst) receives input from a putative motor thalamic nucleus, the ventrointermediate area (VIA), which receives input from the avian globus pallidus, substantia nigra pars reticulata, and deep cerebellar nuclei (Medina *et al.*, 1997) (Figure 3). The avian VIA resembles the motor part of the mammalian ventrobasal thalamic complex (VA/VL) in both its position (located in the lemnothalamus and adjacent to the somatosensory part of the avian ventrobasal complex or DIVA), and its connections (Medina *et al.*, 1997). Both DIVA and VIA project to the frontal part of the hyperpallium (Wild, 1987, 1989; Funke, 1989a, 1989b; Korzeniewska and Güntürkün, 1990; Medina *et al.*, 1997), where somatosensory and motor information may be completely overlapped (Medina and Reiner, 2000) (Figure 3). Of note, as S1 and M1 of mammals, the frontal hyperpallium of birds projects back to the thalamus (including DIVA), to the brainstem and, in some avian species, to the cervical spinal cord (Wild, 1989, 1992; Medina and Reiner, 2000; Wild and Williams, 2000) (Figure 3). In the brainstem and spinal cord, the frontal hyperpallial projections reach precerebellar (including pretectal and rubral nuclei), sensory-relay cell groups (including the dorsal column nuclei and the dorsal horn in the spinal cord), premotor reticulospinal neurons (such as rubrospinal neurons) and, in some birds, a few axons reach the ventral horn of the cervical spinal

cord, where motoneuron pools are located (Wild, 1992; Wild and Williams, 2000). Thus, the frontal hyperpallium contains a somatosensory/somatomotor area that appears at least partially comparable to the overlapped S1+M1 of marsupials, and possibly of early mammals. To know whether these areas are homologous we need to know if a similar sensorimotor field is present in the dorsal pallium of reptiles.

As noted above, in some reptiles (lizards), the frontal part of the dorsal cortex appears to receive somatosensory input from a specific subdivision of the dorsolateral thalamic nucleus, the DLV (Guirado and Dávila, 2002) (Figure 3). Further, this part of the reptilian dorsal cortex has descending projections to diencephalic and midbrain tegmetum (Hoogland and Vermeulen-vanderZee, 1989; Guirado and Dávila, 2002). In the prerubral tegmentum, these cortical projections reach at least the nucleus of the medial longitudinal fascicle, which is a well-known premotor precerebellar and reticulospinal cell group (Figure 3) (ten Donkelaar, 1976; Woodson and Künzle, 1982; Wolters *et al.*, 1986). This feature has been used to suggest that this part of the reptilian dorsal cortex may represent a rudimentary sensorimotor area, partially comparable to that in other amniotes (Medina and Reiner, 2000; Guirado and Dávila, 2002). However, this putative sensorimotor area of the reptilian dorsal cortex does not possess a distinct motor field comparable to M1 of mammals, since its thalamic input does not include a basal ganglia-recipient nor a cerebellar-recipient nucleus. No part of the dorsolateral thalamic nucleus and no part of the reptilian dorsal thalamus receives direct basal ganglia input (Reiner *et al.*, 1984, 1998; Medina and Smeets, 1991) nor input from the deep cerebellar nuclei (Künzle, 1985). Further, in some reptilian species (including the pond turtle) the descending projections of the dorsal cortex are rather modest and do not reach rubral and, perhaps, not even prerubral levels (Zhu *et al.*, 2005). Thus, at present it is unclear whether ancestral reptiles had a rudimentary somatomotor area in the dorsal cortex, and more data are needed in other reptilian species before any conclusion can be reached. If a rudimentary somatomotor area was present in the dorsal cortex of reptiles, this area lacked many of the connections that characterize the true somatomotor cortical area found in birds and mammals (including basal ganglia and cerebellar indirect input, or output to additional precerebellar and reticulospinal fields and to the spinal cord), meaning that these features likely evolved independently in the avian and mammalian radiations.

13.3.5 Other Functional Areas in the Pallium of Birds and Reptiles and Comparison to Mammals

In birds and reptiles, there are other sensory (visual, somatosensory, and auditory) areas in the pallium that are located in the DVR (Karten and Hodos, 1970; Dubbeldam *et al.*, 1981; Bruce and Butler, 1984b; Wild, 1987, 1994; Wild *et al.*, 1993, 1997; Guirado *et al.*, 2000; reviewed by Karten and Shimizu, 1989; Butler, 1994b; Reiner, 2000). These sensory areas are mainly located in the ventral pallial part of the DVR (called nidopallium in birds; Reiner *et al.*, 2004) and receive visual, somatosensory, or auditory input from specific nuclei of the collothalamus or directly from the brainstem (see above-cited references; this is described in detail in Evolution of the Nervous System in Reptiles, Visual Cortex of Turtles). In birds, some of these DVR areas appear to have a better (more detailed) sensory representation than those present in the hyperpallium, such as the nucleus basalis of the budgerigar, which shows a highly somatotopically organized representation of head and body (Wild and Farabaugh, 1996; Wild *et al.*, 1997). In addition, the caudal part of the DVR in birds (including the caudal nidopallium and the region called arcopallium) and reptiles contains associative and/or motor centers that project to the basal ganglia, hypothalamus, and/or, in birds, also to premotor brainstem centers (Zeier and Karten, 1971; Bruce and Neary, 1995a, 1995b, 1995c; Davies *et al.*, 1997; Dubbeldam *et al.*, 1997; Lanuza *et al.*, 1997, 1998; Kröner and Güntürkün, 1999; Bottjer *et al.*, 2000; Martínez-García *et al.*, 2002). Further, in songbirds and budgerigars, the caudal DVR (arcopallium) contains a specific motor area that projects directly to motor brainstem nuclei, the ambiguus, and/or hypoglossal motor nuclei, which control syringeal, respiratory, and tongue muscles (Nottebohm, 1991; Vicario, 1991a, 1991b; Wild, 1993; Brauth *et al.*, 1994; Striedter, 1994; Durand *et al.*, 1997; see details on this motor pallial area and its connections in The Evolution of Vocal Learning Systems in Birds). In songbirds and budgerigars, this motor area is well developed and plays a key role in vocalization (including vocal learning and vocal production), and apparently evolved independently in songbirds and budgerigars (Striedter, 1994). These sensory, associative, and motor areas of the DVR play very important roles in sensory processing, sensorimotor integration, and motor control, and in birds are also involved in cognitive tasks such as learning, memory, and spatial orientation, and they have been compared to specific areas or specific cell populations of the mammalian temporal,

frontal, and prefrontal neocortex (for example, Karten, 1969, 1997; Morgensen and Divac, 1993; Veenman *et al.*, 1995; Kröner and Güntürkün, 1999; see The Evolution of Vocal Learning Systems in Birds and Humans). Although this makes sense from a functional point of view, the different histogenetic origin of the DVR (lateroventral pallium) and neocortex (dorsal pallium) indicates that the similarities of such sauropsidian DVR and mammalian neocortical areas represent cases of analogy (homoplasy). Consistent with this, the thalamic nuclei that project to the DVR sensory areas are not comparable in location to those that project to the neocortex. Thus, the DVR receives sensory information via thalamic nuclei that are located in the intermediate and ventral tiers of the dorsal thalamus, whereas those that project to the neocortex are generally located in the dorsal tier or lemnothalamus (Dávila *et al.*, 2000, 2002; Puelles, 2001). Further, the avian motor area(s) of the caudal DVR projecting to the premotor and/or motor brainstem are not present in the caudal DVR of reptiles (some of them are not even present in all birds), which means that they evolved as novelties in some birds. Thus, it appears that, in contrast to mammals, the repertory of complex behaviors shown by birds and reptiles depends primarily (although not exclusively) on a large variety of cell groups that develop in the ventrolateral pallial histogenetic division, but the contribution of dorsal pallial areas to these behaviors is likely more modest (especially in reptiles).

13.4 Pallial Lamination in Birds and Mammals: Evidence for Independent Evolution

13.4.1 Different Development and Adult Organization of Neocortical Layers and Hyperpallial Subdivisions

In mammals, the neocortex shows a laminar structure of six layers, and each layer has a similar cytoarchitecture and general pattern of connections throughout all areas, including V1, S1, and M1 (Figure 4). Thus, the dorsal thalamic input mainly contacts cells in neocortical layer 4, a layer that is called granular layer because of its typical granule or stellate cells (Humphrey *et al.*, 1977; Kharazia and Weinberg, 1994). In this layer, thalamic axons contact granule cells as well as apical dendrites of pyramidal neurons located below, in layers 5/6 (Mountcastle, 1997). In addition, thalamocortical axons ending in layer 4 provide collaterals that terminate in layer 5 and/or 6, but other thalamocortical axons terminate in layer 1 (Figure 4) (Jones, 1975; Rausell *et al.*, 1992; Lu and Lin, 1993; Zhang and Deschenes, 1998; Groenewegen and Witter, 2004; Sefton *et al.*, 2004). Layers 2 and 3 are called supragranular layers, located superficially to layer 4, and typically contain small to medium-sized pyramidal neurons involved in corticocortical (associational) projections (Gilbert and Kelly, 1975; Jones and Wise, 1977; Swadlow and Weyand, 1981; Sefton *et al.*, 2004). Layers 5 and 6 are called infragranular layers, located deep to layer 4, and typically contain large pyramidal neurons that show descending projections to the striatum, thalamus, and brainstem (Figure 4). Layer 5 mainly projects to the striatum and brainstem (to the midbrain tectum in the case of V1 and to the brainstem tegmentum and spinal cord in the case of S1 and M1), whereas layer 6 typically projects to the thalamus (Gilbert and Kelly, 1975; Jones and Wise, 1977; Swadlow and Weyand, 1981; Sefton *et al.*, 2004; Tracey, 2004). The pyramidal neurons of the supra- and infragranular layers of the neocortex typically have a long apical dendrite that span the cortical layers above its cell body (Figure 4), which provides one of the anatomical bases for the columnar functional organization of the neocortex (Mountcastle, 1997; Lübke *et al.*, 2000).

In birds, the dorsal pallium (corresponding to the so-called hyperpallium) also shows cytoarchitectonic subdivisions, considered by some authors as the layers of the neocortex (for example, Karten *et al.*, 1973; Shimizu and Karten, 1990; reviewed by Medina and Reiner, 2000). From rostral (frontal) to caudal levels, each hyperpallial subdivision is characterized by a specific pattern of connections which partially resembles the connectivity organization of neocortical layers (Figure 4). For example, the thalamic input ends primarily in an intermediate hyperpallial subdivision called the IHA, in both the visual and somatomotor areas (Karten *et al.*, 1973; Watanabe *et al.*, 1983; Wild, 1987, 1997), resembling neocortical layer 4. Nevertheless, some thalamic axons appear to end in the hyperpallium outside IHA (Figure 4). Further, the descending projections to the striatum, thalamus, and brainstem mainly originate in the apical hyperpallium or HA (Reiner and Karten, 1983; Wild, 1992; Wild and Williams, 1999, 2000), resembling neocortical layers 5–6. Morever, the densocellular hyperpallium (HD) appears to be mainly involved in connections with other pallial and subpallial areas (Veenman *et al.*, 1995; Kröner and Güntürkün, 1999; Wild and Williams, 1999), thus partially resembling neocortical layers 2–3. In addition to the apparently similar laminar organization of both neocortex and hyperpallium, there is evidence suggesting that they also

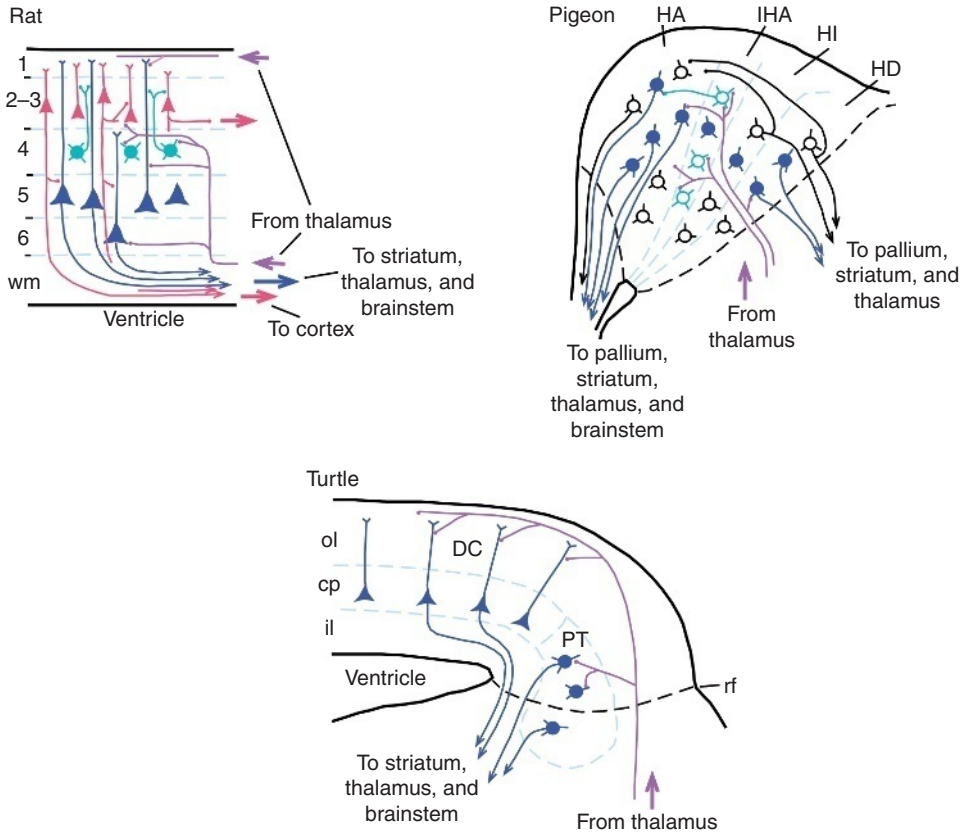

Figure 4 Schematics of the layers/subdivisions, cell types, and major connections of the mammalian neocortex, avian hyperpallium, and reptilian dorsal cortex. The mammalian neocortex shows a six-layered organization, and each layer shows specific cell types and connections. The thalamic input primarily reaches the intermediate layer 4 (also called granular layer, because of its typical granule or stellate cells). In this layer, thalamic axons contact stellate cells as well as apical dendrites of pyramidal neurons located in infragranular layers (layers 5 and 6). Some thalamic axons also reach layers 6 or 1. Infragranular layers contain large pyramidal neurons having apical dendrites that radially span the layers above, and give rise to descending projections to the striatum, thalamus, and brainstem. Supragranular layers contain small to medium pyramidal neurons involved in corticocortical (associational) connections. This anatomical and cellular organization, with radially oriented dendrites that span most layers, constitutes one of the basis of the functional columnar organization of neocortex. In contrast to this organization, the avian hyperpallium shows four mediolateral subdivisions that are formed and organized in a radically different way. These subdivisions contain multipolar or stellate-like neurons having star-like oriented dendrites that do not span adjacent subdivisions (i.e., they lack the translayer, radial dendritic organization typical of neocortex). In contrast to the neocortex, the connections between subdivisions occur by way of tangential projections, instead of the radial connections typical of neocortical columns. Nevertheless, the patterns of connections of hyperpallial subdivisions partially resemble those of neocortical layers. For example, thalamic input primarily ends in an intermediate subdivision (IHA), which is sandwiched between a subdivision (HA) giving rise to descending projections to the striatum, thalamus, and brainstem, and another subdivision (HD) giving rise to pallial projections. However, HA also projects to other pallial areas, whereas HD also projects to the striatum and thalamus, indicating that their similarity with specific neocortical layers in terms of connectivity is only partial. The reptilian dorsal cortex is very simple but resembles, in a very rudimentary way, both the laminar organization of neocortex and the mediolateral subdivisions of hyperpallium. Thus, the reptilian dorsal cortex contains a medial subdivision (dorsal cortex proper or DC) that resembles HA, and a lateral subdivision (pallial thickening or PT) that resembles HD. Further, the reptilian dorsal cortex shows a three-layered structure, with a main cell layer located between superficial and deep cell-sparse layers. The main cell layer contains pyramidal neurons having apical dendrites that span the superficial layer, where they are contacted by incoming thalamic axons. Further, these pyramidal neurons give rise to descending projections to the striatum, thalamus, and brainstem. This basic laminar and cellular organization partially resembles that of the neocortex, with pyramidal neurons located in deeper layers (5/6) giving rise to long descending projections, and thalamic axons contacting the apical dendrites of these cells. Cell types sharing these features and connections were likely present in the dorsal pallium of the common ancestor of mammals, birds, and reptiles (represented in dark blue in schematics). However, many of the cell types present in the mammalian neocortex and avian hyperpallium likely evolved independently in birds and mammals (such as the thalamorecipient stellate or stellate-like cells, or many of the neurons involved in corticocortical connections). cp, main cell layer; DC, reptilian dorsal cortex; HA, apical hyperpallium; HD, densocellular hyperpallium; HI, intercalated hyperpallium; IHA, interstitial nucleus of the apical hyperpallium; il, inner layer; ol, outer layer; PT, pallial thickening; wm, white matter.

share a similar functional columnar organization (Revzin, 1970). Both in the neocortex and the hyperpallium, the sensory input is topographically (retinotopically or somatotopically) organized (Pettigrew and Konishi, 1976; Wilson, 1980; Wild, 1987; Funke, 1989a; Manger *et al.*, 2002). In each single neocortical unit, the excitation reaches layer 4 by way of thalamocortical axons, and then spreads primarily in a columnar way first to supragranular and later to infragranular layers (Petersen and Sakmann, 2001), and this appears to be similar in the avian hyperpallium (Revzin, 1970).

However, the similarity of hyperpallial subdivisions and specific neocortical layers in terms of connectivity is only partial (Veenman *et al.*, 1995; Wild and Williams, 1999). More importantly, developmental and cellular analysis of the avian hyperpallium and mammalian neocortex indicates that the subdivisions of the avian hyperpallium are not true layers (as least, as defined from a developmental point of view, but see Striedter, 2005) and show important organizational differences with neocortical layers (reviewed in Medina and Reiner, 2000). For this reason, the subdivisions of avian hyperpallium have been called pseudolayers, meaning false layers (Medina and Reiner, 2000). This is based on the following facts. First, although the layers of the mammalian neocortex are aligned parallel to the ventricular surface and develop perpendicular to radial glial fibers, the hyperpallial subdivisions are generally organized parallel to radial glial fibers (Striedter and Beydler, 1997; Medina and Reiner, 2000). Since, during development, the majority of neurons migrate from ventricular to mantle positions following radial glial fibers (Rakic, 1972, 1995; Alvarez-Buylla *et al.*, 1988; Striedter and Beydler, 1997), the different disposition of neocortical layers and hyperpallial subdivisions with respect to the radial glial fibers likely reflects that they are formed in radically distinct ways (Medina and Reiner, 2000). Second, as a consequence of their apparently different development, whereas for any single neocortical area the majority of neurons of all layers are born in the same ventricular sector (except interneurons, which immigrate from the subpallium; Anderson *et al.*, 1997), in the avian hyperpallium the neurons of each subdivision (HA, IHA, and HD) are primarily born in different ventricular sectors (Medina and Reiner, 2000). Third, also as a consequence of their development, whereas many layers of the neocortex typically contain pyramidal neurons with an apical dendrite that span the layers above (where they can be contacted by axons of extrinsic origin ending in other layers, as well as by axon collaterals of local neurons of other layers; Mountcastle, 1997), the subdivisions of avian hyperpallium contain neurons showing multipolar or stellate-like morphology, with star-like oriented dendrites that generally do not cross subdivision boundaries (Figure 4) (Watanabe *et al.*, 1983; Tömbol, 1990; Medina and Reiner, 2000). This means that, whereas in the mammalian neocortex, the radial (translayer) disposition of dendrites allows functional integration of layers (and constitutes one of the anatomical basis of functional columns; Lübke *et al.*, 2000), the neuronal communication between subdivisions of the avian hyperpallium is apparently only possible by way of tangential, inter-area axonal connections (Figure 4) (Kröner and Güntürkün, 1999; Medina and Reiner, 2000; Wild and Williams, 2000).

13.4.2 Layers and Subdivisions of the Reptilian Dorsal Cortex. Possibilities and Uncertainties on Dorsal Pallial Evolution

How did the different pallial organizations found in neocortex and hyperpallium evolve and which was the primitive condition in stem amniotes? Extant reptiles have a very simple dorsal pallium, but this shows some features that partially resemble, in a very rudimentary way, both the medial–lateral subdivisions of avian hyperpallium and the lamination of mammalian neocortex (Figure 4). The reptilian dorsal pallium appears to have two parts that show different cytoarchitecture and connections: a medial part or dorsal cortex and a lateral part or pallial thickening (as noted above, only part of the pallial thickening may be part of the dorsal pallium) (Figure 4). In lizards, the thalamic input primarily reaches the pallial thickening, whereas the dorsal cortex gives rise to the descending projections to the striatum and brainstem (see references in previous section; reviewed in Medina and Reiner, 2000). In turtles, thalamic input reaches both the pallial thickening and the dorsal cortex (Mulligan and Ulinski, 1990), and extratelencephalic projections originate in the dorsal cortex (Hall *et al.*, 1977; Ulinski, 1986; Zhu *et al.*, 2005). In lizards and turtles, the pallial thickening shows important intratelencephalic connections (Medina and Reiner, 2000). Thus, the organization of the connections and the relative position of these two divisions in the reptilian dorsal pallium suggests a similarity of the reptilian dorsal cortex and avian HA and the reptilian pallial thickening and avian HD. As the dorsal cortex or pallial thickening, the avian HA and HD also appear to receive a minor direct input from the sensory thalamus (Karten *et al.*, 1973; Watanabe *et al.*, 1983; Wild, 1997; Wild and Williams, 2000). On the other hand, the reptilian dorsal cortex shows a simple three-layered structure, with a main, intermediate cell layer

containing pyramidal-like cells, flanked by a superficial and a deep cell sparse layer (Figure 4) (Reiner, 1993; Medina and Reiner, 2000; Colombe *et al.*, 2004). As neocortical layers, those of the reptilian dorsal cortex are disposed parallel to the ventricular surface and perpendicular to the radial glia. Further, the pyramidal cells of the main cell layer show apical dendrites that span the layer above, where they are contacted by thalamic afferent axons, and they give rise to long descending projections reaching the striatum and brainstem (Figure 4) (Mulligan and Ulinski, 1990; Colombe *et al.*, 2004). Thus, the reptilian dorsal cortex shares with the neocortex some aspects of its laminar and cellular organization. In both the neocortex and reptilian dorsal cortex, the thalamic axons contact the apical dendrites of deep pyramidal neurons, and in both these, deep pyramidal neurons are the source of long descending projections. Of interest, in the neocortex, some thalamic afferent axons travel tangentially in layer 1, where they contact apical dendrites of pyramidal neurons (Rausell *et al.*, 1992), resembling the trajectory of thalamic axons in the reptilian dorsal cortex. Further, analysis of chemically different neurons in the dorsal pallium of mammals, birds, and reptiles indicates that only the cell types present in neocortical layers 5–6 (which contain the deep pyramidal neurons giving rise to long descending projections) are found in birds and reptiles, suggesting that only layers 5–6 were present in the common ancestor (Reiner, 1991). Further, comparative developmental studies suggest that only the subpial layer 1 and the deepest neocortical layers may have been present in the common ancestor of extant reptiles, birds, and mammals (Marín-Padilla, 1998).

All these data together suggest that the pyramidal neurons found in the cell layer of the reptilian dorsal cortex may be homologous to the pyramidal cells of layers 5–6 of the mammalian neocortex, and possibly to some of the multipolar projection neurons of avian HA (at least including the neurons that, in addition to giving rise to long descending projections, receive thalamic input). In contrast, the thalamorecipient granule (or stellate) cells found in neocortical layer 4 have no counterpart in reptiles and are not homologous to the thalamorecipient stellate-like cells found in avian IHA (Figure 4). Stellate cells of neocortical layer 4 and stellate-like cells of IHA apparently evolved independently (and were produced as novelties) in the mammalian and avian radiations. On the other hand, the pyramidal neurons of neocortical layers 2–3 and part of the projection neurons of avian hyperpallium involved in corticocortical connections may also be newly evolved. Finally, it is unclear what part of the mammalian neocortex (if any) is comparable to avian HD and reptilian pallial thickening, both involved in intratelencephalic connections. As noted above, since the pallial thickening and apparently HD receive retinal input, they may be comparable to a lateral part of V1. In relation to this, V1 in rats contains medial and lateral subdivisions which differ in cyto-, myelo-, and chemoarchitecture (Palomero-Gallagher and Zilles, 2004). The medial subdivision represents a monocular subfield, whereas the lateral subdivision represents a binocular subfield. Nonplacental mammals, such as marsupials, also show similar medial–lateral V1 subdivisions in the neocortex, representing areas of either complex or simpler waveform processing (Sousa *et al.*, 1978). Thus, it is possible that such mediolateral subdivisions were present in the origin of mammals and, if so, the lateral V1 part may be comparable to HD/pallial thickening of birds and reptiles. Another possibility is that HD and/or the pallial thickening are not comparable to any part of V1, but rather to a more laterally located cortical or subcortical pallial area, such as the insular cortex (or part of it) or the claustrum (Striedter, 1997). The position of these structures at the lateral extreme of the neocortex, either abutting the lateral pallium or within it, resembles that of both HD and pallial thickening (Figure 2). In contrast to this possibility, the connectivity patterns of HD and pallial thickening are very different from those of the insular cortex or claustrum. For example, in contrast to HD and pallial thickening, neither the insular cortex nor the claustrum receive direct input from the dorsal lateral geniculate nucleus (Clascá *et al.*, 1997; Sefton *et al.*, 2004). More studies will be needed to resolve this issue. If the pallial thickening of reptiles were not homologous (as a field) to any part of V1, it would challenge the existence of a primary visual area in the dorsal pallium of the amniote common ancestor (since in lizards the geniculate projection only reaches the pallial thickening but not the dorsal cortex proper), opening new and important questions on neocortical evolution.

13.5 Functional Properties of the Visual and Somatosensory Areas of Neocortex and Sauropsidian Dorsal Pallium: Do Mammals, Birds, and Reptiles See and Feel the Same?

13.5.1 Visual Area: Retinotopy, Signal Types, Binocularity, and Perception

The mammalian V1 contains a detailed point-to-point retinal map, received through the retinogeniculocortical pathway, which subserves conscious

vision (Kahn *et al.*, 2000; Sefton *et al.*, 2004; Wässle, 2004). Neurons in V1 respond to orientation, direction, or color, and this information reaches the cortex through mostly segregated parallel pathways (Wässle, 2004). This information is then processed and combined (a process that involves higher-order areas), making possible animals' visual perception of the world. Visual signals are first detected by retinal photoreceptors, rods (involved in detection of low light levels), and cones (involved in detection of lights of different wavelengths; i.e., they are color-sensitive). The signals detected at the photoreceptor level are then processed and filtered through a complex retinal system involving several cell types (including horizontal, bipolar, amacrine, and ganglion cells), connected through specific circuitries (Lee, 2004; Wässle, 2004). At the end of this process, different types of retinal ganglion cells respond to orientation, direction, motion, or color, and this information is then transmitted to the brain through the retinofugal pathways (one of which is the retinogeniculate system). In primates, achromatic retinofugal signals mainly reach the visual cortex by way of the magnocellular layer of the geniculate nucleus, whereas chromatic retinofugal signals reach V1 mainly via either the koniocellular or the parvocellular geniculate cells, and each pathway mainly ends on a separate layer or sublayer in V1 (Chatterjee and Callaway, 2003; Lee, 2004).

In V1, orientation and direction signals represent a first step for analysis of form or movement, in which higher-order visual areas participate (Sincich and Horton, 2005; Saul *et al.*, 2005; Shmuel *et al.*, 2005; van Hooser *et al.*, 2005). Neurons responsive (or sensitive) to orientation or direction appear to be present in V1 of a large variety of mammals (including placental and marsupial species; Murphy and Berman, 1979; Parnavelas *et al.*, 1981; Crewther *et al.*, 1984; Orban *et al.*, 1986; Vidyasagar *et al.*, 1992; Ibbotson and Mark, 2003; Priebe and Ferster, 2005), and many mammals appear to have at least a second visual area (V2) involved in higher-order processing (Kaas, 2004; Sefton *et al.*, 2004), suggesting that some basic aspects of form and movement perception are common to all mammals. Nevertheless, in some mammals (such as marsupials) only a low percentage of V1 neurons respond to motion (Ibbotson and Mark, 2003), whereas other mammals possess multiple higher-order visual areas, one of which (V5/MT of primates) is specially involved in motion perception (Riecansky, 2004; Sincich *et al.*, 2005; Silvanto *et al.*, 2005). Thus, it appears that some mammals have a better visual perception of movement and form than others. Further, in mammals (such as primates, cats, and rats, as well as marsupials), some or many neurons of V1 are characterized by binocular convergence (depending on the degree of orbital convergence, which is maximal in primates), and are involved in perception of depth (stereoscopic vision) (Vidyasagar *et al.*, 1992; Barton, 2004; Grunewald and Skoumbourdis, 2004; Heesy, 2004; Menz and Freeman, 2004; Read, 2005). But again, some mammals show higher binocular convergence and have more visual cortical areas involved in its analysis, indicating that some species apparently have better depth perception than others. Nevertheless, in many mammals, several noncortical areas (including pretectum, superior colliculus, and other subcortical areas) are involved in motion processing (Ibbotson and Price, 2001; Price and Ibbotson, 2001; Sefton *et al.*, 2004). The superior colliculus appears to be involved in the spatial localization of biologically significant stimulus rather than its recognition (where it is rather than what it is) (Schneider, 1969), and can influence head/eye movements and guidance toward or away from a stimulus (reviewed by Sefton *et al.*, 2004). The visual cortex (with the participation of higher-order areas) appears to be involved in perception of both what the stimulus is (form and pattern discrimination) and where it is, among other aspects of visual perception.

Regarding color perception, the majority of mammals appear to have dichromatic vision, whereas – among placental mammals – only some primates have trichromatic vision. Among nonplacental mammals, it appears that some Australian marsupials may also have trichromatic vision. This depends on the pigment (opsin) variety found in retinal cone photoreceptors, and in the existence of color opponent systems. It appears that most mammals have two cone types: a majority of cones are sensitive to medium or long wavelengths (M/L-cones, sensitive to green or red), depending on the species; and a minority of cones are sensitive to short wavelengths (S-cones, sensitive to blue or ultraviolet (UV)), depending on the species (Peichl and Moutairou, 1998; Yokoyama and Radlwimmer, 1998, 2001; Shi and Yokoyama, 2003; Gouras and Ekesten, 2004). Among placental mammals, only some primates (including squirrel monkeys, New World monkeys, and humans) have a trichromatic color vision and their retina contains cones sensitive to green, red, or blue. Many marsupials also have M/L- and S-cones (Deeb *et al.*, 2003; Strachan *et al.*, 2004), and it seems that they were present in the retina of ancestral vertebrates well before the emergence of mammals (Shi and

Yokoyama, 2003). It has been suggested that the green-sensitive and red-sensitive cones present in mammals evolved from a single M/L cone present in the common ancestor well before the origin of mammals (Yokoyama and Radlwimmer, 1998). However, several species of Australian marsupials do have trichromatic retinas, with cones sensitive to short, medium, or long wavelengths (Arrese *et al.*, 2002, 2005), which appears to be due to retention from the ancestor (see below). It seems that the retina of ancestral placental mammals became dichromatic when these animals adopted nocturnality and some primates, subsequently, re-evolved trichromacy (Arrese *et al.*, 2002).

It seems that color perception involves a comparison of the relative activities of different cones by way of an opponent process, which starts in the retina and is conveyed to the visual cortex by parallel, anatomically segregated color-opponent systems (Dacey, 2000). In mammals having a dichromatic retina, only one opponent system exists, a blue–yellow system, in which signals from blue cones are opposed to signals from red or green cones. For trichromatic retinas (such as those of some primates), there are two color opponent systems, one for a red–green system, in which signals from red- and green-sensitive cones are opposed, and another one for a blue–yellow system, in which signals from blue cones are opposed to a combined signal from red and green cones (Dacey, 2000; Chatterjee and Callaway, 2003). Specific retinal ganglion cells exist for each color system. The blue–yellow information is conveyed to V1 through the koniocellular geniculate pathway, whereas the red–green information is conveyed by the parvocellular geniculate pathway (Lee, 2004). The information reaching V1 is later combined in higher-order visual areas. It is likely that ancestral placental mammals only had the blue–yellow system, and that the anatomical substrate for the red–green system evolved as a novelty in primates (Dacey, 2000; Lee, 2004).

In reptiles and birds, the retina contains cell types (including rod and cone photoreceptors, as well as horizontal, bipolar, amacrine, and ganglion cells) and circuitries in general similar to many of those present in mammals (Fernández *et al.*, 1994; Kittila and Granda, 1994; Ammermüller and Kolb, 1995; Haverkamp *et al.*, 1997, 1999; Luksch and Golz, 2003). Ganglion cells responsive to direction, motion, or color are found in the retina of both birds and reptiles, and cells responsive to orientation are also found in birds (Granda and Fulbrook, 1989; Guiloff and Kolb, 1994; Ammermüller *et al.*, 1995; Borg-Graham, 2001; Wilke *et al.*, 2001; Jones and Osorio, 2004). Retinal information is then conveyed to the dorsal pallium by way of a retinotopically organized retinogeniculodorsal pallial pathway (Bravo and Pettigrew, 1981; Miceli and Repérant, 1982; Ehrlich and Mark, 1984; Mulligan and Ulinski, 1990). This suggests that the dorsal pallium of birds and reptiles may be involved in some aspects of visual perception similar to those processed by V1 in mammals. Consistent with this, the visual hyperpallium of some birds (such as owls) contains neurons showing selectivity for orientation and movement direction (Pettigrew and Konishi, 1976), and has been shown to be involved in form discrimination, including some complex aspects such as subjective contour discrimination (Nieder and Wagner, 1999). In other birds (chicks or pigeons), the hyperpallium is involved in motion processing, far-field pattern discrimination, spatial discrimination acquisition, and in sun-compass associative learning (Gusel'nikov *et al.*, 1977; Leresche *et al.*, 1983; Britto *et al.*, 1990; Budzynski *et al.*, 2002; Watanabe, 2003; Budzynski and Bingman, 2004). Among birds, the complexity of visual processing by the hyperpallium appears to be higher in owls (which are frontal-eyed birds) than in other birds. In fact, the hyperpallium of owls shows a larger size, a more detailed retinotopic map, a much higher binocular convergence, and a more complex visual processing than that of lateral-eyed birds, such as pigeons (Pettigrew and Konishi, 1976; Nieder and Wagner, 1999, 2000, 2001; Liu and Pettigrew, 2003). Thus, the visual hyperpallium of owls is involved in depth perception and detection of visual illusions (subjective contours), exhibiting a functional complexity analogous to that of higher-order visual areas of highly visual mammals such as primates and cats (Nieder and Wagner, 1999, 2000, 2001; Liu and Pettigrew, 2003; van der Willigen *et al.*, 2003). In contrast, binocularity in pigeons is low (Martin and Young, 1983; McFadden and Wild, 1986; Holden and Low, 1989). Further, although the hyperpallium in pigeons is involved in motion perception and far-field discrimination, other brain areas, such as the optic tectum and the areas involved in the tectothalamo-DVR pathway, also play very important roles in motion processing or in other aspects of visual discrimination (Gusel'nikov *et al.*, 1977; Leresche *et al.*, 1983; Macko and Hodos, 1984; Britto *et al.*, 1990; Wang *et al.*, 1993; Laverghetta and Shimizu, 1999; Crowder *et al.*, 2004; Nguyen *et al.*, 2004). Among these areas, the thalamic nucleus rotundus and its DVR target play an important role in processing of ambient illumination, near-field discrimination, spatial-pattern vision, motion, and color (Wang *et al.*, 1993).

In reptiles, the visual dorsal cortex shows a coarse retinotopic map (Mulligan and Ulinski, 1990), and it appears involved in some aspects of visual processing, such as motion, discrimination acquisition, and spatial learning, but not in brightness discrimination (Reiner and Powers, 1983; Grisham and Powers, 1989, 1990; Prechtl, 1994; Prechtl *et al.*, 2000; Nenadic *et al.*, 2002). However, as in pigeons, other brain areas of reptiles, such as those involved in the tectothalamo-DVR pathway, play a more important role in brightness and pattern discrimination than the dorsal cortex (Morenkov and Pivovarov, 1975; Reiner and Powers, 1983).

Regarding color perception, the retina of birds and reptiles also supports color vision, but this appears to be more complex than in mammals. Thus, it appears that the retina of many diurnal birds and reptiles contains four types of cones, sentitive to red, green, blue, or UV or near-UV light (Ammermüller *et al.*, 1995; Bowmaker *et al.*, 1997; Kawamura *et al.*, 1999; Ventura *et al.*, 2001; Smith *et al.*, 2002). The cones of many diurnal birds and reptiles also contain colored oil droplets, which act as filters and apparently enhance color discrimination (Bowmaker *et al.*, 1997; Vorobyev, 2003). Parallel opponent retinal pathways have been shown in some species of reptiles, suggesting the existence of tetrachromatic color vision in these animals (Ammermüller *et al.*, 1995; Ventura *et al.*, 2001). In turtles, the opponent color systems described in the retina include a blue–yellow system, a red–green system, and a UV–blue system, among other possibilities (Ventura *et al.*, 2001). It is unclear whether all these systems are present in other reptiles or in birds. As noted above, the blue–yellow opponent system is apparently present in most mammals and may have been present in stem amniotes. However, the anatomical substrate of the red–green pathway of turtles is likely nonhomologous to that found in some primates. As noted above, birds and reptiles possess a retinotopically organized retinogeniculodorsal pallial pathway comparable (likely homologous) to the retinothalamo-V1 of mammals, suggesting that the avian and reptilian dorsal pallium may be involved in color vision processing. However, only a few aspects of color vision (if any) may be processed in the dorsal pallium of birds, and it appears that color vision in birds and possibly reptiles is mainly (if not only) processed by other brain areas and pathways, such as the tectothalamo (rotundal)-DVR pathway (Güntürkün, 1991; Chaves *et al.*, 1993; Wang *et al.*, 1993; Chaves and Hodos, 1997, 1998).

All of these data together indicate that, although the visual area of the reptilian dorsal cortex, avian hyperpallium, and mammalian V1 are involved in some similar basic aspects of visual perception, many complex functions shown by the visual hyperpallium of some birds and by V1 of highly visual mammals, such as depth perception (associated to binocularity) and subjective contour discrimination, among others, likely evolved independently. Consistent with this, the anatomical substrate for the binocularity is different in birds and mammals (Casini *et al.*, 1992; Medina and Reiner, 2000). Further, the role of V1 in color processing and the anatomical pathways related to it may have evolved only in mammals. Regarding motion, the dorsal pallial visual area of reptiles (at least turtles), birds, and mammals appears involved in its processing, and this may have characterized the dorsal pallial visual area of stem amniotes. All these data suggest that the retinogeniculodorsal pallial pathway found in birds and reptiles is mainly comparable to part of the magnocellular retinogeniculocortical pathway of mammals, but not to the parvocellular pathway (conveying mainly chromatic information of the red–green system) nor possibly the koniocellular pathway (conveying mainly chromatic information of the blue–yellow system).

In reptiles and many birds, the retinotectothalamo (rotundal)-DVR pathway is more developed than the retinogeniculodorsal pallial pathway, and appears to play an important role in some aspects of visual processing, such as motion, color, and pattern discrimination (perhaps important for knowing both what the stimulus is and where it is). This general pattern may have characterized stem amniotes. It appears that early mammals were nocturnal animals, which may explain why many extant mammals have dichromatic vision (instead of the tetrachromatic vision that characterizes many birds and reptiles). Perhaps this was accompanied by a regression in visual perception abilities and their anatomical substrate, and an improvement of other sensory systems, such as the somatosensory and the auditory systems. The evolution of new mammalian species living in diurnal niches was likely accompanied by the great development of the retinothalamodorsal pallial pathway, and by the development of more visual neocortical areas (Husband and Shimizu, 2001). An increase in size and complexity of the retinothalamodorsal pallial pathway also occurred in birds, but this was particularly important in some frontal-eyed birds (such as the owl). Did this involve the development of higher-order visual areas in the dorsal pallium of owls? As noted above, the visual hyperpallium of birds is involved in highly complex visual functions comparable to those carried out by higher-order

visual areas of the mammalian neocortex. However, physiological studies have not analyzed the existence of multiple visual areas in the hyperpallium of owls. A recent study has shown the existence of at least two somatosensory representations in the frontal hyperpallium of owls (Manger *et al.*, 2002), and it is likely that more than one visual representation exists in the large hyperpallium of owls.

Finally, regarding the question of whether mammals, birds, and reptiles see the same, it is clear that not all mammals have the same degree of depth, color, and/or form perception, and this is also true in birds. Regarding color vision, although many diurnal reptiles and birds appear to have tetrachromatic vision and most mammals have dichromatic vision, there are examples of color-blind or trichromatic animals within mammals. Further, a few mammals (such as mouse and rat) and many birds and reptiles detect UV light, whereas most mammals (including humans) do not. Thus, the question of whether mammals, birds, and reptiles see the same is nonsense since visual perception differs among mammals, among birds, and possibly among reptiles. Nevertheless, some basic aspects of visual perception appear to be similar between many amniotes. Of particular interest is the fact that some complex visual functions related to form and depth perception appear to be similar between frontal-eyed birds (such as owls) and some highly visual mammals such as cats and some primates. As noted above, the anatomical substrate for the complex visual processing by the dorsal pallium found in these animals likely evolved independently. Further, in birds and reptiles, many aspects of visual perception (including color perception) appear to be processed in the DVR (ventrolateral pallium), rather than the dorsal pallium.

13.5.2 Somatosensory Area: Somatotopy, Signal Types, Perception, and Multiple Maps

In mammals, S1 contains a somatotopically organized map of the whole body (contralateral side) (Tracey, 2004). The information received by S1 via the ventrobasal thalamic complex includes tactile (touch, pressure), vibration, and proprioceptive (postural) signals, as well as pain and temperature. The somatosensory information reaching the frontal hyperpallium in birds by way of DIVA is also somatotopically organized, and includes at least tactile (light touch and pressure signals) and vibration information, mostly from the contralateral body surface (Wild, 1987; Funke, 1989a). Based on the external cuneate (which receives proprioceptive information from extraocular and wing muscles; Wild, 1985; Hayman *et al.*, 1995) and spinal inputs to DIVA (Schneider and Necker, 1989; Wild, 1989), it is likely that the frontal hyperpallium also receives proprioceptive, pain, and temperature signals. However, in contrast to mammals, mainly the body (including the neck) appears to be represented in the frontal hyperpallium in several avian species (Wild, 1987, 1997), although some studies have also reported representation of the beak (Korzeniewska, 1987; discussed in Wild, 1989). In birds, it appears that the head somatosensory information is mostly represented in another pallial area, called nucleus basalis, located in the DVR (Berkhoudt *et al.*, 1981; Dubbeldam *et al.*, 1981; Wild *et al.*, 1997). The somatosensory information reaches this DVR nucleus by way of a direct, somatotopically organized projection from the principal sensory trigeminal nucleus (Dubbeldam *et al.*, 1981; Wild and Zeigler, 1996; Wild *et al.*, 2001). Further, in some birds, such as the budgerigar, the nucleus basalis of the DVR includes not only head but also body representation, and this appears to be more detailed than that in the hyperpallium (Wild *et al.*, 1997). Thus, it appears that birds possess two different systems for pallial somatosensory representation, which show different degrees of development depending on the species. As noted above, only the hyperpallial representation appears comparable to S1 of mammals. The frontal dorsal cortex of reptiles also appears to receive somatosensory information of the body (in lizards, turtles, and possibly crocodiles) and, at least in some lizards, also the head (Desfilis *et al.*, 1998). More studies are needed to know whether this pattern is common in other reptiles. It is unclear whether the somatosensory information reaching the frontal dorsal cortex in reptiles is or is not topographically organized. Although it seems likely that the primary somatosensory area observed in the dorsal pallium of extant birds, reptiles, and mammals evolved from a homologous area present in their common ancestor (stem amniotes), the scarcity of data in reptiles does not allow any suggestion on the specific features of this primitive area. In any case, this area was likely very small, and likely lacked many of the attributes (in terms of anatomical organization, connections, and functional complexity) found in S1 of mammals and in the frontal hyperpallium of birds. As noted above, the cytoarchitectural organization and intrinsic columnar circuitry shown by the neocortex and hyperpallium evolved independently. Since in birds there is a small overlap of the primary visual and somatosensory areas in the hyperpallium (Deng and Wang, 1992), it is possible that a partial

ovelap of sensory areas characterized the dorsal pallium of stem amniotes (Figure 3).

Of interest, the neocortex of extant mammals contains multiple somatosensory representations, many of which (including S1, a secondary somatosensory area or S2, and the parietal ventral area) appeared to be already present in the origin of mammals (Krubitzer, 1995; Kaas, 2004). This provides an idea of the importance and high quality of somatosensory perception in these animals, and this great development may be related to the fact that ancestral mammals were nocturnal animals, primarily relying on senses other than vision. Further, somatosensory representation is even more complex in some mammals, such as primates, in which S1 contains four subdivisions (areas 3a, 3b, 1, and 2), each one showing a complete body representation (Tracey, 2004). In other mammals, including rodents, S1 has a single body representation, possibly comparable to area 3b of primates (Northcutt and Kaas, 1995). In birds having a large hyperpallium (such as the owl), two separate somatosensory representations of the claw have been observed, each showing a detailed somatotopic organization (Manger *et al.*, 2002). Thus, it appears that at least some birds have a more complex somatosensory representation in the hyperpallium, which may mean that they have a more elaborated analysis of this information and a more sophisticated somatosensory perception. Since the somatosensory area of the dorsal cortex of reptiles is apparently very small, it seems unlikely that multiple somatosensory representations were present in the dorsal pallium of stem amniotes. This means that the additional somatosensory hyperpallial area found in owls likely evolved independently and cannot be compared to any of the multiple S1 areas found in primates, to S2, nor to other somatosensory areas of mammalian neocortex (Manger *et al.*, 2002). Another interesting aspect of somatosensory representation in the neocortex of mammals is its activity-dependent plasticity, which is important for behavior modification and adaptation as a result of sensory experience (Kaas, 1995; Tracey, 2004). It appears that plasticity also characterizes the somatosensory hyperpallial area of owls (Manger *et al.*, 2002).

Regarding the question of whether mammals, birds, and reptiles feel the same, based on the number of pallial representations and variety of somatosensory receptors found in mammals (Kaas, 2004; Tracey, 2004), it appears that in general mammals have a much better somatosensory perception than reptiles and most birds. But again, it appears that somatosensory perception differs among mammals, as well as among birds and maybe among reptiles. One of the reasons is that the number of somatosensory representations and higher-order areas varies between species (Kaas, 2004). Another reason may be the existence of differences in peripheral receptors (in terms of quality, quantity, and/or location). For example, the complex receptor type found in owl claws (Manger *et al.*, 2002) may not be present in the claws of other birds, or may be present at a low number/area ratio. Further, different parts of the body and head have a different representation (in terms of relative size) in the neocortex/dorsal pallium in different species, which depends on their specific behavior. For example, the S1 of humans has a very large (or relatively large) representation of digits (which is related to the great tactile discrimination and exploratory and manipulatory use of our fingers), whereas in S1 of mouse and rat, the digits are not so well represented but the area related to the whiskers (barrel field area) is relatively large (which relates to the great importance of vibrissae in exploratory behavior and texture discrimination in rodents; reviewed by Waite, 2004). This rule also appears to be true for somatosensory areas in pallial regions other than the dorsal pallium. An example of this is found in the nucleus basalis of the budgerigar, which shows a larger size and more extensive representation of areas such as the beak, highly used by these animals (Wild *et al.*, 1997). Similarly, the claw of barn owl, used for perching and grasping prey and containing an elaborated tactile sensory receptor, likely has a larger representation in the frontal hyperpallium of this animal (including two areas, as noted above; Manger *et al.*, 2002) than that of the pigeon or the canary.

13.6 Conclusions

The neocortex contains specific sensory, associative, and motor areas that allow mammals to obtain a detailed map of the world and to adapt their behavior to it. Available data suggest that at least two such areas, the primary visual area and the primary somatosensory area, are also present in the dorsal pallium of birds and reptiles, and likely evolved from similar areas found in stem amniotes. However, these dorsal pallial areas present in the common ancestor likely had a very simple cytoarchitecture (possibly including a rudimentary three-layered structure plus at least two mediolateral subdivisions), and possessed fewer cell types and connections than those found in the mammalian neocortex and avian hyperpallium. For example, the complex six-layered organization of neocortex and the four mediolateral subdivisions of hyperpallium evolved independently in mammals or

birds. Further, the columnar functional organization of neocortex and the columnar-like organization of hyperpallium also evolved independently. In addition, these primitive areas of stem amniotes were likely involved in few aspects of visual or somatosensory perception. The role of the visual area in complex aspects of form and pattern discrimination or in depth perception (associated to binocularity) likely evolved independently in mammals and some birds, and its role in color perception (and the anatomical substrate related to it) apparently evolved only in the mammalian radiation. Finally, available data suggest that the dorsal pallium of stem amniotes may have lacked a true somatomotor area, and this evolved independently in birds and mammals (see History of Ideas on Brain Evolution, Phylogenetic Character Reconstruction, Evolution of the Nervous System in Reptiles, The Evolution of Vertebrate Eyes, How Can Fossils Tell us About the Evolution of the Neocortex?, The Origin of Neocortex: Lessons from Comparative Embryology, Reconstructing the Organization of the Forebrain of the First Mammals, The Evolution of Motor Cortex and Motor Systems).

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14 The Evolution of Vocal Learning Systems in Birds and Humans

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Glossary

anterior vocal pathway Pathway in the anterior part of the forebrain that includes a pallial (cortical) region, striatal region, and thalamic region and that controls vocal learning and complex aspects of vocal production.

arcopallium Means arched pallium; a subdivision of the avian telencephalon with a border that is shaped like an arch along other pallial regions. It is a major output region of the telencephalon.

auditory learning The ability to make novel associations with sounds heard.

hyperpallium Means upper pallium; one of the dorsal most subdivisions of the avian telencephalon. It has input, output, and intratelencephalic functions.

mesopallium Means middle pallium; a subdivision of the avian telencephalon that is located in between the nidopallium ventral to it and the hyperpallium dorsal to it. It is a major intratelencephalic region.

nidopallium Means nested pallium; a subdivision of the avian telencephalon that is shaped like a nest for the pallium regions dorsal to it and sits on top of the subpallium, or basal ganglia ventral to it. It has both input and intratelencephalic functions.

pallium Means mantle or covering. The pallium of vertebrates is the part of the embryonic brain that gives rise to all cortical regions in mammals and pallial named areas in birds.

posterior vocal pathway Pathway in the mid to posterior part of the forebrain, that includes pallial (cortical) regions, midbrain and medulla regions, and that controls production of learned vocalizations.

vocal learning The ability to modify acoustic and/or syntactic structure of sounds produced, including imitation and improvisation.

14.1 What is Vocal Learning

Vocal learning is the ability to modify acoustic and/or syntactic structure of sounds produced, including imitation and improvisation. It is distinct from auditory learning, which is the ability to make associations with sounds heard. Most, if not all, vertebrates are capable of auditory learning (see The Evolution of the Primate and Human Auditory System, Shared and Convergent Features of the Auditory System of Vertebrates), but few are capable of vocal learning. The latter has been found to date only in four distantly related groups of mammals (humans, bats, cetaceans, and recently elephants) and three distantly related groups of birds (parrots, hummingbirds, and songbirds) (Figure 1) (Nottebohm, 1972; Jarvis *et al.*, 2000; Poole *et al.*, 2005). Vocal learning is the behavioral substrate for spoken human language. An example helps in understanding the distinction between vocal learning and auditory learning. A dog can learn the meaning of the human words 'sit' (in English), 'sientese' (in Spanish), or 'osuwali' (in Japanese) or of a sentence ('come here boy'). Dogs are not born with this knowledge of human words or syntax. They acquire it through auditory learning. However, a dog cannot imitate the sounds 'sit', 'sientese', or 'osuwali'. A human, parrots, and some songbirds can. This is vocal learning, and though it depends upon auditory learning (Konishi, 1965), it is distinct from it.

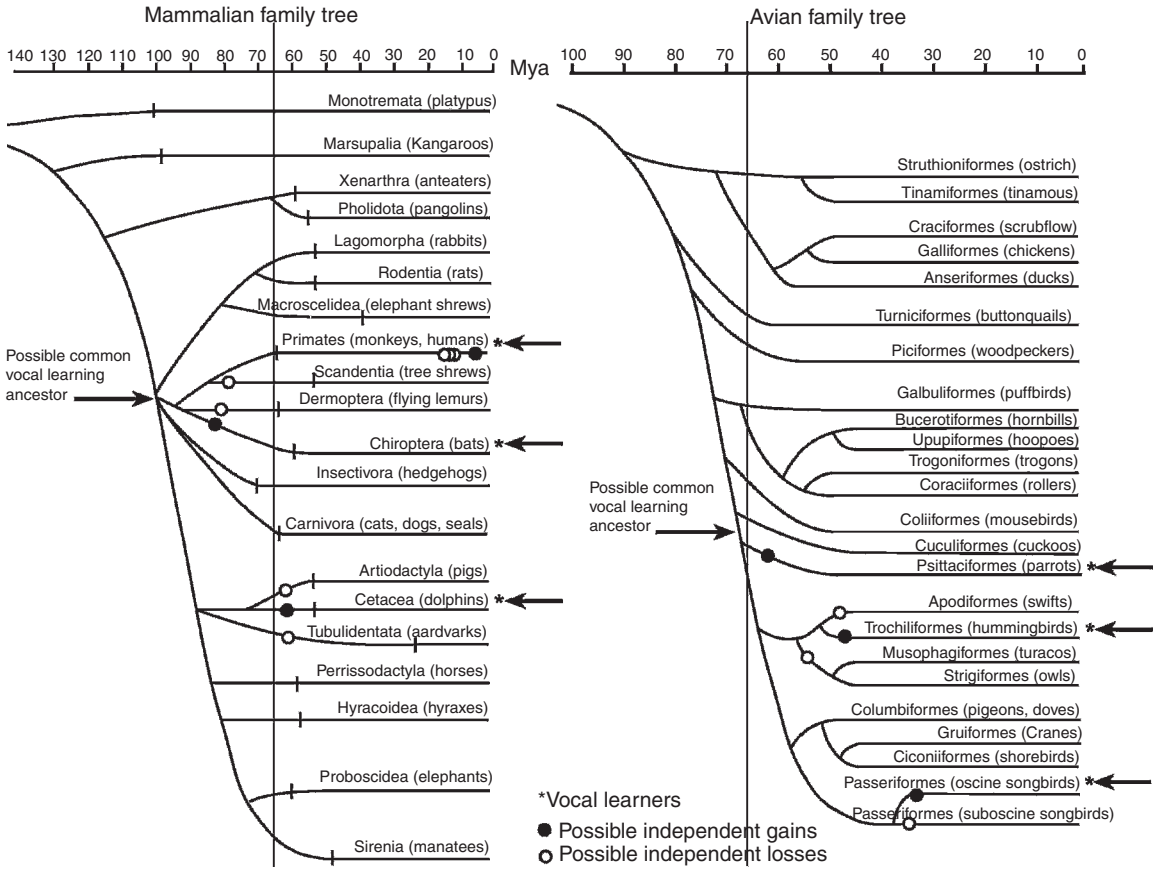

Figure 1 Family trees of living mammalian and avian orders. The mammalian tree is derived from the morphological analysis by Novacek (1992, 2001); horizontal lines indicate extant of geologic evidence from fossils. The avian tree was derived from DNA DNA hybridization analysis by Sibley and Ahlquist (1990, p. 838). The Latin name of each order is given along with examples of common species. Passeriformes are divided into its two suborders, suboscine and oscine songbirds. The vertical line down the trees indicates the cretaceous tertiary boundary; Mya millions of years ago. Open and closed circles show the minimal ancestral nodes where vocal learning could have either evolved independently or been lost independently. Independent losses would have at least required one common vocal learning ancestor, located by the right facing arrows. Within primates, there would have to be least seven independent losses (tree shrews, prosimians, New and Old World monkeys, apes, and chimps) followed by the regain of vocal learning in humans (assuming that all nonhuman primates are vocal nonlearners). The trees are not meant to present the final dogma of mammalian and avian evolution, as there are many differences of opinion among scientists. Reproduced from Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. *Ann. NY Acad. Sci.* 1016, 749 777, with permission.

Most vocal learners only imitate sounds of their own species, and not all vocal learning species have vocal abilities to the same degree. Humans are the most prolific vocal learners, as they learn to produce a seemingly infinite number of combinations of learned vocalizations. Not as prolific are some parrots, corvid songbirds, starlings, and mockingbirds, where they produce hundreds if not thousands of calls and/or learned warble/song combinations. Finally, less prolific are very stereotyped songbirds and hummingbirds, where they produce only one distinct song type with little variation (Catchpole and Slater, 1995; Farabaugh and Dooling, 1996; Ferreira *et al.*, 2006). Each of the vocal learning avian and mammalian groups has close vocal nonlearning relatives (Figure 1). Thus, it has been argued that vocal learning has evolved independently of a common ancestor in the three vocal learning bird groups (Nottebohm, 1972) and presumably in the four vocal learning mammalian groups (Jarvis, 2004). The question thus arises, is there something special about the brains of these animals that can imitate sounds.

14.2 Consensus Brain Systems of Vocal Learners

There is something special about the brain systems of vocal learners. Only vocal learners, songbirds, parrots, hummingbirds, and humans, have brain regions in their cerebrums (or telencephalon) that control vocal behavior (Jurgens, 2002; Jarvis *et al.*, 2000). Vocal control brain regions have not yet

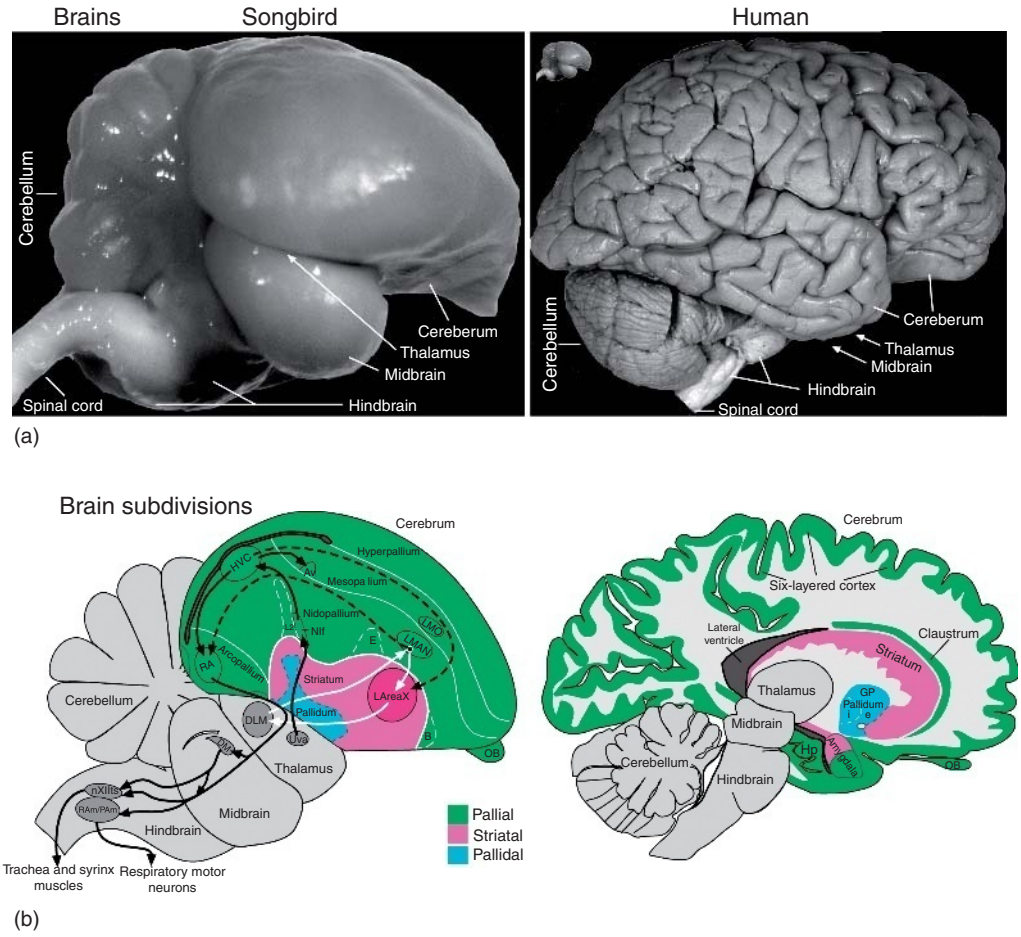

Figure 2 Avian and mammalian brain relationships. a, Side view of a songbird (zebra finch) and human brain to represent avian and mammalian species. In this view, the songbird cerebrum covers the thalamus and the human cerebrum covers the thalamus and midbrain. Inset (left) next to the human brain is the zebra finch brain to the same scale. b, Sagittal view of brain subdivisions according to the modern understanding of avian and mammalian brain relationships (Reiner *et al.*, 2004b; Jarvis *et al.*, 2005). Solid white lines are lamina (cell-sparse zones separating brain subdivisions). Large white areas in the human cerebrum are axon pathways called white matter. Dashed white lines separate primary sensory neuron populations from adjacent regions. Abbreviations in Table 1. Human brain image in (a), reproduced from, courtesy of John W. Sundsten, Digital Anatomist Project, Dept. of Biological Structure, University of Washington, with permission. b, Reprinted by permission from Macmillan Publishers Ltd: *Nat. Rev. Neurosci.* (Jarvis, E. D., Gunturkun, O., Bruce, L., *et al.* 2005. Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* 6, 151 159.), copyright (2005).

been investigated in cetaceans, bats, and elephants. Nonvocal learners, including nonhuman primates and chickens, only have midbrain and medulla regions that control innate vocalizations (Wild, 1997). Using this knowledge, it has been possible to generate a consensus vocal pathway, much like generating a consensus DNA sequence from comparing comparable genes of different species, by comparing vocal brain regions of different vocal learning and vocal nonlearning species. This comparison is facilitated by a recent revision to the nomenclature and understanding of avian brain organization relative to mammals and other vertebrates (Reiner *et al.*, 2004b; Jarvis *et al.*, 2005). Like mammals, birds have pallidal, striatal, and pallial subdivisions in their cerebrums. However, the pallial subdivision in mammals is layered in its cellular organization, whereas in birds it is nuclear (Figure 2). Even with this major difference, connectivity and other functional properties are similar. Below, the consensus brain regions, connectivity, and function studies of vocal learning species are described.

14.2.1 Brain Regions and Connectivity

Remarkably, all three vocal learning bird groups have seven comparable cerebral vocal brain nuclei: four posterior forebrain nuclei and three anterior forebrain nuclei (Figures 3a–3c; abbreviations in Table 1) (Jarvis *et al.*, 2000). These brain nuclei have been given different names in each bird group because of the possibility that each evolved their

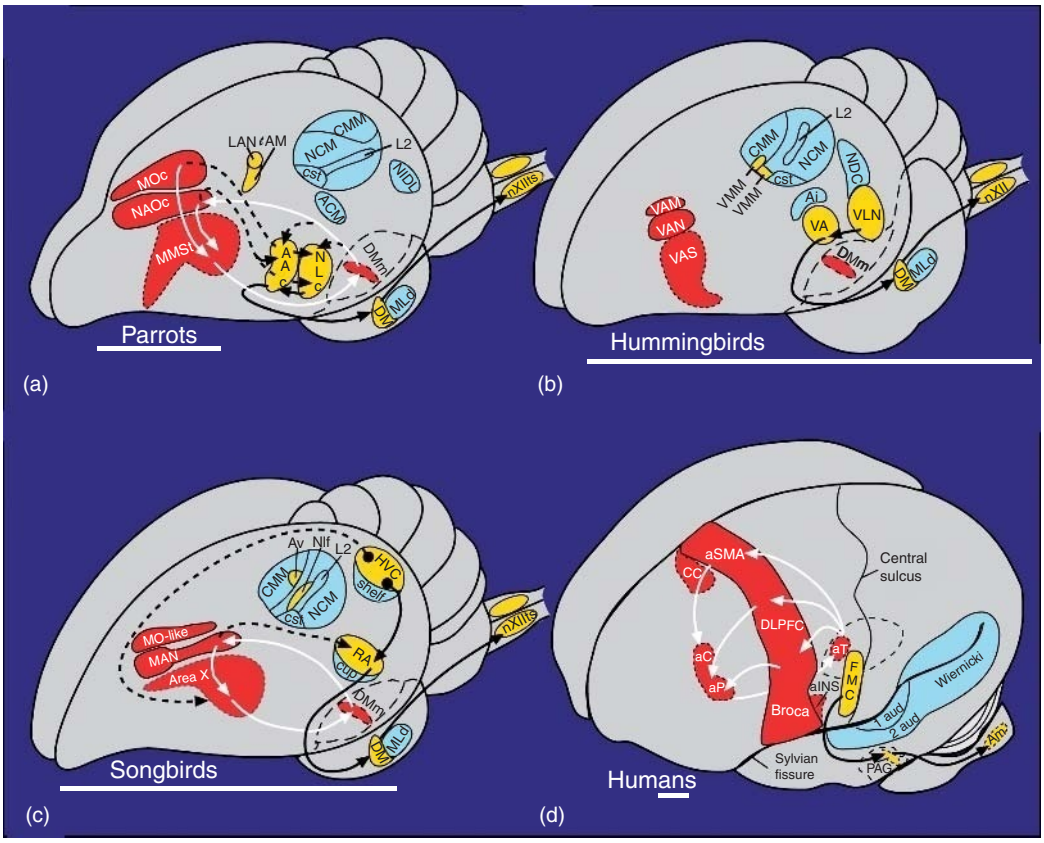

Figure 3 Proposed comparable vocal and auditory brain areas among vocal learning birds (a c) and humans (d). Left hemispheres are shown, as this is the dominant side for language in humans and for song in some songbirds. Yellow regions and black arrows indicate proposed posterior vocal pathways; red regions and white arrows indicate proposed anterior vocal pathways; dashed lines indicate connections between the two vocal pathways; blue indicates auditory regions. For simplification, not all connections are shown. The globus pallidus in the human brain, also not shown, is presumably part of the anterior pathway as in nonvocal pathways of mammals. Basal ganglia, thalamic, and midbrain (for the human brain) regions are drawn with dashed-line boundaries to indicate that they are deeper in the brain relative to the anatomical structures above them. The anatomical boundaries drawn for the proposed human brain regions involved in vocal and auditory processing should be interpreted conservatively and for heuristic purposes only. Human brain lesions and brain imaging studies do not allow one to determine functional anatomical boundaries with high resolution. Scale bar: ~7 mm. Abbreviations are in Table 1. Modified from Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. *Ann. NY Acad. Sci.* 1016, 749 777, with permission.

vocal nuclei independently of a common ancestor with such nuclei (Striedter, 1994; Jarvis *et al.*, 2000). In all three bird groups, the posterior nuclei form a posterior vocal pathway that projects from a nidopallial vocal nucleus (HVC, NLC, VLN) to the arcopallial vocal nucleus (RA, AAC dorsal part, VA), to the midbrain (DM) and medulla (nXIIts) vocal motor neurons (Figures 3a–3c, black arrows) (Striedter, 1994; Durand *et al.*, 1997; Vates *et al.*, 1997; Gahr, 2000); nXIIts projects to the muscles of the syrinx, the avian vocal organ. Vocal nonlearning birds have DM and nXIIts for production of innate vocalizations, but they appear not to have projections to these nuclei from the arcopallium (Wild *et al.*, 1997). The anterior nuclei (connectivity examined only in songbirds and parrots) are part of an anterior vocal pathway loop, where a pallial vocal nucleus (MAN, NAO) projects to the striatal vocal nucleus (area X, MMSt), the striatal vocal nucleus to a nucleus in DLM of the dorsal thalamus (DLM, DMM), and the dorsal thalamus back to the pallial vocal nucleus (MAN, NAO) (Figures 3a and 3c, white arrows) (Durand *et al.*, 1997; Vates *et al.*, 1997). The parrot pallial MO vocal nucleus also projects to the striatal vocal nucleus (MMSt) (Durand *et al.*, 1997). Connectivity of the songbird MO analogue has not yet been determined.

The major differences among vocal learning birds are in the connections between the posterior and anterior vocal pathways (Jarvis and Mello, 2000). In songbirds, the posterior pathway sends input to the anterior pathway via HVC to area X; the anterior pathway sends output to the posterior pathway via lateral MAN (LMAN) to RA and medial MAN (mMAN) to HVC (Figures 3c and 4a). In contrast,

Table 1 Some of the abbreviations used in this article

AAC	Central nucleus of the anterior arcopallium
AACd	Central nucleus of the anterior arcopallium, dorsal part
AACv	Central nucleus of the anterior arcopallium, ventral part
aCC	Anterior cingulate cortex
aCd	Anterior caudate
ACM	Caudal medial arcopallium
aDLPFC	Anterior dorsal lateral prefrontal cortex
Ai	Intermediate arcopallium
aINS	Anterior insula cortex
Am	Nucleus ambiguous
aP	Anterior putamen
area X	Area X of the striatum
aST	Anterior striatum
aT	Anterior thalamus
Av	Avalanch
CM	Caudal mesopallium
CSt	Caudal striatum
DLM	Medial nucleus of dorsolateral thalamus
DM	Dorsal medial nucleus of the midbrain
DMM	Magnocellular nucleus of the dorsomedial thalamus
FMC	Face motor cortex
HVC	A letter based name
L2	Field L2
MAN	Magnocellular nucleus of anterior nidopallium
MLd	Mesencephalic lateral dorsal nucleus
MMSt	Magnocellular nucleus of the anterior striatum
MOc	Oval nucleus of the mesopallium complex
NAOc	Oval nucleus of the anterior nidopallium complex
NCM	Caudal medial nidopallium
NDC	Caudal dorsal nidopallium
NIDL	Intermediate dorsal lateral nidopallium
NIf	Interfacial nucleus of the nidopallium
NLC	Central nucleus of the lateral nidopallium
nXIIts	Tracheosyringeal subdivision of the hypoglossal nucleus
Ov	Nucleus oviodalis
PAG	Periaqueductal grey
pre-SMA	Presupplementary motor area
RA	Robust nucleus of the arcopallium
St	Striatum
Uva	Nucleus uvaeformis
VA/VL	Ventral anterior/ventral lateral nuclei of the mammalian thalamus.
VAM	Vocal nucleus of the anterior mesopallium
VAN	Vocal nucleus of the anterior nidopallium
VAS	Vocal nucleus of the anterior striatum
VA	Vocal nucleus of the arcopallium
VLN	Vocal nucleus of the lateral nidopallium
VMM	Vocal nucleus of the medial mesopallium
VMN	Vocal nucleus of the medial nidopallium

in parrots, the posterior pathway sends input into the anterior pathway via ventral AAC (AACv, parallel of songbird RA) to NAO (parallel of songbird MAN) and MO; the anterior pathway sends output to the posterior pathway via NAO to NLC (parallel of songbird HVC) and AAC (Figures 3a and 4b) (Durand *et al.*, 1997).

In humans, imaging and lesion studies have revealed they have cortical and striatal regions that are active and necessary for learning and production of language (reviewed in Jarvis, 2004). These include what Jarvis has proposed is a lateral-to-medial strip of premotor cortex – from the anterior insula (aINS), the Brocas area, the anterior dorsal lateral prefrontal cortex (aDLPFC), the presupplementary motor area (pre-SMA), and the anterior cingulate (aCC) – below this level of cortex – an anterior region of the striatum and a posterior region of cortex – the face motor cortex (Figure 3d), as well as anterior parts of the thalamus. To date, these areas have not been found to be required for vocal behavior in vocal nonlearning mammals, including nonhuman primates. However, the anterior cingulate is required for voluntary control of when to vocalize, but not of the acoustic structure of vocalizations in vocal nonlearning mammals (Jurgens, 2002).

Ethical and practical issues prevent connectivity tract-tracing experiments in humans and thus the connectivity of vocal learning pathways is not known for any mammal. However, studies have been performed on the cerebrums of nonvocal learning mammals. Therefore, it is possible to make connectivity comparisons between vocal learning pathways in vocal learning birds with nonvocal pathways in vocal nonlearning mammals. In this regard, the avian posterior vocal pathways are similar in connectivity to mammalian motor corticospinal pathways (Figure 4). Specifically, the projecting neurons of songbird RA and parrot AACd are similar to pyramidal tract (PT) neurons of lower layer 5 of mammalian motor cortex (Matsumura and Kubota, 1979; Glickstein *et al.*, 1985; Karten and Shimizu, 1989; Keizer and Kuypers, 1989; Reiner *et al.*, 2003). The latter send long axonal projections out of the cerebrum through pyramidal tracts to synapse onto brainstem and spinal cord premotor or α-motor neurons that control muscle contraction and relaxation. The projection neurons of parrot NLC and the RA-projecting neurons of songbird HVC are similar to layer 2 and 3 neurons of mammalian cortex, which send intrapallial projections to layer 5 (Figure 4) (Aroniadou and Keller, 1993; Capaday *et al.*, 1998). Mammalian parallels to songbird NIf and Av are less clear.

The only connectivity determined among cerebral vocal brain areas of humans (Kuypers, 1958a) is the finding of face motor cortex projection to nucleus ambiguous (Am) of the medulla; Am projects to the muscles of the larynx, the main mammalian vocal organ (Zhang *et al.*, 1995; Jurgens, 1998) and is thus the mammalian parallel of avian nXIIts. This connectivity from face motor cortex in humans was

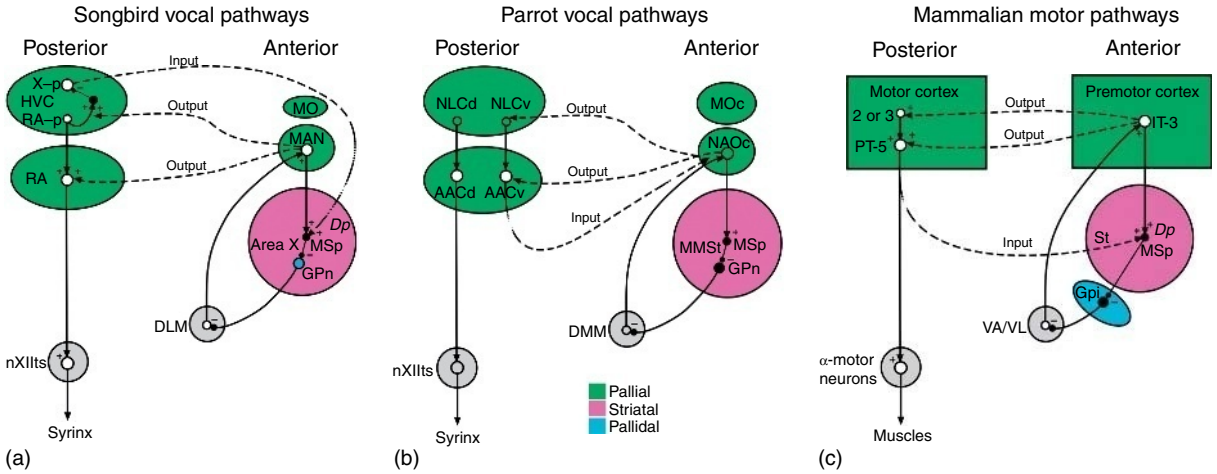

Figure 4 Comparative and simplified connectivity of posterior and anterior vocal motor pathways in (a) songbirds and (b) parrots and motor pathways in (c) mammals. Dashed lines: connections between anterior and posterior pathways; inputs and outputs are labeled relative to anterior pathways. Output from songbird MAN to HVC and RA are not from the same neurons; medial MAN (mMAN) neurons project to HVC, lateral MAN (LMAN) neurons project to RA. ○: excitatory neurons; •: inhibitory neurons; +: excitatory glutamate neurotransmitter release; : inhibitory GABA release. MSp, medium spiny neuron; GPn, globus pallidus-like neuron in songbird area X and parrot MMSt. Only the direct pathway through the mammalian basal ganglia (St to GPi) is shown, as this is the one most similar to area X connectivity (MSp to GPn) (Reiner *et al.*, 2004a). X-p, X-projecting neuron of HVC; RA-p, RA-projecting neuron of HVC; PT-5, pyramidal tract neuron of motor cortex layer 5; IT-3, intratelencephalic projecting neuron of layer 3. Connections that need validation for this model to be correct are whether collaterals of the same neurons of mMAN project to mAreaX and to HVC, as opposed to different neurons, whether input from HVC into area X is onto the area X MSp neurons, whether the microcircuitry in parrot MMSt is the same as in songbirds, whether the collaterals of single IT-3 neurons of mammal cortex send branches to both layers 3 and 5 of motor cortex or just to one layer per IT-3 neuron. Abbreviations are in Table 1. Modified from Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. *Ann. NY Acad. Sci.* 1016, 749 777, with permission.

determined using silver staining of degenerated axons in patients who had had vascular strokes to brain areas that included but were not limited to face motor cortex (Kuypers, 1958a). Kuypers (1958b) reproduced similar lesions in macaque monkeys and chimpanzees and found that their face motor cortex projects minimally, if at all, to Am, but it does project massively to the hypoglossal nucleus and to all other brainstem cranial motor nuclei as found in humans. The hypoglossal nucleus in mammals and the nontracheosyringeal part of nXII in birds controls muscles of the tongue (Wild, 1997). In this manner, the pallial nuclei of the songbird and parrot posterior vocal pathways combined are more similar to the human face motor cortex than to any other part of the human pallium.

The avian anterior vocal pathways are similar in connectivity to mammalian corticobasal ganglia-thalamic-cortical loops (Figure 4) (Bottjer and Johnson, 1997; Durand *et al.*, 1997; Jarvis *et al.*, 1998; Perkel and Farries, 2000). Specifically, the projection neurons of songbird MAN and parrot NAO (Vates and Nottebohm, 1995; Foster *et al.*, 1997; Durand *et al.*, 1997) are similar to intratelencephalic (IT) neurons of layer 3 and upper layer 5 of mammalian premotor cortex, which send two collateral projections, one to medium spiny neurons of the striatum ventral to it and the other to other cortical regions, including motor cortex (Figure 4) (Avendano *et al.*, 1992; Reiner *et al.*, 2003). Unlike mammals, the spiny neurons in both songbird area X, and presumably parrot MMSt, project to pallidal-like cells within area X and MMSt instead of to a separate structure consisting only of pallidal cells (Durand *et al.*, 1997; Perkel and Farries, 2000; Reiner *et al.*, 2004a). This striatal–pallidal cell intermingling may be a general trait of the anterior avian striatum (Farries *et al.*, 2005). The projection of the pallidal-like cells of songbird area X and parrot MMSt are similar to the motor pallidal projection neurons of the internal globus pallidus (GPi) of mammals, which project to the ventral lateral (VL) and ventral anterior (VA) nuclei of the dorsal thalamus (Figure 4) (Alexander *et al.*, 1986). Like songbird DLM and parrot DMM projections to LMAN and NAO, mammalian VL/VA projects back to layer 3 neurons of the same premotor areas, closing parallel loops (Jacobson and Trojanowski, 1975; Alexander *et al.*, 1986; Luo *et al.*, 2001).

Because connections between the posterior and anterior vocal pathways differ between songbirds and parrots, comparisons between them and mammals will also differ. In mammals, the PT-layer 5 neurons of motor cortex have axon collaterals, where one projects into the striatum and the other

projects to the medulla and spinal cord (Figure 4c) (Alexander and Crutcher, 1990; Reiner *et al.*, 2003). This is different from the songbird where a specific cell type of HVC, called X-projecting neuron, projects to the striatum separately from neurons of RA of the arcopallium that projects to the medulla. This is also different from the parrot, where AAC of the arcopallium has two anatomically separate neuron populations, AACd that projects to the medulla and AACv that projects to anterior pallial vocal nuclei NAO and MO (Durand *et al.*, 1997). Output of mammalian anterior pathways are proposed to be the collaterals of the IT–layer 3 and IT–upper layer 5 neurons that project to other cortical regions (Figure 4c) (Reiner *et al.*, 2003; Jarvis, 2004).

Taken together, the above analysis suggests that there are gross similarities between the connectivity of the consensus bird brain system for learned vocalizing and the nonvocal motor pathways (a posterior-like pathway) and cortical-basal-ganglia-thalamic-cortical loops (an anterior-like pathway) of mammals (Figures 4a–4c). Differences in connectivity between birds and mammals appear to be in the details, particularly with the pallidal cell types within the avian striatum and with connectivity between posterior and anterior pathways. Functions of these brain regions are now compared from lesion studies.

14.2.2 Brain Lesions

There are some gross similarities in behavioral deficits following lesions in specific brain areas of vocal learning birds (experimentally placed) and of humans (due to stroke or trauma). Lesions to songbird HVC and RA (Nottebohm *et al.*, 1976; Simpson and Vicario, 1990), on the left side in canaries, cause deficits similar to those found after damage to left human face motor cortex, this being muteness for learned vocalizations, i.e., for speech (Valenstein, 1975; Jurgens *et al.*, 1982; Jurgens, 2002). Innate sounds, such as crying and screaming, can still be produced. When the lesions are unilateral, both birds and patients often recover some vocal behavior, because the opposite hemisphere appears to take over some function; likewise, recovery is better when the canary is a juvenile or the patient a child (Nottebohm, 1977; Rey *et al.*, 1988; Hertz-Pannier *et al.*, 2002). Lesions to parrot NLC cause deficits in producing the correct acoustic structure of learned vocalizations, particularly for learned speech (Lavenex, 2000). The symptoms are similar to that of dysarthria in humans after recovery from damage to the face motor cortex. Lesions to the face motor cortex in chimpanzees and other nonhuman primates do not affect their ability to produce vocalizations (Kuypers, 1958b; Jurgens *et al.*, 1982; Kirzinger and Jurgens, 1982). Lesions to avian nXIIts and DM and mammalian Am and PAG result in muteness in both vocal learners and nonlearners (Brown, 1965; Seller, 1981; Nottebohm *et al.*, 1976; Jurgens, 1994, 1998; Esposito *et al.*, 1999). One difference is that lesions to songbird NIf or parrot LAN of the posterior pathway do not prevent production of learned vocalizations or cause dysarthric-like vocalizations, but lead to production of more varied syntax or impaired vocal imitation (Hosino and Okanoya, 2000; Plummer and Striedter, 2002).

Lesions to songbird MAN (Nottebohm *et al.*, 1990; Scharff and Nottebohm, 1991; Foster and Bottjer, 2001) cause deficits that are most similar to those found after damage to anterior parts of the human premotor cortex, this being disruption of imitation and/or inducing sequencing problems. In birds and humans, such lesions do not prevent the ability to produce learned song or speech. In humans, these deficits are called verbal aphasias and verbal amusias (Benson and Ardila, 1996). Damage to the left side often leads to verbal aphasias, whereas damage to the right can lead to verbal amusias (Berman, 1981). The deficits in humans, however, are more complex. Specifically, lesions to songbird LMAN (Bottjer *et al.*, 1984; Scharff and Nottebohm, 1991; Kao *et al.*, 2005) and lesions to the human insula and the Brocas area (Mohr, 1976; Dronkers, 1996; Benson and Ardila, 1996) lead to poor imitation with sparing or even inducing more stereotyped song or speech. However, in addition, lesions to the Brocas area and/or DLPFC (Benson and Ardila, 1996) lead to poor syntax production in construction of phonemes into words and words into sentences. Lesions to DLPFC also result in uncontrolled echolalia imitation, whereas lesions to pre-SMA and anterior cingulate result in spontaneous speech arrest, lack of spontaneous speech, and/or loss of emotional tone in speech, but with imitation preserved (Nielsen and Jacobs, 1951; Barris *et al.*, 1953; Rubens, 1975; Valenstein, 1975; Jonas, 1981). Lesions to songbird mMAN lead to a decreased ability in vocal learning and some disruption of syntax (Foster and Bottjer, 2001), as do lesions to the Brocas area.

Lesions to songbird area X and to the human anterior striatum does not prevent the ability to produce song or speech, but does result in disruption of vocal learning and disruption of some syntax in birds (Scharff and Nottebohm, 1991; Sohrabji *et al.*, 1990; Kobayashi *et al.*, 2001) or verbal aphasias and amusias in humans (Mohr, 1976; Bechtereva

et al., 1979; Leicester, 1980; Damasio *et al.*, 1982; Alexander *et al.*, 1987; Speedie *et al.*, 1993; Cummings, 1993; Lieberman, 2000). Specifically, songbirds do not crystallize onto correct syllable structure and syntax heard, and as adults they can stutter (Scharff and Nottebohm, 1991; Sohrabji *et al.*, 1990; Kobayashi *et al.*, 2001). Humans can have a combination of symptoms (Mohr, 1976) perhaps because, as in nonhuman mammals, large cortical areas send projections that converge onto relatively smaller striatal areas (Beiser *et al.*, 1997). Not many cases have been reported of lesions to the human globus pallidus leading to aphasias (Strub, 1989), but the fact that this can occur suggests some link with a striatal vocal area in humans.

Similar to a preliminary report on songbird DLM (Halsema and Bottjer, 1991), damage to anterior portions of the human thalamus (VA, VL, and A) leads to verbal aphasias (Graff-Radford *et al.*, 1985). In humans, thalamic lesions can lead to temporary muteness followed by aphasia deficits that are sometimes greater than after lesions to the anterior striatum or premotor cortical areas. This greater deficit may occur perhaps because there is further convergence of inputs from the striatum into the thalamus (Beiser *et al.*, 1997). However, the interpretation of thalamic lesions in humans is controversial (Benson and Ardila, 1996), perhaps because of small but important differences in lesion locations between patients among studies. The thalamus concentrates many functions into adjacent small nuclei, and thus, a relatively small variance in the location of a lesion may lead to a large difference in the brain function affected.

The lesions in birds and in humans can affect more than one modality. For example, lesions to LMAN or HVC in songbirds (Scharff *et al.*, 1998; Burt *et al.*, 2000) and to the Brocas area and anterior striatum in humans (Freedman *et al.*, 1984; Benson and Ardila, 1996) lead to decreased abilities in song/speech perception and discrimination. The perceptual deficits, however, are usually not as great as the motor deficits.

Taken together, the above evidence is consistent with the presence in humans of a posterior-like vocal motor pathway and an anterior-like vocal premotor pathway that are similar to the production and learning pathways of vocal learning birds. The relative locations of the brain regions in humans appear to be comparable to the relative location of the pathways in birds. The clearest difference between birds and humans appears to be the greater complexity of the deficits found after lesions in humans. Function of brain regions from activation studies is now compared.

14.2.3 Brain Activation

Brain activation includes changes in electrophysiological activity (recorded in both birds and in humans during surgery of patients), electrical stimulation (birds and humans), motor- and sensory-driven gene expression (birds and nonhuman mammals), and PET and magnetic resonance imaging of activated brain regions (in humans). In vocal learning birds, such studies have revealed that all seven comparable cerebral nuclei display vocalizing-driven expression of immediate early genes (Figure 5) (Jarvis and Nottebohm, 1997; Jarvis *et al.*, 1998, 2000 Jarvis and Mello, 2000); these are genes that are responsive to changes in neural activity. In deafened songbirds, these nuclei still display vocalizing-driven expression (Jarvis and Nottebohm, 1997), indicating that motor-driven gene activation is independent of hearing. Likewise, premotor neural firing has been found in HVC, RA, NIf, LAreaX, and LMAN when a bird sings (McCasland, 1987; Yu and Margoliash, 1996; Hessler and Doupe, 1999a; Hahnloser *et al.*, 2002). In deafened birds, similar singing-associated activity still occurs when a bird sings, at least for LMAN (Hessler and Doupe, 1999a). The firing in HVC and RA correlates with sequencing of syllables and syllable structure, respectively, whereas firing in LAreaX and LMAN is much more varied and in LMAN it correlates with song variability. In addition, neural firing and gene expression in LAreaX, LMAN, as well as RA differ depending upon the social context in which singing occurs (Jarvis *et al.*, 1998; Hessler and Doupe, 1999b); they are moderate when a bird sings directly facing another bird and high when a bird sings in an undirected manner. No difference has been observed between right (the dominant) and left HVC activity during singing in zebra finches, but in song sparrows activity in the left and right HVC is associated with production of specific sequences of song syllables (Nealen and Schmidt, 2002). Stimulation with electrical pulses to HVC during singing temporarily disrupts song output, i.e., song arrest (Vu *et al.*, 1998).

In humans, the brain area most comparable to songbird HVC and RA is one that is always activated (as measured with PET and fMRI) with all speech tasks: the face motor cortex (Figure 5) (Petersen *et al.*, 1988; Rosen *et al.*, 2000; Gracco *et al.*, 2005). Similar to other songbird vocal nuclei, other human vocal brain areas appear to be activated or not activated depending upon the context in which speech is produced. Production of verbs and complex sentences can be accompanied by activation in all or a subregion of the strip of cortex

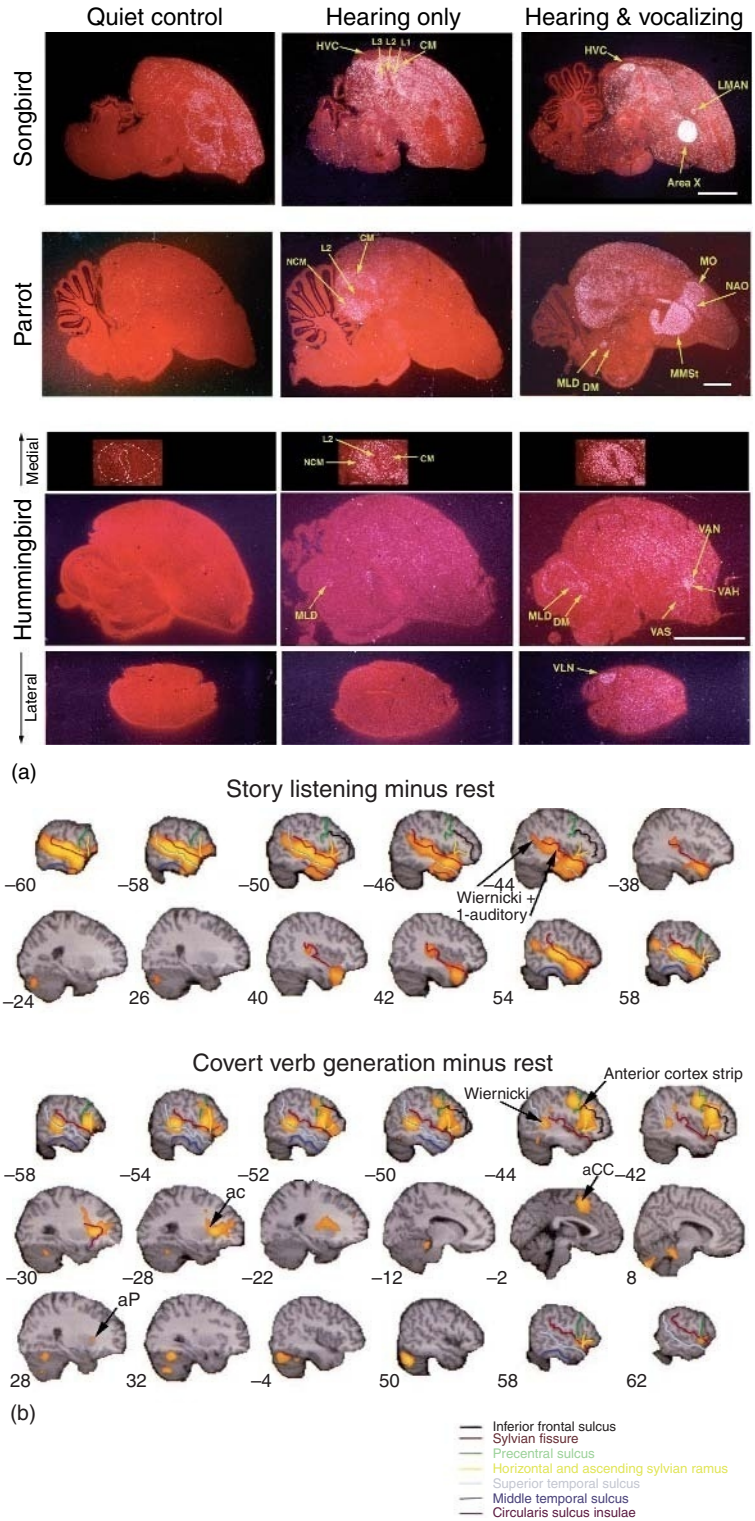

Figure 5 Hearing and vocalizing-driven brain activation patterns in vocal learning species. a, Example brain activation patterns in some brain regions of vocal learning birds (songbird-canary; parrot-budgerigar; hummingbird-sombre), as seen with hearing and vocalizing-driven *egr-1* gene expression (white). Shown are darkfield emulsion dipped sagittal sections reacted by *in situ* hybridizations to the *egr-1* gene; anterior is to the right, dorsal is toward the top. Red is cresyl violet staining. b, Example brain activation patterns in some brain regions of humans, as seen with hearing and vocalizing-driven PET signals minus rest. Shown are PET signals superimposed on sagittal slices, labeled with millimetric *x*-axis coordinates. The brighter the yellow signal the higher the activation. In all groups, different hearing and vocalizing tasks can differentially activate different brain areas. Not all activated brain areas are represented in these images. a, Figures based on Jarvis and Nottebohm (1997), Jarvis and Mello (2000), and Jarvis *et al.* (2000). b, Modified from Papathanassiou, D., Etard, O., Mellet, E., Zago, L., Mazoyer, B., and Tzourio-Mazoyer, N. 2000. A common language network for comprehension and production: A contribution to the definition of language epicenters with PET. *Neuroimage* 11, 347 357, Elsevier.

anterior to the face motor cortex: the anterior insula, Brocas area, DLPFC, pre-SMA, and anterior cingulate (Petersen *et al.*, 1988; Price *et al.*, 1996; Poeppel, 1996; Wise *et al.*, 1999; Crosson *et al.*, 1999; Papathanassiou *et al.*, 2000; Rosen *et al.*, 2000; Palmer *et al.*, 2001; Gracco *et al.*, 2005). Activation in the Brocas area, DLPFC, and pre-SMA is higher when speech tasks are more complex, including learning to vocalize new words or sentences, sequencing words into complex syntax, producing nonstereotyped sentences, and thinking about speaking (Hinke *et al.*, 1993; Poeppel, 1996; Bookheimer *et al.*, 2000; Buckner *et al.*, 1999). The left brain vocal areas show more activation than their right counterparts (Price *et al.*, 1996; Poeppel, 1996; Papathanassiou *et al.*, 2000; Rosen *et al.*, 2000). Like vocal nuclei in birds, premotor speech-related neural activity has been found in the Brocas area (Fried *et al.*, 1981). Further, low-threshold electrical stimulation to the face motor cortex, the Brocas area, or the anterior supplementary areas cause speech arrest or generation of phonemes or words (Jonas, 1981; Fried *et al.*, 1991; Ojemann, 1991, 2003).

In noncortical areas, speech production is accompanied by activation of the anterior striatum and the thalamus (Wallesch *et al.*, 1985; Klein *et al.*, 1994; Wildgruber *et al.*, 2001; Gracco *et al.*, 2005). Low-threshold electrical stimulation to ventral lateral and anterior thalamic nuclei, particularly in the left hemisphere, leads to a variety of speech responses, including word repetition, speech arrest, speech acceleration, spontaneous speech, anomia, and verbal aphasia (but also auditory aphasia) (Johnson and Ojemann, 2000). The globus pallidus can also show activation during speaking (Wise *et al.*, 1999). In nonhuman mammals and in birds, PAG and DM, Am, and nXII display premotor vocalizing neural firing (Larson, 1991; Larson *et al.*, 1994; Zhang *et al.*, 1995; Dusterhoft *et al.*, 2004) and/or vocalizing-driven gene expression (Jarvis *et al.*, 1998, 2000; Jarvis and Mello, 2000).

The cerebral vocal areas can also show neural firing during hearing, and this depends upon hearing task and species. In awake male zebra finches, firing is minimal in vocal nuclei (all the way down to nXIIts) when a bird hears playbacks of song, but greater when it is anesthetized or asleep and presented with playbacks of its own song (Williams and Nottebohm, 1985; Dave and Margoliash, 2000; Nealen and Schmidt, 2002; Cardin and Schmidt, 2003). In song sparrows, the reverse occurs: robust firing is observed in HVC when an awake bird hears playbacks of its own song, and this response is diminished when it is anesthetized (Nealen and Schmidt, 2002). In both species, the level or number of neurons firing in vocal nuclei during hearing is lower than that during singing. In humans, the face motor cortex, the Brocas area, and/or the DLPFC often show increased activation when a person hears speech or is asked to perform a task that requires thinking in silent speech (Hinke *et al.*, 1993; Price *et al.*, 1996; Poeppel, 1996; Wise *et al.*, 1999; Crosson *et al.*, 1999; Papathanassiou *et al.*, 2000; Rosen *et al.*, 2000; Palmer *et al.*, 2001). The magnitude of activation is usually lower during hearing than that seen during actual speaking. The anterior insula, the Brocas area, and DLPFC can also be activated by other factors, such as by engaging working memory (MacLeod *et al.*, 1998; Zhang *et al.*, 2003), which is a short-term memory that is formed before committing it to long-term storage. It is unclear, however, if this activation occurs in overlapping brain regions activated by speech or a speech perceptual task or whether the working memory tasks require language processing, or if there are separate but adjacent brain areas that are activated.

Taken together, the brain activation findings are consistent with the idea that songbird HVC and RA are more similar in their functional properties to face motor cortex than to any other human brain area and that songbird MAN, area X, and the anterior part of the dorsal thalamus are more similar in their properties to parts of the human premotor cortex, anterior striatum, and ventral lateral/anterior thalamus, respectively.

14.3 Consensus Auditory System

The above discussion focused solely on the motor component of vocal learning systems. This is because the motor component is what is specialized in vocal learners, whereas the auditory component is common among vocal learners and vocal nonlearners. Thus, the auditory component is only briefly discussed here; more detail is given in Jarvis (2004). Birds, reptiles, and mammals have relatively similar auditory pathways (Figure 6) (Webster *et al.*, 1992; Vates *et al.*, 1996; Carr and Code, 2000). The pathway begins with ear hair cells that synapse onto sensory neurons, which project to cochlea and lemniscal nuclei of the brainstem, which in turn project to midbrain (avian MLd, reptile torus, mammalian inferior colliculus) and thalamic (avian Ov, reptile reunions, mammalian medial geniculate) auditory nuclei. The thalamic nuclei in turn project to primary auditory cell populations in the pallium (avian L2, reptile caudal pallium, mammalian layer 4 of primary auditory cortex). Avian L2

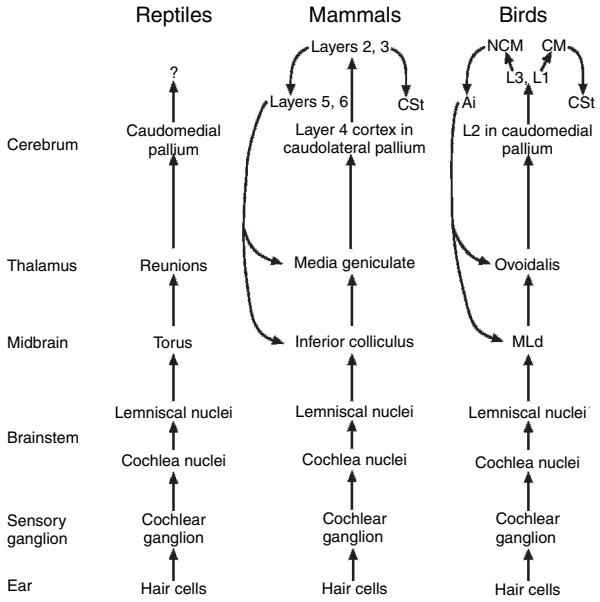

Figure 6 Comparative and simplified connectivity among auditory pathways in reptiles, mammals, and birds, placed in order from left to right of the most recently evolved. The connectivity from CM to CSt in birds needs verification by retrograde tracing. Abbreviations are in Table 1. Reproduced from Jarvis, E. D. 2004. Learned birdsong and the neurobiology of human language. *Ann. NY Acad. Sci.* 1016, 749 777, with permission.

then projects to other pallial cell (L1, L3, NCM, CM) and striatal (CSt) populations that form a complex network. Mammalian layer 4 cells then project to other layers of primary auditory cortex and to secondary auditory regions. Cerebral pathway connectivity is not known for reptiles. In terms of connectivity, avian L1 and L3 neurons are similar to mammalian layers 2 and 3 of primary auditory cortex, the latter of which receive input, like L2, from layer 4 (Karten, 1991; Wild *et al.*, 1993). Avian NCM and CM are also similar to layers 2 and 3 in that they form reciprocal intrapallial connections with each other and receive some input from L2.

The source of auditory input into the vocal pathways of vocal learning birds is unclear. Proposed routes include the HVC shelf into HVC, the RA cup into RA, Ov or CM into NIf, and from NIf dendrites in L2, in songbirds (Wild, 1994; Fortune and Margoliash, 1995; Vates *et al.*, 1996; Mello *et al.*, 1998). However, the location of the vocal nuclei relative to the auditory regions differs among vocal learning groups. In songbirds, the posterior vocal nuclei are embedded in the auditory regions; in hummingbirds, they are situated more laterally, but still adjacent to the auditory regions; in parrots, they are situated far laterally and physically separate from the auditory regions (Figures 3a–3c).

In humans, the primary auditory cortex information is passed to secondary auditory areas, which includes the Wernickes area (Figure 3d). When damaged, this area leads to auditory aphasias, sometimes call fluent aphasia. A patient can speak words relatively well, but produces nonsense highly verbal speech. One reason for this is that the vocal pathways may no longer receive feedback from the auditory system. Information from the Wernickes area has been proposed to be passed to the Brocas area through arcuate fibers that traverse a caudal–rostral direction (Geschwind, 1979), but this has not been demonstrated experimentally. Bilateral damage to primary auditory cortex and Wernickes area also leads to full auditory agnosia, the inability to consciously recognize any sounds (speech, musical instruments, natural noises, etc.) (Benson and Ardila, 1996).

No one has tested whether lesions to avian secondary auditory areas result in fluent song aphasias. Yet lesions to songbird NCM and CM result in a significant decline in the ability to form auditory memories of songs heard (MacDougall-Shackleton *et al.*, 1998). It is difficult to ascertain how nonhuman animals, including birds, perceive sensory stimuli, and therefore it is difficult to make comparisons with humans in regard to perceptual auditory deficits.

14.4 Evolution of Vocal Learning Systems from a Common Motor Pathway

Given that auditory pathways in avian, mammalian, and reptilian species are similar, whether or not a given species is a vocal learner, this suggests

that the auditory pathway in vocal learning birds and in humans was inherited from their common stem-amniote ancestor, thought to have lived approximately 320 Mya (Evans, 2000). Having a cerebral auditory area would explain why nonhuman mammals, including a dog, exhibit auditory learning, including learning to understand the meaning of human speech, although with less facility than a human. For vocal learning pathways, because the connections of the anterior and posterior vocal pathways in vocal learning birds bear some resemblance to those of nonvocal pathways in both birds and mammals, pre-existing connectivity was presumably a genetic constraint for the evolution of vocal learning (Durand *et al.*, 1997; Farries, 2001; Lieberman, 2002; Jarvis, 2004). In this manner, a mutational event that caused descending projections of avian arcopallium neurons to synapse onto nXIIts or mammalian layer 5 neurons of the face motor cortex to synapse onto Am may be the only major change that is needed to initiate a vocal learning pathway. Thereafter, other vocal brain regions could develop out of adjacent motor brain regions with pre-existing connectivity. Such a mutational event would be expected to occur in genes that regulate synaptic connectivity of upper pallial motor neurons to lower α-motor neurons. This hypothesis requires that avian nonvocal motor learning systems have up to seven areas distributed into two pathways in at least six brain subdivisions (the mesopallium, nidopallium, arcopallium, striatum, pallidal-like cells in the striatum, and dorsal thalamus). It would also require that mammalian nonvocal motor learning systems have brain regions distributed in two pathways involving at least four brain subdivisions (the six layers of the cortex, the striatum, the pallidum, and the dorsal thalamus). Not apparent in this view is the question of whether there is a genetic constraint for auditory information entering vocal learning pathways.

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15 The Evolution of the Amygdala in Vertebrates

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Glossary

agonistic behaviors Behaviors expressed during confrontation with conspecifics for resources, territory, or mates. This usually leads to aggressive/defensive behaviors different from prey predator interactions. In territorial species, encounters with adult conspecifics can be either agonistic or reproductive.

conditioning, instrumental (or operant) This is a type of associative learning in which an unlikely motor response to a given clue (such as pressing a lever when the animal sees it) is associated with a reward (e.g., food) or to absence of punishment. The chances of showing the motor response augment due to its association with the reward. This can be interpreted as a stimulus response association (detection of the lever pressing it) due to reinforcement (food consumption).

conditioning, Pavlovian (or classical) As opposed to instrumental conditioning, in this kind of associative learning two stimuli called unconditioned (US) and conditioned stimulus (CS), are associated. The US renders an automatic response (usually not voluntary or not conscious), which is part of the repertoire of behaviors specific to that species (for instance, salivation, increased blood pressure). This is called unconditioned response. Repeated presentation of the CS together with the US leads to an association of the

former to the latter that results in a conditioned response, for example, the CS alone provokes the response that was elicited by the US.

fear and anxiety — Fear is an acute reaction to potentially harming stimuli that consists of behavioral (e.g., freezing, potentiated startle), vegetative (e.g., increased blood pressure, tachycardia) and endocrine responses (surge in epinephrine/norepinephrine, adrenocorticotropic hormone, and corticosteroid levels). Anxiety is a sustained reaction in advance to a potentially harming but ill defined outcome.

hodology — Study of pathways. In neurobiology, hodology is used to define the study of the interconnections of brain cells and areas.

homeotic genes — A set of genes characterized by a sequence (homeobox) whose product constitutes a DNA binding homeodomain. This binding to DNA mediates their ability to modulate or control transcription of other genes (in other words, homeotic genes code transcription factors). The expression of homeotic genes during early development determines the main positional coordinates of the different body parts, thus being fundamental for morphogenesis.

pheromone — Substance secreted or excreted by an animal that has an intrinsic biological significance for conspecifics, thus inducing an unconditioned behavioral or neuroendocrine response. Although it is assumed that pheromones are mainly detected by the vomeronasal organ, it has not been demonstrated in every instance. Moreover, the vomeronasal organ also detects signals from common predators or preys, which obviously cannot be considered pheromones. Finally, some pheromones have been shown to be detected by the main olfactory system.

reinforcement, reward — Etymologically, reinforcement means strengthening. In psychological terms, reinforcement designates the increase in the probability of expressing a given behavior induced by the positive outcome that this behavior renders, in the context of instrumental conditioning. Reinforcers (stimuli reinforcing behaviors) are usually perceived as pleasant or rewarding, so that in many instances reward ing is used as synonymous with reinforcing.

stimulus reward association — Associative learning in which an initially neutral stimulus that precedes or is presented together with a reinforcer, becomes rewarding (secondary or conditioned reinforcer). After this association, the animal would work for the secondary reward.

topography — Physical relationships of regions in the adult brain. For example, *A* is topographically dorsal to *B* if, in histological sections of the adult brain, *A* occupies a location nearer the dorsum than *B*.

topology — In terms of anatomy, this refers to physical relationships of regions based upon their developmental history. Thus, when a structure *A* is said to be topologically deep to structure *B*, it is means that *A* and *B* derive from the same region of the embryonic neuroepithelium. Topology and topography may not coincide: a structure may be topologically lateral to another, but, due to a flexure of the mediolateral axis, appears (topographically) medial to it in the adult brain.

Abbreviations: Mammals

AA — Anterior amygdaloid area (AAD: dorsal; AAV: ventral).

AB — Accessory basal (or basomedial) nucleus of the amygdala (ABa: anterior; ABp: posterior).

Acb — Nucleus accumbens.

AHA — Amygdalohippocampal area (or posterior nucleus of the amygdala).

APir — Amygdalopiriform transition area.

AStr — Amygdalostriatal transition area.

B	Basal (or basolateral) nucleus of the amygdala (Ba: anterior; Bp: posterior; Bv: ventral).
BAOT	Nucleus of the accessory olfac tory tract.
BST	Bed nucleus of the stria terminalis.
Ce	Central amygdala.
CeC	Capsular or paracapsular divi sion of the central amygdala.
CeL	Lateral central amygdala.
CeM	Medial central amygdala.
Cl	Claustrum.
COAa	Anterior cortical amygdala.
COApl	Posterior lateral cortical amyg dala or periamygdaloid cortex.
COApm	Posterior medial cortical amygdala.
CPu	Caudate putamen (striatum).
CxA	Corticoamygdaloid transition.
DEn	Dorsal endopiriform nucleus.
DG	Dentate gyrus.
EA	Extended amygdala (CeEA: cen tral; MeEA: medial).
I	Intercalated nuclei of amygdala.
IPAC	Interstitial nucleus of the poster ior limb of the anterior commissure.
L	Lateral nucleus of the amygdala.
LGP	Lateral globus pallidus.
lot	Lateral olfactory tract.
LOT	Nucleus of the lateral olfactory tract.
Me	Medial amygdala.
MeA	Anterior medial amygdala.
MeP	Posterior medial amygdala (MePV: ventral; MePD: dorsal).
MGm	Medial division of the medial geniculate nucleus.
Pir	Piriform cortex.
PMv	Ventral premammillary nucleus.
SI	Substantia innominata.
SN	Substantia nigra.
st	Stria terminalis.
TR	Postpiriform transition area.
VEn	Ventral endopiriform nucleus.
VMH	Ventromedial nucleus of the hypothalamus.
VTA	Ventral tegmental area.

Abbreviations: Reptiles

ac	Anterior commissure.
Acb	Nucleus accumbens.
ADVR	Anterior dorsal ventricular ridge.
Amb	Nucleus ambiguus.
aot	Accessory olfactory tract.
BAOT	Nucleus of the accessory olfac tory tract.
BST	Bed nucleus of the stria terminalis.
BSTl	Dorsolateral (supracommisural) division of the BST.
BSTm	Ventromedial (subcommissural) division of the BST.
DC	Dorsal cortex.
DLA	Dorsolateral amygdaloid nucleus.
dLC	Deep lateral cortex.
DMX	Dorsal motor nucleus of the vagus.
DSt	Dorsal striatum.
GP	Globus pallidus.
LA	Lateral amygdala.
LC	Lateral cortex.
LCc	Caudal lateral cortex.
lfb	Lateral forebrain bundle.
LHN	Lateral posterior hypothalamic nucleus.
lot	Lateral olfactory tract.
MA	Medial amygdala.
MC	Medial cortex.
NAOT	Nucleus of the accessory olfac tory tract.
NLOT	Nucleus of the lateral olfactory tract.
NS	Nucleus sphericus.
PB	Parabrachial nucleus.
PDVR	Posterior dorsal ventricular ridge (PDVRv: ventral; PDVRdl: Dorsolateral; PDVRdm: dorsomedial).
PMv	Ventral premammillary nucleus.
S	Septum.
SAT	Striatoamygdaloid transition area (SATm: medial; SATl: lateral).
sm	Stria medullaris.
sol	Nucleus of the solitary tract.
st	Stria terminalis.
VAA	Ventral anterior amygdala.
Vds	Nucleus descendens nervi trigemini.
VMH	Ventromedial nucleus of the hypothalamus.
VP	Ventral pallidum.
VPA	Ventral posterior amygdala.
zl	Zona limitans.

Abbreviations: Birds

AA	Anterior arcopallium (anterior archistriatum).
Acb	Nucleus accumbens (medial asp ect of the lobus paraolfactorius).
AD	Dorsal arcopallium (dorsal intermediate archistriatum).
AM	Medial arcopallium (medial archistriatum).

APH	Parahippocampal area.
AV	Ventral arcopallium (ventral intermediate archistriatum).
Bas	Nucleus basorostralis pallii.
BSTl	Lateral bed nucleus of the stria terminalis (called BST or nucleus accumbens depending on the authors).
BSTm	Medial bed nucleus of the stria terminalis.
CDL	Area corticoidea dorsolateralis.
CPi	Cortex piriformis.
E	Entopallium.
FA	Tractus frontoarcopallialis (frontoarchistriatalis).
GP	Globus pallidus (paleostriatum primitivum).
H	Hyperpallium.
Hp	Hippocampus.
INP	Intrapeduncular nucleus.
L	Field L of the NC.
LAD	Lamina arcopallialis dorsalis.
lfb	Lateral forebrain bundle.
LM	Lamina mesopallialis.
LPS	Lamina palliosubpallialis.
LSt	Lateral striatum (paleostriatum augmentatum).
M	Ventral mesopallium (hyper striatum ventrale).
MSt	Medial striatum (lateral aspect of the lobus paraolfactorius, just medial to the paleostriatum).
N	Nidopallium (neostriatum).
NC	Caudal nidopallium, usually divided into lateral (NCL) and medial divisions (NCM).
OB	Olfactory bulb.
OM	Occipitomesencephalic tract.
OMH	Occipitomesencephalic tract, hypothalamic part.
PoA	Posterior nucleus of the pallial amygdala (posterior archistriatum).
Rt	Nucleus rotundus.
S	Septum.
SpA	Subpallial amygdala (ventral paleostriatum).
TnA	Nucleus teniae of the amygdala.
TPO	Area temporoparieto occipitalis of the cerebral hemispheres.
tsm	Tractus septopalliomesencephalicus.
VP	Ventral pallidum.

Abbreviations: Amphibians

Aa	Anterior amygdala.
BST	Bed nucleus of the stria termina lis (BSTr: rostral; BSTc: caudal).
Ca	Central amygdala.
Dp	Dorsal pallium.
La	Lateral amygdala.
lfb	Lateral forebrain bundle.
Lp	Lateral pallium.
Ls	Lateral septum.
Ma	Medial amygdala.
Mp	Medial pallium.
PLa	Posterior lateral amygdala.
POA	Anterior preoptic area.
Str	Striatum.

Other Abbreviations

AChase	Acetylcholinesterase.
ChAT	Choline acetyltransferase.
CRF	Corticotropin releasing factor.
NT	Neurotensin.
SP	Substance P.
SS	Somatostatin.

15.1 Introduction

The name amygdala (from the Latin–Greek *amygdala*, almond) was coined by Burdach (cited by Swanson and Petrovich, 1998) to designate an almond-shaped structure deep in the temporal lobe of the human brain. The amygdala is easy to identify macroscopically in the brain of many mammals as a smooth bump in the caudal ventral cerebral hemispheres (e.g., rat, lamb; Figure 1). In both lissencephalic and gyrencephalic mammals the amygdala is ventral to the rhinal fissure and apparently connected with the lateral olfactory tract (*lot*), which reflects the olfactory and vomeronasal function of some of its components.

A closer look at the mammalian amygdala reveals, however, that it is an extremely complex and anatomically heterogeneous structure. Thus, in the first comparative approach to the anatomy of the amygdala of vertebrates, Johnston (1923) proposed that the amygdala includes pallial (basolateral and cortical divisions) and subpallial derivatives (central and medial amygdala), a view demonstrated by Swanson and Petrovich (1998). Moreover, the amygdala is not just a component of the chemosensory (olfactory and vomeronasal) systems, but also includes nonchemosensory areas of diverse embryological origin displaying distinct anatomical and neurochemical features. This has led Swanson and Petrovich to conclude that "terms such as 'amygdala' . . . combine cell groups arbitrarily rather than according to the structural and functional units to which they seem to belong. The amygdala is neither a structural nor a functional unit."

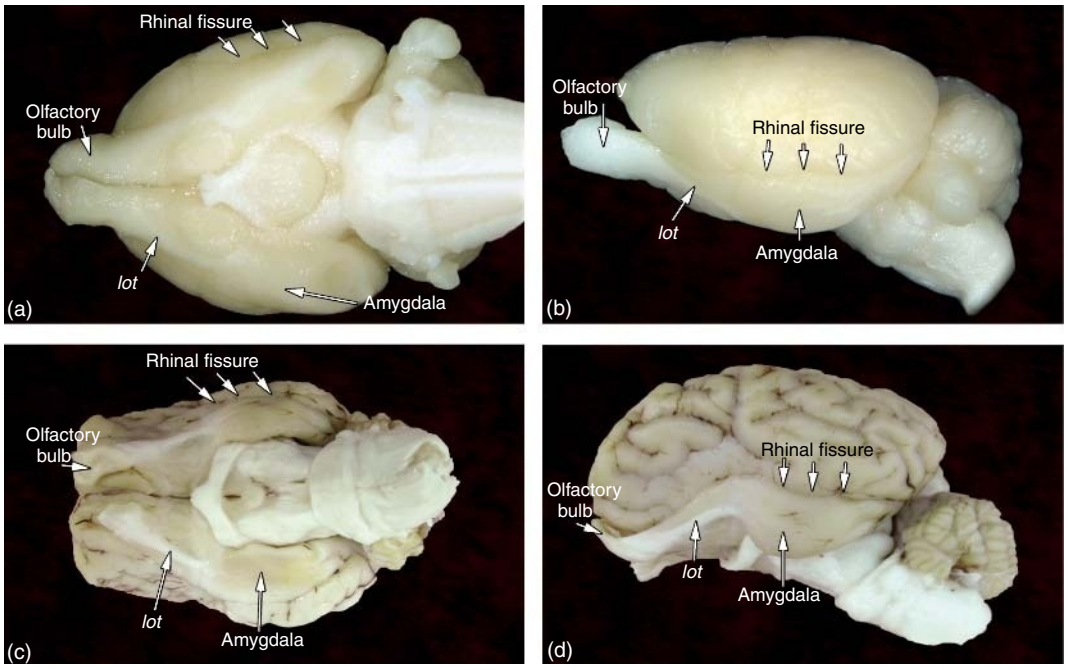

Figure 1 Gross anatomy of the mammalian amygdala. The amygdala can be easily identified in the brains of lissencephalic (e.g., the rat; ventral view (a); lateral view (b)) and gyrencephalic mammals (e.g., the lamb; ventral (c); lateral (d)). In both cases, the lateral olfactory tract (*lot*) can be followed from the olfactory bulbs up to a basal bump in the caudal cerebral hemispheres that corresponds to the amygdala.

The extreme morphological and functional complexity of the mammalian amygdala makes identifying its diverse components in nonmammals a very demanding task. This is further rendered difficult by the strong differences in the anatomical organization of the cerebral hemispheres of mammals and nonmammals, derived from the development of a huge isocortex in the mammalian forebrain. Therefore, a previous step to the comparative study of the amygdala of vertebrates is to characterize the divisions of the mammalian amygdala using developmental approaches (including expression of homeotic genes) as well as neurochemical and hodological data in the adult. Once the mammalian amygdala is fully characterized, we will identify the divisions of the amygdala of reptiles and birds using the same criteria. Then, we will discuss the evolutionary origins of the amniote amygdala and the possible influence that the amygdaloid function might have had on the evolution of the cerebral hemispheres.

15.2 Anatomical Heterogeneity of the Mammalian Amygdala

As a previous step to the study of the anatomy of the mammalian amygdala, and to understand its position within the cerebral hemispheres, we briefly discuss the identity and characteristic features of the main divisions of the vertebrate telencephalon: the pallium, striatum, and pallidum (Figure 2).

15.2.1 Organization of the Cerebral Hemispheres

In 1975, two groups independently observed that the nucleus accumbens (Acb) and olfactory tubercle showed a set of connections with the midbrain tegmentum that recalled those of the caudate putamen (CPu: Heimer and Wilson, 1975; Swanson and Cowan, 1975). Based on this evidence, Heimer and Wilson (1975) suggested that the Acb, olfactory tubercle, and fundus striatum were just the ventral portion of the striatum, the dorsal one being the CPu. Recent studies have revealed further similarities between the dorsal and ventral portions of the striatum, pertaining to their intrinsic organization, extrinsic connections, and neurochemistry. The common pattern of organization shared by all the striatal structures includes a massive glutamatergic input from areas of the cortex (archicortex – hippocampal formation – for the ventral striatum, isocortex for the dorsal striatum), as well as direct and indirect (through the ventral pallidum (VP) and globus pallidus (GP)) efferent projections to tegmental centers (ventral tegmental area (VTA) and substantia nigra (SN)), arising

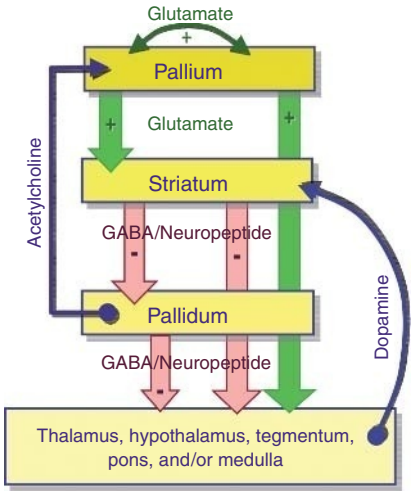

Figure 2 Organization of the vertebrate cerebral hemispheres. The telencephalon of vertebrates is composed of the pallium (cortex and other nuclear pallial centers) and subpallium (striatum and pallidum). The pallium gives rise to glutamatergic (excitatory) intrapallial and descending projections that reach the striatum and several extratelencephalic targets. The subpallium is engaged in a striatopallidotegmentostriatal loop in which the descending projections are GABAergic and peptidergic, whereas the tegmentostriatal pathway is dopaminergic. Both the pallium and subpallium project (directly or indirectly) to extratelencephalic executive centers. Whereas the pallium has an excitatory influence on them, the subpallium probably affects behavior by disinhibition. Based on Swanson and Risold (1999) and Lanuza *et al.* (2002).

from spiny stellate, GABAergic (and peptidergic) cells. In turn, the tegmental targets of the striatum give rise to dopaminergic projections back to their striatal input areas (Figure 2).

Recent studies have revised and expanded this view. Thus, Swanson and Risold (1999) have reinterpreted the lateral septum as a portion of the striatum that they call the medial striatum (MSt), following a suggestion by Ramón y Cajal (1901). Like the striatum proper, the lateral septum receives a dense glutamatergic input from parts of the cortex (hippocampal formation *sensu lato*). Moreover, the spiny stellate cells of the lateral septum originate a GABAergic projection to pallidal (medial septum and diagonal band complex, which is thus considered the medial pallidum) and tegmental structures (VTA) from which they receive, in turn, a dopaminergic afferent. This scheme has been further extended to the cerebral hemispheres of all amniote vertebrates (Lanuza *et al.*, 2002). As indicated in Figure 2, the corticostriatopallidal pathway (including the striatotegmental loop) seems to be the basic circuit of the cerebral hemispheres. In addition to the hodological and neurochemical features mentioned above, this circuit is further characterized by the presence of dense (glutamatergic) corticocortical connections including, in some cases, commissural projections. Cortical connections also include descending projections to the thalamus, hypothalamus, pons, tegmentum, and/or brainstem that bypass the striatopallidum. Finally, a sparse population of cholinergic cells (and some GABAergic ones; Kohler *et al.*, 1984; Zaborszky *et al.*, 1999; Sarter and Bruno, 2002) located in the striatopallidum provides a feedback to the cortex.

The organization of the mammalian amygdala and its diverse anatomical components can be studied by using this simple model of the cerebral hemispheres that considers them to be composed of pallial structures, derived of the embryonic pallium, and subpallial ones, which are the adult derivatives of the ganglionic eminences. Although tangential migration during embryonic development (Marin and Rubenstein, 2003) might have partially blurred this scheme, it is still a useful framework to describe the anatomical heterogeneity of the amygdaloid complex.

15.2.2 The Pallial Amygdala

The definition of the pallial territories in the adult brain is a complex issue, as it is to delineate its different regions or compartments. This is so because not all the pallial territories use the same developmental program. Parts of the pallium develop in a very organized way, according to which those neurons produced in different times migrate following a neurogenetic gradient, either inside-out (isocortex) or outside-in (e.g., hippocampal fascia dentate; Jacobson, 1991). This leads to the formation of a cortex, a superficial structure showing a layered cytoarchitectonic organization that makes it easy to identify. Other pallial derivatives, however, the claustrum (Cl) and the endopiriform nucleus being good examples, show not a cortical but rather a nuclear organization, with no apparent stratification. We call them nuclear pallial structures. Since the amygdala occupies the latero-ventral edge of the pallium, the nuclear pallial derivatives of the amygdala are adjacent to subpallial ones that are equally nonlayered, so that the palliosubpallial boundary becomes especially difficult to trace within the amygdaloid complex.

Two kinds of additional data can be helpful to delineate the palliosubpallial boundary. First, the pallium and subpallium express different sets of homeotic genes during intermediate embryonic development. Thus, the maps of expression of these genes in embryos are used to cartograph the pallial and subpallial territories of the amygdala (Puelles *et al.*, 2000; Medina *et al.*, 2004). However, during late embryonic stages, the

amygdala undergoes a topologically complex development that makes it difficult to interpret the fate of the embryonic labeled territories. This may be further complicated by tangential migration similar to that reported to occur from subpallial to pallial territories (Marin and Rubenstein, 2003).

Therefore, data on the neurochemistry and neuronal morphology of the adult brain are also helpful in this respect. As Swanson and Petrovich (1998) pointed out, pallial structures are characterized by originating excitatory (mostly glutamatergic) extrinsic projections to the subpallium and brainstem (as well as intrapallial projections), whereas subpallial structures give rise to descending GABAergic projections. Whereas labeling of GABAergic cells is relatively easy by using immunohistochemistry for GABA or glutamic acid decarboxylase (GAD), and/or *in situ* hybridization for the detection of GAD mRNA, the histochemical mapping of glutamatergic cells is not as easy. Nevertheless, it is well known that a subpopulation of the glutamatergic cells of the cerebral hemispheres are rich in zinc (Frederickson *et al.*, 2000) and can be visualized using a variety of histochemical techniques. The maps of the expression of GAD (Pare and Smith, 1993; Swanson and Petrovich, 1998) and of the distribution of zinc-rich (glutamatergic) cells in the amygdala of rats (Christensen and Geneser, 1995; Brown and Dyck, 2004) are nicely complementary and delineate quite a clear pallio-subpallial boundary. These data indicate that the subpallial amygdala includes the medial and central nuclei of the amygdala and the BST (at least its intra-amygdaloid portion). The remaining nuclei, including all the cortical amygdala and the AHA, as well as the whole basolateral division of the amygdala (lateral, basal, or basolateral and basal accessory or basomedial nuclei) are pallial derivatives (Figures 3 and 4). The presence of zinc-laden cells in the amygdalostriatal area (Brown and Dyck, 2004) suggests that some pallial cells may have migrated into putative striatal territories.

15.2.2.1 The cortical and basolateral divisions of the amygdala The pallial amygdala is composed of two kinds of structures. Some of them are superficial and show a layered organization, thus constituting the cortical amygdala. Topologically deep to these structures (and to the adjacent piriform cortex, see below) one finds a series of cell groups with nuclear configuration that conform the basolateral division of the amygdala. Some additional nuclei, not included in the basolateral amygdala, such as the AHA are also deep nuclear pallial structures.

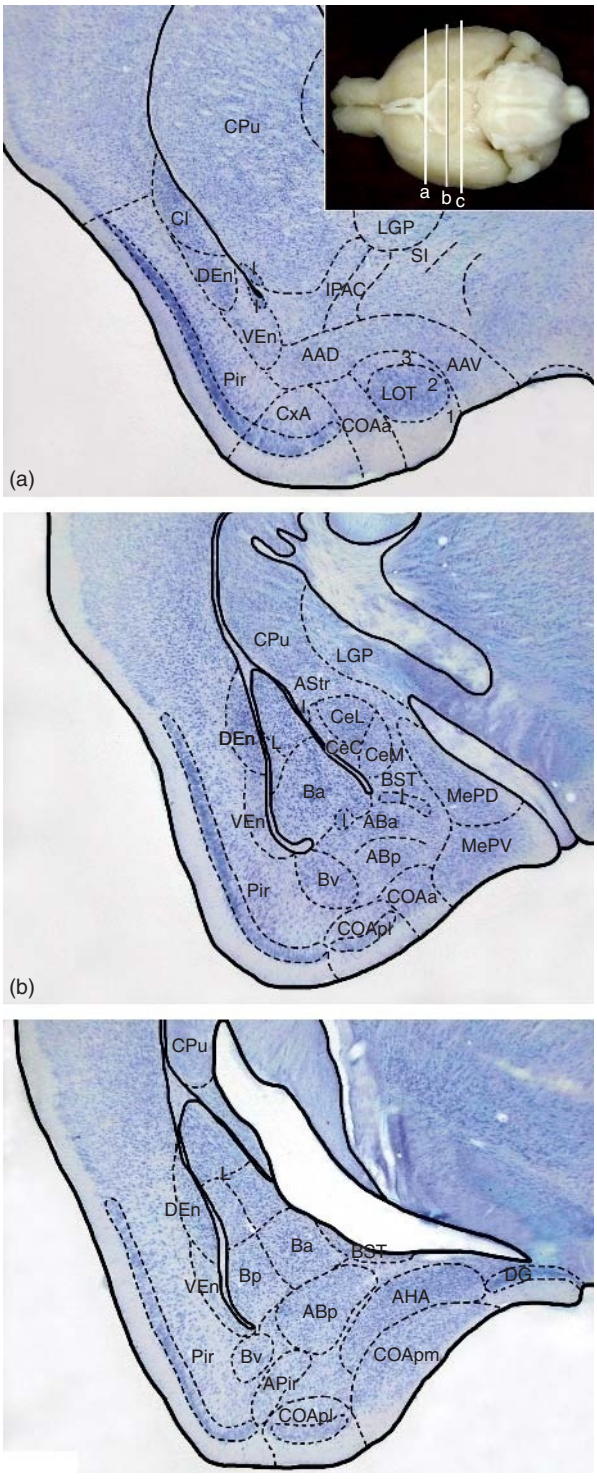

Figure 3 Cytoarchitecture of the amygdala of the mouse. Nissl-stained frontal sections through the left cerebral hemisphere of a mouse at about commissural (a); anterior postcommissural (b); and caudal levels of the telencephalon (c). The approximate levels of the sections are indicated on a ventral view of the brain in the inset. The cytoarchitectonic boundaries of the different amygdaloid nuclei and adjoining structures are delineated on the sections (for abbreviations, see 'Glossary').

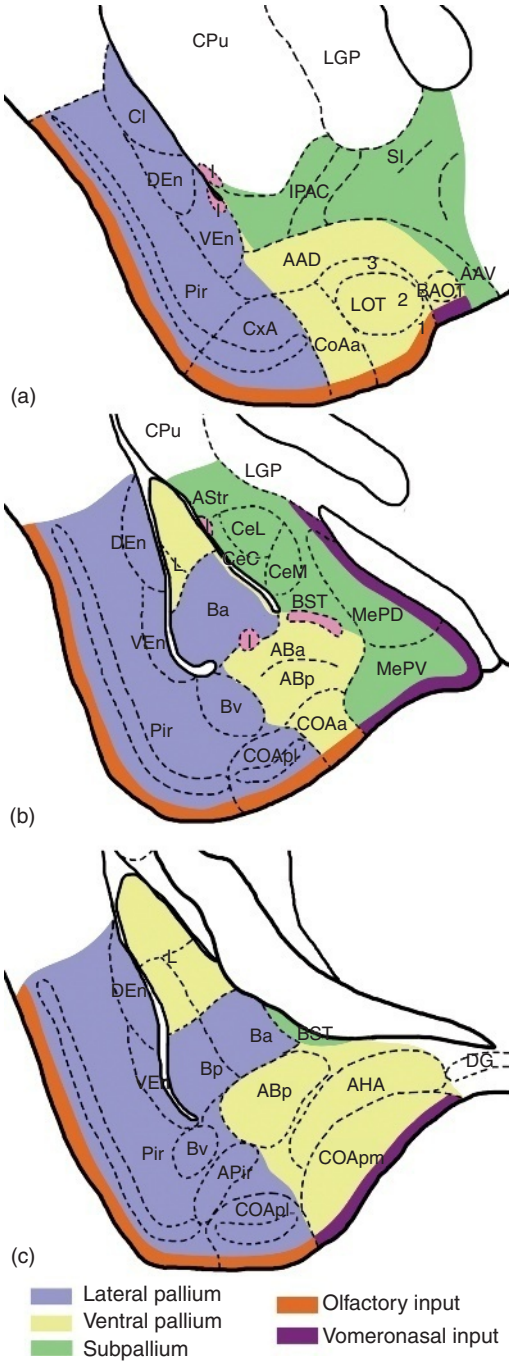

Figure 4 Pallial and subpallial territories of the mammalian amygdala. A schematic diagram of the mammalian amygdala, based on the Nissl-stained sections shown in Figure 3, shows the palliosubpallial boundary and the extent of the latero- and ventropallial territories within the pallial amygdala. The main olfactory bulb projects to entire superficial lateral pallium plus a small portion of the ventral pallium. In contrast, as indicated, the accessory olfactory bulbs project exclusively to ventropallial and subpallial structures. The intercalated cell masses (pink) and the amygdaloid capsule connect the deepest (L) with the more superficial (AB, COApm) parts of the ventropallial amygdala. This gives topological congruence to the proposed picture, since the deep lateral pallium (Ba) is separated from the subpallium (Ce and intra-amygdaloid BST) by a ventropallial bridge.

The cortical amygdala is composed of several areas on the ventral surface of the caudal cerebral hemispheres. These include the nuclei of the accessory (BAOT) and lateral olfactory tract (LOT), as well as the anterior cortical amygdala (COAa) rostrally, and the posterior cortical amygdala (the medial part, COApm, and the lateral part, COApl; also called periamygdaloid cortex by some authors) caudally. To these structures, some authors add rostral (corticoamygdaloid, CxA) and caudal (amygdalopiriform, APir; postpiriform, TR) transitional areas. All these structures show a similar (though not equally neat) layering with a (molecular) layer 1 that receives a superficial projection from the main (LOT, COAa, COApl) or accessory olfactory bulb (BAOT, COApm) and a deeper commissural-associative afferent (McDonald, 1998), thus resembling the piriform cortex.

The nuclear pallial amygdala includes at least the three nuclei that conform the basolateral division of the amygdala: the basal (B, or basolateral), accessory basal (AB, or basomedial) and lateral (L) nuclei. In addition, some of the structures rostral and caudal to these nuclei and deep to the cortical amygdala and/or the piriform cortex also belong to the pallial noncortical amygdala. Thus, the AHA (included in the posterior nucleus of the amygdala by Swanson and collaborators; Canteras *et al.*, 1992a) seems to be a caudomedial continuation of the AB. On the other hand, the dorsal portion of the anterior amygdaloid area (AA), which is immediately deep to the COAa and LOT, seems a rostral continuation of the AB.

15.2.2.2 Compartments of the pallium: Lateropallial and ventropallial portions of the mammalian amygdala Pallial derivatives occupy the dorsal surface of the cerebral hemispheres from the midline to the lateral ventricular sulcus. It is generally assumed (although this division is not based on solid experimental evidence) that the cortex is composed of three wide areas, derived from the medial, dorsal, and lateral pallia. In mammalian neuroanatomy, this roughly fits the traditional classification of the cortex into three cytoarchitectonically distinct regions: from medial to lateral, (1) the archicortex with a single cell layer sandwiched between two plexiform layers (hippocampal formation); (2) the isocortex (or neocortex) with 5–6 cell layers plus a molecular layer on top of the white matter; and (3) the paleocortex (olfactory cortex), characterized by a superficial molecular layer (I) and two cell layers (plus the endopiriform nucleus). Since the boundaries between these three areas are quite fuzzy, the existence of transitional cortical areas should be

taken into consideration. It is evident that the lateralmost regions of the pallium impinge on the amygdala.

The molecular and developmental bases for such a division of the pallium are not yet clear. Nevertheless, the expression of developmental genes in vertebrate embryos reveals an unexpected heterogeneity within the lateral aspect of the pallium (Puelles *et al.*, 2000; Medina *et al.*, 2004) that has led to the identification of a fourth pallial region, the ventral pallium (previously considered by Smith-Fernandez *et al.*, 1998, as an intermediate zone between the pallium and the subpallium). The ventral pallium was defined by a pattern of genetic expression that includes several pallial markers (Tbr-1 and a juxtaventricular rim of Pax-6) but excludes Emx-1 (Puelles *et al.*, 2000). *In situ* hybridization in 15-day-old mouse embryos indicates that the amygdala includes ventropallial derivatives together with portions of the lateral pallium (Puelles *et al.*, 2000; Puelles, 2001). Medina *et al.* (2004) further refined this analysis and demonstrated that during late embryonic development the lateral and ventral pallial territories display a differential pattern of genetic expression (lateral pallium: *Cadherin 8* and *Emx-1*; ventral pallium: *Dbx-1*, *Neurogenin*, and *Semaphorin 5A*).

From a comparative viewpoint, assigning each one of the areas and nuclei of the pallial amygdala to either the lateral or ventral pallium constitutes a key issue. Namely, derivatives of the embryonic ventral pallium of mammals can only be homologous to ventropallial structures of nonmammals, and the same is valid for the lateropallial derivatives. Data on the expression of homeotic genes during embryonic development, derived from the above-cited studies (Puelles, 2001; Medina *et al.*, 2004), indicate that the only lateropallial derivatives of the amygdala are the basal nucleus (B) and posterolateral cortical amygdala (COApl). In addition, it is likely that the transitional territories located between the cortical amygdala and the piriform/entorhinal cortex (CxA, APir, TR) also belong to the lateral pallium.

An analysis of the topology of the mammalian amygdala indicates that the remaining areas and nuclei of the pallial amygdala are ventropallial, since they are adjacent to striatal territories. Thus, the ventropallial division of the cortical amygdala consists of the LOT, COAa, BAOT, and COApm. In addition, the L and AB constitute the deep ventropallial amygdala. Thus, the L is adjacent to the striatal derivatives such as the CPu, the striatoamygdaloid transition, and the central amygdala. On the other hand, the AB and maybe its rostral (dorsal AA) and caudal neighbors (AHA) are adjacent to the Me (anterior or posterior divisions) and to the intra-amygdaloid portion of the BST. Topology demands that the B (lateropallial) and the Ce (striatal) be separated by a rim of ventropallial territory. This is likely constituted by the amygdaloid capsule and the adjoining posterior paracapsular intercalated cell masses of the amygdala (Medina *et al.*, 2004).

This renders a scheme of the mammalian pallial amygdala (Figure 4) in which every nucleus or area belongs to a compartment depending on its superficial or deep position as well as its ventropallial or lateropallial nature.

15.2.3 The Subpallial Amygdala

As discussed above, the subpallial telencephalon develops from two structures of the embryonic cerebral hemispheres, namely the lateral and medial ganglionic eminences. It is generally assumed that the pallidum of the adult telencephalon (including the GP, VP, and medial pallidum or medial septum, according to Swanson and Risold, 1999) derives from the embryonic medial ganglionic eminence. On the other hand, the Acb, olfactory tubercle, and CPu (striatal structures) are supposedly the adult derivatives of the lateral ganglionic eminence.

15.2.3.1 Striatal and pallidal compartments within the subpallial amygdala In the caudal cerebral hemispheres the striatal or pallidal identity of the subpallial structures becomes confused. These include the BST (both their intra- and extra-amygdaloid portions), the central amygdala (Ce), and the medial amygdala (Me) and several adjoining structures. The Ce is composed of three subnuclei, namely the medial (CeM) and lateral (CeL) divisions, plus the lateral-most cell group in touch with the amygdaloid capsule, the capsular or paracapsular central amygdala (CeC). Some authors consider that the CeC includes a ventral portion of the caudate, recognized by other authors as an independent structure called the amygdalostriatal transition (Cassell *et al.*, 1999). The Me is usually divided into anterior (MeA) and posterior parts, the latter divided in turn into a dorsal (MePD) and a ventral division (MePV). With regard to the extra-amygdaloid BST, it includes the supracapsular part plus a myriad of subnuclei within the BST proper, most of which receive topographical names. A detailed description of the BST is beyond the scope of this review. The interested reader is referred to Moga *et al.* (1989) and Dong *et al.* (2001). To sum up, an anterior and a posterior division of the BST are generally recognized. The anterior BST is

composed of several cell groups surrounding the anterior limb of the anterior commissure caudal to the Acb. The posterior division consists of several cell groups caudal to the anterior commissure, which apparently impinge on the preoptic hypothalamus.

Data on the neurochemistry, connections, neuronal morphology, and the pattern of expression of homeotic genes during embryonic development should be used to trace the pallidostriatal boundary at the level of the subpallial amygdala. Swanson and Petrovich (1998) delineated the palliosubpallial boundary by studying the expression of GAD mRNA. Topology requires that those subpallial structures that are in contact with pallial ones be striatal derivatives. Thus, these researchers proposed that the medial and central amygdaloid nuclei constitute the caudal tip of the (ventral) striatum. In addition, it is generally agreed that the BST constitutes a part of the pallidal complex related to the septal or amygdaloid formations (Swanson and Risold, 1999), since it derives from the medial ganglionic eminence. Taking these considerations into account, the projections from the basolateral division of the amygdala to the central and medial amygdala (Dong *et al.*, 2001) should be interpreted as corticostriatal projections (palliostriatal, glutamatergic), whereas the well-known projections from the central and medial amygdaloid nuclei to the anterior and posterior (roughly) portions of the BST (Dong *et al.*, 2001) would represent striatopallidal projections (GABAergic). However, as discussed in the next section, a closer analysis of the available evidence indicates a more complex panorama.

15.2.3.2 The extended amygdala: A striatopallidal structure or a third subpallial compartment? On the one hand, the basolateral amygdala projects not only to the Ce and/or Me (corticostriatal intraamygdaloid pathways), but also portions of the BST (Adamec, 1989; Dong *et al.*, 2001). This projection is rich in zinc (Perez-Clausell *et al.*, 1989), thus glutamatergic, so that it represents a corticopallidal glutamatergic pathway that would contravene the scheme proposed for the organization of the cerebral hemispheres (but see Naito and Kita, 1994). On the other hand, the subpallial amygdala, including the BST, displays bidirectional intrinsic connections: as expected, the putative striatal compartments (Me and Ce) project to presumptive pallidal structures (anterior and posterior BST), but there are also important projections from the BST back to the Ce and Me (Ottersen, 1980; Coolen and Wood, 1998; McDonald *et al.*, 1999; Shammah-Lagnado and Santiago, 1999; Dong *et al.*, 2000; Shammah-Lagnado *et al.*, 2000; Dong and Swanson, 2003, 2004a, 2004b), which would represent pallidostriatal pathways. Therefore, the above-described general scheme on the organization of the cerebral hemispheres does not fit the hodological and histochemical features of the subpallial amygdala.

The exceptional properties of this region of the cerebral hemispheres have generated the idea that they belong to a third area of the subpallial telencephalon known as the extended amygdala (EA) (Alheid and Heimer, 1988; Olmos and Heimer, 1999; Shammah-Lagnado *et al.*, 1999), usually considered to be composed of two divisions, the central and medial EA (Alheid *et al.*, 1995). The medial EA is composed of the Me and the portions of the BST with which the Me is interconnected (roughly the posterior BST), plus a few intervening cell groups within the sublenticular substantia innominata and supracapsular BST (Shammah-Lagnado *et al.*, 2000). On the other hand, the Ce plus the anterior BST, together with a ring of additional cell groups linking these two structures both above (supracapsular BST) and below the internal capsule (fundus striatum or interstitial nucleus of the posterior limb of the anterior commissure (IPAC), within the sublenticular substantia innominata) make up the central EA. Data are more abundant and convincing for the existence of the central rather than the medial EA.

Although the internal capsule divides the EA into two apparently unconnected poles (Ce/Me amygdala and BST proper), the connective and histochemical properties of both poles of the EA are similar, thus suggesting a functional unity and a structural continuity (Roberts *et al.*, 1982). Thus, the Ce and those divisions of the anterior BST with which it is interconnected show a similar pattern of distribution of cells co-expressing different neuropeptides, such as corticotropin-releasing factor (CRF), neurotensin (NT), and enkephalin or substance P (SP)/somatostatin (SS) (Shimada *et al.*, 1989; Day *et al.*, 1999). In turn, the two main components of the medial EA, the Me and posteromedial BST, possess similar populations of vasopressinergic cells that are sexually dimorphic and display similar projections (Wang *et al.*, 1993).

Indeed, if they are interpreted as striatal or pallidal structures, the medial and central EA are also atypical in other respects besides the existence of intrinsic bidirectional connections and the presence of massive palliopallidal glutamatergic projections. Thus, the EA only receives a scarce tegmental innervation (compared with other

striatal compartments) that reaches presumed pallidal territories as well (BST; Hasue and Shammah-Lagnado, 2002). Moreover, the EA (at least its central division) displays long descending projections directed to the hypothalamus, periaqueductal gray, monoaminergic cell groups in the midbrain and brainstem, parabrachial region, and dorsal vagal complex (see below). This contrasts with the rest of the striatopallidal system, whose descending projections reach specifically the tegmental cell groups that originate their dopaminergic innervation.

The existence of the EA, or the usefulness of the concept of EA, is at the center of an intense debate (Canteras *et al.*, 1995; Swanson and Petrovich, 1998), from which two alternative views for the nature of the subpallial amygdala emerge: either it is viewed as a third subpallial compartment (neither striatal, nor pallidal), the EA, or it is envisaged as a patchwork of distinct striatal (Me and Ce) and pallidal territories (most of the BST). However, McDonald (2003) has recently put forward a third hypothesis that somewhat reconciles both views. According to his view, a certain mixing up of the striatal and pallidal cells has occurred in the caudal cerebral hemispheres. For instance, during embryonic development cells derived from the medial and lateral ganglionic eminences migrate tangentially (as has been shown to happen for the GABAergic cells in the pallium; Marin and Rubenstein, 2003), thus generating a series of structures where the striatopallidal boundaries are very fuzzy. This mixing up of striatal and pallidal cells may have generated special properties for the resulting cell groups, which constitute the above-mentioned distinctive features of the EA.

In line with this view, Cassell *et al.* (1999), using data on the cellular morphology, neurochemistry, and connections of the Ce, have proposed that it is composed of a striatal portion with a core-shell configuration (paracapsular and lateral divisions) and a pallidal one (the medial Ce). McDonald studied the morphology of Golgi-impregnated neurons in the Ce (McDonald, 1982b) and BST (McDonald, 1983). In both nuclei, he found a mixture of typical striatal medium-sized, spiny stellate cells (more abundant in the lateral aspect of both nuclei), with neurons displaying a pallidal morphology (long, thick, sparsely branched dendrites with few or no spines) more abundant in the medial Ce and posterior lateral BST. McDonald (2003) interprets these data as suggestive of a striatal nature of the lateral Ce and dorsolateral BST and of a pallidal or pallidostriatal nature of the rest of both nuclei. It is interesting to note that his drawings (McDonald, 1982a, 1983) show several cases of dendrites arising from cell bodies in the putative striatal compartment of the central EA that cross the boundaries to extend into the presumed pallidal (or striatopallidal) compartment (and vice versa). This dendritic exchange explains why the pallial and striatal projection fields overlap extensively within the central EA. A similar intermingling of striatal and pallidal cells has been suggested for nucleus X of the subpallium of songbirds (Perkel *et al.*, 2002).

This view is easy to test, since it predicts that inputs from pallial regions to the EA (e.g., zinc-positive fibers from the basolateral amygdala) should synapse onto (spiny) dendrites of striatal cells, whereas intrinsic connections within the EA should arise from striatal neurons (medium-sized spiny stellate cells) and terminate on pallidal ones.

15.3 Functional Neuroanatomy of the Mammalian Amygdala

Current ideas on the role of the mammalian amygdala in physiology and behavior are based on deep knowledge of its connections and on solid experimental evidence using techniques of functional neuroanatomy (lesion experiments, electrophysiology, and the study of the expression of immediate-early genes). In summary, it is generally accepted that the amygdala receives sensory information from many sources (brainstem, thalamus, olfactory bulbs, and different areas of the cortex) and gives rise to three main output pathways by which it modulates different aspects of behavior and physiology. First, the descending pathways of the central EA to hypothalamic and brainstem centers are involved in the generation of fear/anxiety responses (Davis, 2000; Ledoux, 2000), including motor, vegetative, and endocrine components (although the medial amygdala may also be involved in the endocrine responses to emotional stressors; Dayas *et al.*, 1999). Second, projections from the medial EA (and portions of the pallial amygdala) to hypothalamic centers probably constitute the pathway that mediates the neuroendocrine (and maybe some behavioral) responses to chemical cues (e.g., pheromones) in relation to reproduction (Newman, 2002; Halpern and Martinez-Marcos, 2003) but also to agonistic behaviors (Meredith and Westberry, 2004). Finally, the massive amygdalostriatal projections arising from the basolateral division of the amygdala and terminating in the ventral (but also in the dorsal) striatum seem involved in reward-related processes (Baxter and Murray, 2002). In this

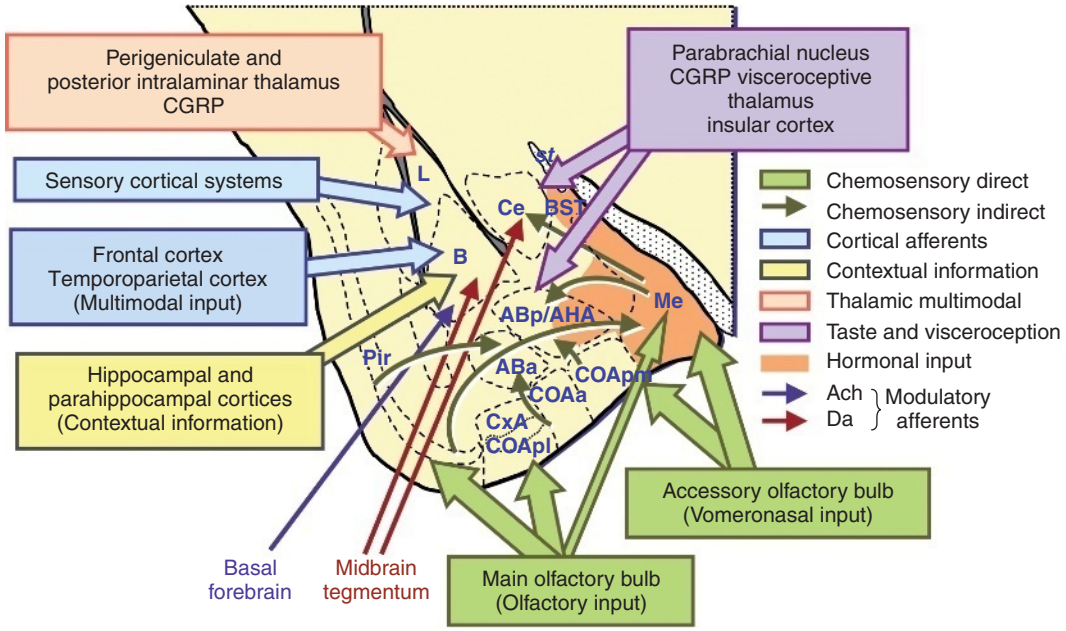

Figure 5 Inputs to the mammalian amygdala. Besides the olfactory and vomeronasal inputs to the corticomedial amygdala from the olfactory bulbs, the deep nuclei of the amygdala are targeted by brainstem, thalamic, and cortical afferents. The cortical inputs arise from different portions of the isocortex, including secondary and tertiary sensory areas, the frontal and temporal associative isocortex, the archicortex (hippocampal formation, *sensu lato*) and the paleocortex (see green arrows arising from the piriform cortex). The latter projection, together with the intra-amygdaloid projections from the corticomedial amygdala, makes the basolateral amygdala an olfactory vomeronasal-associative center. As detailed in the text, most of the nonolfactory afferents reach both the basolateral and the central amygdala. Among them, it should be noticed that part of the thalamic and brainstem (parabrachial) sensory afferents contains the peptide CGRP. Several modulatory afferents reach the amygdala from many different sources, among which the cholinergic and dopaminergic ones (arising from the basal forebrain and the ventral tegmentum, respectively) converge on the B nucleus.

context, the intricate intrinsic circuitry of the amygdala apparently provides a basis for convergence of different stimuli onto amygdaloid neurons (especially in the basolateral amygdala, Pitkanen, 2000), which by means of synaptic plasticity (Blair *et al.*, 2001) would lead to the establishment of learned responses (e.g., conditioned fear or secondary reward).

In order to review the functional anatomy of the amygdala we will first describe the neural pathways that convey sensory information to the amygdala. Other afferents with a less clear sensory significance but having a modulatory role will be described. Moreover, different cell groups in the amygdala express receptors to steroid hormones, thus providing endocrine inputs to the amygdala, which will also be reviewed. Finally, the outputs of the amygdala will be analyzed on the context of their role in the expression of behavior and physiologically related processes.

15.3.1 Inputs to the Amygdala

In addition to its direct olfactory and vomeronasal inputs, sensory information reaches the amygdala from three different relay stations (Figure 5). First, some brainstem centers receiving relatively direct projections from the sensory organs project to the amygdala. Second, nuclei of the dorsal thalamus provide the amygdala with unimodal or multimodal sensory afferents. Finally, several parts of the cortex convey highly processed sensory information to the amygdala.

15.3.1.1 The amygdala as part of the olfactory and vomeronasal systems The amygdala is the recipient of several afferents from primary or secondary chemosensory centers. The most direct and massive ones arise from the main and accessory olfactory bulbs and terminate in the cortical and medial amygdala. Thus, olfactory information reaches directly the LOT, CxA, COAa, and COApl (although, according to Scalia and Winans (1975), the LOT shows only fiber but not terminal degeneration after lesions of the main olfactory bulbs). On the other hand, the accessory olfactory bulbs provide a direct vomeronasal input to the BAOT, COApm, Me, and portions of the BST (Broadwell, 1975; Scalia and Winans, 1975). Thus, whereas the

main olfactory system includes several cortical areas (parts of the cortical amygdala plus the piriform and entorhinal cortices), there is a single vomeronasal cortex, the COApm. In fact, not only does it receive a dense input from the accessory olfactory bulb but also it projects back to it (Canteras *et al.*, 1992a; Martinez-Marcos and Halpern, 1999). Other connections of the COApm include projections to olfactory centers (piriform cortex and endopiriform nucleus) and to its contralateral counterpart, via the anterior commissure (Canteras *et al.*, 1992a).

However, the amygdala is also a tertiary olfactory center. In fact, the piriform cortex projects to the cortical amygdala (a projection that recalls the associative connections within the olfactory cortex), to parts of the AB and to the Me (McDonald, 1998). Moreover, other cortical areas receiving olfactory projections, such as the CxA (Shammah-Lagnado and Santiago, 1999), and the COApl (or periamygdaloid cortex; Majak and Pitkanen, 2003) do project to the deep pallial amygdaloid structures, such as the anterior AB, posterior B and L nuclei. In addition, the LOT projects massively and bilaterally to the B and parts of the L (Santiago and Shammah-Lagnado, 2004). There is an additional projection, which has important functional implications, from the COApl to the COApm (Canteras *et al.*, 1992a; Majak and Pitkanen, 2003).

The data reveiwed above suggest that the Me is an associative olfactory–vomeronasal center. This associative role of the Me is in agreement with the existence of direct projections from the main olfactory bulb to the anterior Me reported in several mammals, including rabbits, rats, and several species of opossum (Scalia and Winans, 1975; Shammah-Lagnado and Negrao, 1981), as well as the Madagascan hedgehog tenrec (Kunzle and Radtke-Schuller, 2000). Therefore, there is anatomical evidence for an olfactory–vomeronasal convergence in both the Me and COApm. The latter has been confirmed electrophysiologically (Licht and Meredith, 1987).

In a similar way, the amygdaloid nuclei receiving projections from the accessory olfactory bulb also give rise to intrinsic amygdaloid projections. Thus, the Me projects to parts of the Ce and to the medial part of the BST, but also to parts of the basolateral amygdala, including the posterior AB, the AHA, and parts of the L (Gomez and Newman, 1992; Canteras *et al.*, 1995). In addition, the COApm displays a set of projections to the subpallial amygdala, including the Me and portions of the BST, but also to pallial regions such as the AB (Canteras *et al.*, 1992a) and the posterior part of the B (our unpublished results in mice). Finally, the AHA receives a strong input from the BAOT (in addition to the projections already described from parts of the Me).

In conclusion, although the amygdala is usually considered to contain distinct, nonoverlapping olfactory and vomeronasal territories, most of the vomeronasal amygdala (MeA and COApm) receives convergent inputs from the main and accessory olfactory bulbs and it projects, in turn, to several secondary olfactory centers. In addition, intra-amygdaloid connections (superficial to deep), including an intricate set of interconnections within the basolateral division of the amygdala (Pitkanen *et al.*, 1997), allow the association of both modalities of chemosensory information in discrete nuclei and subnuclei of the basolateral division of the amygdala.

15.3.1.2 Brainstem sensory afferents: The amygdala as part of the gustatory, viscerosensory, and nociceptive systems Other kinds of chemosensory information also reach the amygdala quite directly. Thus, brainstem gustatory/visceroceptive centers, namely the nucleus of the solitary tract and parabrachial pons (Ricardo and Koh, 1978; Saper and Loewy, 1980; Cechetto and Saper, 1987; Halsell, 1992), as well as the gustatory/visceroceptive thalamus (parvicellular division of the ventroposterior nucleus, central medial, interanteromedial, and paraventricular thalamic nuclei; Turner and Herkenham, 1991) project to different parts of the amygdala. These projections not only terminate in the pallial (nuclear) amygdala (mainly AB and COAa) but also in the subpallial amygdala (CeL and parts of the BST; Zardetto-Smith and Gray, 1987; Volz *et al.*, 1990; Turner and Herkenham, 1991).

It is interesting to note that part of the projection from the parabrachial nucleus to the amygdala contains calcitonin gene-related peptide (CGRP) in humans (de Lacalle and Saper, 2000) and rodents (Schwaber *et al.*, 1988), where this peptide has been shown to coexist with SP (Yamano *et al.*, 1988b) and NT (Yamano *et al.*, 1988a). This projection rich in CGRP/NT/SP, seems to convey specifically nociceptive stimuli (Bernard *et al.*, 1993). In this respect, the CGRPergic innervation of the amygdala consists of a dense fiber plexus in the CeL and CeC, which extends to the so-called amygdalostriatal transition (Figure 6e) and to most of the anterior BST (central EA), but not to the medial EA (Kawai *et al.*, 1985; Kruger *et al.*, 1988; Yasui *et al.*, 1991). As we will see, part of the thalamus also seems to contribute to the CGRP innervation of the central EA and neighboring areas (Yasui *et al.*, 1991).

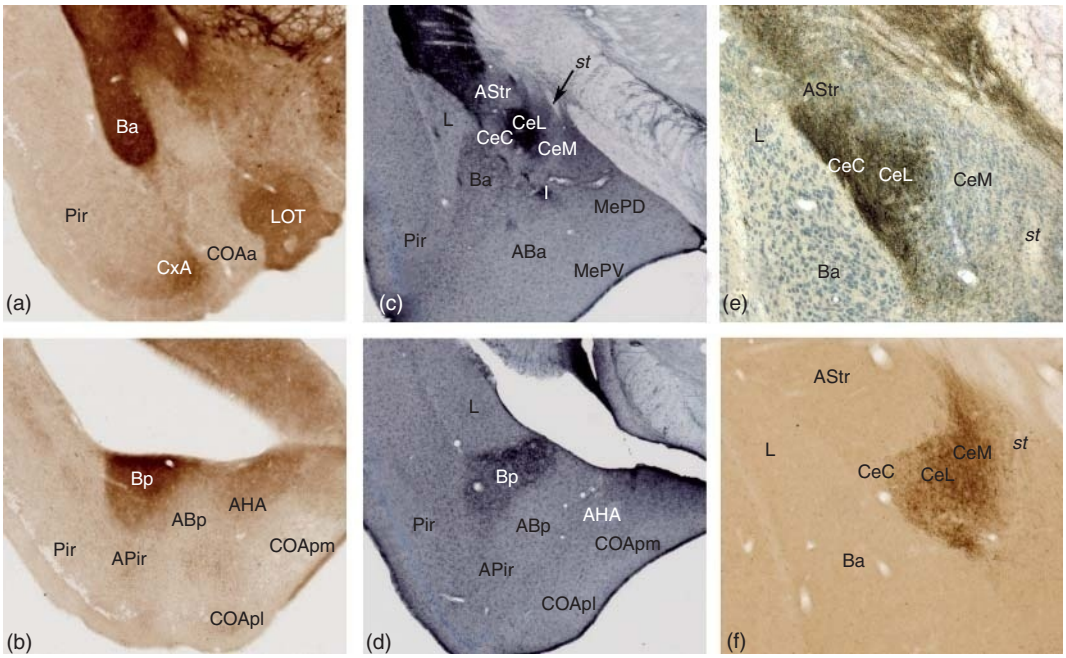

Figure 6 Histochemical features of the amygdala of mammals. a and b, Histochemical detection of AChase reveals a dense cholinergic innervation of the basal nucleus (both anterior, Ba; and posterior parts, Bp), as well as the LOT. c and d, The B (especially the Bp) also receives a distinctive dopaminergic innervation, as revealed by the immunohistochemical detection of dopamine transporter. e and f, The immunohistochemical detection of the peptides CGRP (e) and CRF (f) reveal the presence of two main compartments within the Ce. Thus, the capsular (CeC) and lateral (CeL) parts of the central amygdala display a dense innervation by CGRP-immunoreactive fibers (e). In contrast, most of the CRFergic cells are located in the medial aspect of the nucleus (CeM), although the CeL also displays a few CRFergic cells and fibers (f).

15.3.1.3 Thalamic inputs to the amygdala The amygdala is the target for several ascending pathways from the thalamus. As we have already described, the parvicellular division of the ventroposterior nucleus, central medial, inter-anteromedial, and paraventricular thalamic nuclei relay gustatory and viscerocceptive information to the amygdala. In addition, there are reports of afferents to the amygdala from the magnocellular (medial and dorsal) portions of the medial geniculate nucleus, posterior intralaminar, supra-geniculate, subparafascicular, and parataenial nuclei (Turner and Herkenham, 1991; Doron and Ledoux, 1999). The posterior intralaminar thalamus is the relay station for the main somatosensory pathways (dorsal column and spinothalamic pathways; Kunzle, 1994) and also receives visual and auditory information from the superior and inferior colliculi (LeDoux *et al.*, 1987; Linke, 1999; Linke *et al.*, 1999). Therefore, the posterior intralaminar thalamus conveys a mixture of somatosensory (mainly nociceptive and thermoceptive), visual, and auditory information to the central and basolateral amygdala (Bordi and Ledoux, 1994). This projection reaches both the pallial (Doron and Ledoux, 1999) and subpallial amygdala (Ce plus amygdalostriatal transition; Turner and Herkenham, 1991). Although most of the thalamic projections to the amygdala are glutamatergic (LeDoux and Farb, 1991), part of the projection from the posterior intralaminar thalamus also contains CGRP (Yasui *et al.*, 1991).

15.3.1.4 Highly processed sensory inputs: The cortical afferents to the pallial and subpallial amygdala All three main areas of the cortex project to the amygdala: (1) isocortex; (2) paleocortex (piriform cortex and adjacent areas); and (3) archicortex (hippocampus or Hp). For a thorough review of the corticoamygdaloid connections, the reader is referred to McDonald (1998). The pallial amygdala stands as the endpoint of a cascade of projections from the sensory areas of the isocortex. As a rule, projections to the amygdala from primary sensory cortical areas are scarce, if at all present, whereas secondary and associative sensory cortical areas display massive projections to the amygdala. This is true for the auditory temporoperirhinal cortex (Vaudano *et al.*, 1991; Mascagni *et al.*, 1993; Romanski and Ledoux, 1993; Shi and Cassell, 1999) and for the somatosensory parietal posterior insular cortex (Shi and Cassell, 1998a). The

gustatory visceroceptive anterior–posterior insular cortex (Shi and Cassell, 1998b) is exceptional, since both primary and secondary associative areas display important projections to the amygdala. Visual cortical areas also show a cascade-like projection to the amygdala, which is especially clear in cats and primates, where the bulk of the visual inputs to the amygdala arise from visual areas in the inferior temporal lobe, involved in complex higher-order visual processing (Shi and Davis, 2001). Cortical sensory inputs mainly reach the basolateral amygdala (L and B nuclei), though many of the cortical areas also show relatively minor projections to the lateral Ce (McDonald, 1998).

In addition, high-order associative areas of the cortex, such as the hippocampal and parahippocampal cortices (including the subiculum and entorhinal areas) project heavily to the amygdala (Brothers and Finch, 1985; Aggleton, 1986; Canteras and Swanson, 1992; McDonald and Mascagni, 1997). These connections are usually interpreted as providing the amygdala with information about the spatial and temporal context in which events take place (Ergorul and Eichenbaum, 2004). This is demonstrated by the fact that hippocampal lesions impair the capacity of acquiring fear to a context (test cage) where a foot shock is systematically given to the animals, but not to a pure tone with which the foot shock is paired (Phillips and Ledoux, 1992). Hippocampal/parahippocampal projections target the basolateral amygdala (mainly the L, B, and AHA). In addition, parts of the hippocampal and parahippocampal cortex (especially the entorhinal cortex; McDonald and Mascagni, 1997) also project massively to the CeL, Me, and intra-amygdaloid BST (Canteras and Swanson, 1992).

Finally, with minor differences, all the mammals studied (monkeys, cats, and rats; McDonald, 1998) display robust, topographically organized prefronto-amygdaloid projections. These include not only the aforementioned inputs from the anterior insular cortex, but also a set of projections from the medial (prelimbic, infralimbic, and anterior cingulate) and ventrolateral (orbitary) prefrontal areas (Carmichael and Price, 1995; McDonald *et al.*, 1996; Ghashghaei and Barbas, 2002). The prefrontal afferents terminate massively in the basolateral amygdala (B, AB, and L nuclei) but also in parts of the cortical (COAa and COApl) and the subcortical amygdala (CeL, MeA, and parts of the BST). Convergence of all the prefrontal afferents seems to occur, especially upon the B (and to a lesser extent the AB). Instead of providing information on the spatial, temporal, or spectral configuration of specific stimuli, or on the chemical nature of odorants (and pheromones), the prefrontal cortex seems to convey information on the outcomes of detecting incoming stimuli in terms of reward/aversion. In other words, the connections of the prefrontal cortex and amygdala are the substrate for emotional tagging of incoming stimuli by establishing, maintaining, or modifying associations of stimuli with reward (Gaffan *et al.*, 1993; Rolls, 2000) or aversion (Garcia *et al.*, 1999; Morrow *et al.*, 1999). In this context, it has been shown that lesions of the medial prefrontal cortex of rats interfere with the extinction of conditioned fear to a tone (Morgan *et al.*, 1993; Lebron *et al.*, 2004). This indicates that extinction of conditioned fear is an active process that involves modulation of the activity of neurons in the amygdala by prefrontal inputs (Milad and Quirk, 2002).

15.3.1.5 Redundant sensory pathways to the amygdala? All these anatomical data indicate that sensory information reaches the amygdala using two different gateways, namely thalamic (plus direct brainstem) afferents and cortical pathways. In this respect, conditioned fear to a simple tone is acquired by rats with either lesions of the medial division of the medial geniculate body (MGm) or of the auditory cortex (including temporal and perirhinal areas), but not by rats with combined cortical and thalamic lesions (Romanski and Ledoux, 1992). This suggests that the thalamic and cortical sensory pathways to the amygdala are redundant (for an alternative interpretation, see Shi and Davis, 2001). Nevertheless, it is likely that both pathways convey different kinds of information. Thus, the thalamic route provides the amygdala with crude sensory information that, nevertheless, can be biologically meaningful, such as loud noises, big moving objects or shadows (e.g., looming objects), pain, visceral sensations or sweet, salty, or bitter taste. In contrast, the cortical sensory pathway provides the amygdala with highly processed sensory information mediating recognition of stimuli with complex spatial (visual stimuli; Tanaka, 1996) or spectrotemporal configuration (e.g., species-specific vocalizations; Wang and Kadia, 2001), multimodal contextual information (hippocampal and parahippocampal (APH) areas), or information about the possible outcomes (reward/aversion) of the incoming stimuli (prefrontal cortex). Although both pathways can be used to elicit responses to simple stimuli (a pure tone), responding to complex stimuli surely requires the intervention of the cortical loop.

This is supported by physiological studies in different species indicating that neurons in the amygdala robustly respond to vocalizations of conspecifics and to biologically relevant sounds emitted by related species (Sawa and Delgado, 1963; Kling *et al.*, 1987), as well as to meaningful visual stimuli such as faces from conspecifics or closely related species (AB nucleus of monkeys; Leonard *et al.*, 1985) or sexually arousing images (humans; Hamann *et al.*, 2004). Interspecies differences are expected on the kind of sensory cortical processing performed (thus, in the cortical areas involved). Thus, primates are specialized in using visual and auditory stimuli for intra- and interspecies recognition, whereas most of the remaining mammals would use auditory or olfactory/vomeronasal cues instead.

15.3.1.6 Modulatory afferents: Cholinergic projections from the basal forebrain and tegmental monoaminergic afferents Afferents to the amygdala also include several neurochemically identified afferents. This has both functional and comparative implications. On the one hand, these afferents are surely playing a modulatory role of the amygdaloid function. On the other hand, using (immuno)histochemical tools, the terminal fields of these afferents are easily revealed and help to identify and delineate some of the amygdaloid nuclei (chemoarchitecture). Concerning this, the cholinergic and dopaminergic afferents are especially useful from a comparative viewpoint.

The innervation of pallial derivatives by basal forebrain cholinergic cell groups (Ch1–Ch4, according to the classification by Mesulam *et al.*, 1983) is part of the fundamental circuit of the cerebral hemispheres of, at least, tetrapod vertebrates (Medina and Reiner, 1994; Marin *et al.*, 1997c; Lanuza *et al.*, 2002) and seems to be present in some teleost species (Rodriguez-Moldes *et al.*, 2002; Mueller *et al.*, 2004). Therefore, cholinergic innervation is a neurochemical feature with an added value for comparative neuroanatomy. In this respect, the mammalian amygdala is richly innervated by cholinergic fibers (Amaral and Bassett, 1989), immunoreactive for choline acetyltransferase (ChAT), and positive for acetylcholinesterase (AChase histochemistry). The densest patches of cholinergic innervation are found in the B nucleus and the LOT (Figures 6a and 6b), which represent dense afferents from the Ch4 cholinergic cell group, namely the nucleus basalis–substantia innominata complex (Hecker and Mesulam, 1994).

Second, all the vertebrates studied show important ascending projections to the forebrain from dopaminergic tegmental cell groups (Smeets and Reiner, 1994). This consists of tegmentostriatal projections arising from groups A9 (SN) and A10 (VTA) that mainly terminate in the dorsal and ventral striatum respectively. Indeed, some of the putative striatal components of the mammalian amygdala, such as the Ce (Figure 6c) and the anterior and posterolateral BST (Freedman and Cassell, 1994; Freedman and Shi, 2001) display dopaminergic fibers. These fibers arise from the dorsocaudal A10 group, e.g., dopaminergic cells caudal to the VTA and medial to the SN, within the cytoarchitectonic boundaries of the dorsal raphe nucleus and periaqueductal gray (Hasue and Shammah-Lagnado, 2002). In addition, portions of the pallium are specifically innervated by dopaminergic tegmental cell groups, as is the case for the prefrontal cortex (mesocortical pathway) and the basolateral amygdala. Specifically, a dense plexus of dopaminergic fibers, mainly arising from A10, innervates the caudal aspect of the B nucleus (Fallon *et al.*, 1978; Brinley-Reed and McDonald, 1999; Figures 6c and 6d).

In addition, the amygdala displays distinct serotonergic and adrenergic innervations (Emson *et al.*, 1979; Sadikot and Parent, 1990; Canteras *et al.*, 1992a; Asan, 1998) arising from the raphe complex, and the locus coeruleus and A1/C1 A2/C2 medullary adrenergic cell groups respectively (Myers and Rinaman, 2002). Some of the adrenergic projections from the A1 group also contain neuropeptide Y (Zardetto-Smith and Gray, 1995).

Finally, the amygdala receives projections from diverse hypothalamic nuclei, such as the ventromedial (VMH; Canteras *et al.*, 1994) and anterior nuclei (Risold *et al.*, 1994), the lateral hypothalamic area (a projection that contains dynorphin; Zardetto-Smith *et al.*, 1988) and the ventral premammillary nucleus (PMv; Canteras *et al.*, 1992b). These nuclei receive strong inputs from parts of the amygdala (see below). The VMH displays diffuse projections to the medial, central, and basolateral amygdala whereas the PMv is interconnected with the principal nucleus of the BST, the MePD, as well as the AHA and adjacent portions of the COApm.

15.3.1.7 Hormonal inputs to the amygdala Besides receiving inputs from numerous sensory and modulatory centers of the brain, the amygdala is also the target for the action of steroid hormones. In fact, together with the hypothalamus, the medial EA (Me and posteromedial BST) is the area of the brain with the highest density of cells concentrating both estrogens and androgens (Pfaff and Keiner, 1973; Warembourg, 1977).

Immunohistochemical and *in situ* hybridization studies on the distribution of androgen and (α and β) estrogen receptors (Simerly *et al.*, 1990; Lu *et al.*, 1998; Mitra *et al.*, 2003; Perez *et al.*, 2003) reveal that sexual steroids influence not just the medial EA but also the remaining telencephalic centers projecting to the medial preoptic and ventrolateral aspect (shell) of the ventromedial hypothalamus. Thus, the ventral lateral septum and the AHA are among the nuclei displaying the highest concentration of steroid-sensitive cells in the forebrain. Since, at least in rodents, the VMH and medial preoptic nucleus are known to control feminine (Blaustein and Erskine, 2002) and masculine (Hull *et al.*, 2002) sexual behaviors, the receptors to gonadal steroids in the amygdala and septum are thought to be part of the mechanism for the endocrine control of copulatory behavior. However, other parts of the amygdala that do not project substantially to these hypothalamic centers also show a high (COAa) or moderate density (COApl, and, to a lesser degree, the COApm) of neurons expressing sexual steroid receptors. This suggests additional roles of sexual steroids in the modulation of amygdala-mediated behavioral and/or physiological processes.

Corticosteroids may also influence amygdaloid function. In fact, most of the peptidergic cells in the central EA display receptors to corticosteroids (Cintra *et al.*, 1991; Honkaniemi *et al.*, 1992). This is supposed to mediate stress-induced changes in the expression of peptides by the projection cells of the central EA, as part of the stress-adaptive changes (Palkovits, 2000). The medial EA is also rich in neurons expressing glucocorticoid receptors (Honkaniemi *et al.*, 1992) and may participate in stress responses (Dayas *et al.*, 1999). In addition, the basolateral amygdala displays low levels of receptors to glucocorticoids for which a role in modulating memory acquisition has been proposed (Roozendaal and McGaugh, 1997).

15.3.2 Outputs of the Amygdala

The influence of the amygdala on behavior is mediated by amygdaloid projections to diverse neural centers (Figure 7). Attaining their sites of

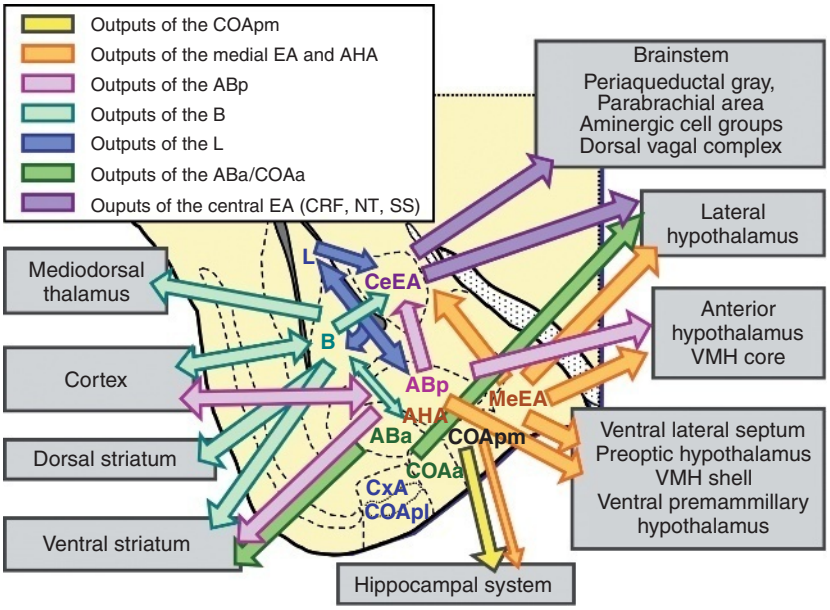

Figure 7 Schematic view of the main outputs of the mammalian amygdala. As a part of the pallium, the basolateral amygdala is engaged in connections with the rest of the pallium, the striatum, and the thalamus. In this respect the B nucleus constitutes the main (but not the only) source of outputs from the amygdaloid pallium. In contrast, the L is almost exclusively engaged in intra-amygdaloid connections. Since it is targeted by many sensory cortical and subcortical inputs, it is usually considered the sensory interface of the basolateral amygdala. The CeEA gives rise to long-distance descending projections to different centers of the hypothalamus, tegmentum, pons, and medulla. These projections seem to constitute the anatomical substrate of fear/anxiety reactions. Massive projections from the basolateral to the central (extended) amygdala allow fear reactions to different stimuli. The medial EA, together with the AHA, project to a sexually dimorphic forebrain system, including parts of the septum and the preoptic tuberal and premammillary hypothalamus. This seems to allow reproductive/agonistic behaviors elicited by vomeronasal-detected pheromones. In addition, the medial EA and AHA project to other parts of the hypothalamus (anterior nucleus and VMH core) involved in the expression of defensive behaviors. The basolateral amygdala, more specifically the posterior part of the AB, also projects to the circuit of defensive behavior.

origin and of termination, amygdaloid projections can be classified into five main pathways. Most of the pallial regions of the amygdala, and some subpallial centers, give rise to projections to striatal territories, including the dorsal and ventral striatum (*sensu stricto*) and parts of the lateral (striatal) septum. Second, parts of the pallial amygdala project to the ventromedial hypothalamus through the stria terminalis, and to the lateral hypothalamus through the ansa lenticularis. Third, the EA also projects to the several hypothalamic centers using both strial and nonstrial pathways. In addition, the central EA gives rise to long-distance projections directed to the pons and medulla (long descending projections of the amygdala). Finally, the cortical afferents to the pallial amygdala are reciprocated by a series of amygdalocortical pathways. This is complemented with projections from the pallial amygdala to the discrete regions of the dorsal thalamus.

15.3.2.1 Projections of the pallial amygdala to the basal telencephalon Virtually all the nuclei in the pallial amygdala project to striatal territories. The main amygdalostriatal pathway originates in the basolateral division of the amygdala and terminates in a continuum of structures within the ventral striatopallidum, which extends from the Ce caudally, to the shell of the Acb rostrally, and roughly defines the central EA. This projection mainly arises from the B and AB (Kelley *et al.*, 1982; Brog *et al.*, 1993; Petrovich *et al.*, 1996; Wright *et al.*, 1996; Dong *et al.*, 2001).

In addition, the basal nucleus (B), and, to a much lesser extent, the AB and L, are the origin of a massive projection to the dorsal striatum (CPu; Kelley *et al.*, 1982; Wright *et al.*, 1996). In contrast to the amygdaloid projection to ventral striatal territories, the projection from the B to the CPu is bilateral and is organized so that each portion of the amygdala projects to equivalent points of the striatum in both hemispheres.

Projections from the basolateral amygdala to the ventral striatum have been implicated in stimulus–reward associations (Everitt *et al.*, 1999) and, in fact, the basolateral amygdala is a focus for self-stimulation (through implanted electrodes) with a low-intensity threshold (Kane *et al.*, 1991a, 1991b).

The AHA displays a distinctive pattern of projections to the basal telencephalon (Canteras *et al.*, 1992a). Thus, in contrast to the B and AB, the AHA does not project to the central EA but rather to the medial EA (Me and posteromedial BST). This projection continues further rostrally to reach the ventral lateral septum and, to a lesser degree, the shell of the Acb, the olfactory tubercle, and the substantia innominata.

15.3.2.2 Projections of the extended amygdala to the striatopallidal telencephalon One of the features of the EA, in which it differs from the striatopallidum, is the presence of important reciprocal connections between the BST and the intra-amygdaloid EA (Me and Ce; see above). The Me gives rise to additional projections to the basal forebrain. First, the Me and the posteromedial BST also project to two striatal territories, the medial shell of the Acb and parts of the olfactory tubercle, as well as to the ventral aspect of the lateral septum (Canteras *et al.*, 1995). These projections mainly arise from the MeA. The projection of the Me to the lateral septum is known to contain vasopressin and shows a clear sexual dimorphism (Wang *et al.*, 1993).

As expected, the Me is interconnected with the posteromedial BST, a connection that defines the medial EA. However, the ventral aspect of the MeA also projects to the central EA (Canteras *et al.*, 1995; Shammah-Lagnado *et al.*, 1999; Dong *et al.*, 2001). This connection from the medial to the central EA is unidirectional. In fact, although the Ce shows minor intra-amygdaloid projections (Jolkkonen and Pitkanen, 1998), it projects neither to the Me nor to the posteromedial BST (Dong *et al.*, 2001). The functional significance of these connections is not properly understood. Nevertheless, since the Me is dominated by chemosensory inputs (mainly vomeronasal), its projections to the central EA and lateral septum are likely involved in orchestrating fear/anxiety (Ce) and other behavioral reactions (ventral lateral septum) to chemical cues derived from conspecifics (agonistic encounters and territorial behavior: Compaan *et al.*, 1993; Kollack-Walker *et al.*, 1997) or from predators (Meredith and Westberry, 2004). This is further discussed at the end of this article.

15.3.2.3 Projections from the pallial amygdala to the hypothalamus The second major output pathway for the amygdala is the stria terminalis. Although this is usually viewed as a single, homogeneous tract, it is indeed composed of fibers from different pallial and subpallial centers. The pallial component of the stria terminalis arises from cells within the boundaries of the posterior AB (ABp) and the AHA (Price *et al.*, 1991). The pallial stria terminalis in fact includes two different projections. The ABp projects to the core of the VMH (Petrovich *et al.*, 1996). On the other hand, the AHA (plus maybe the deepest parts of the COApl) projects the

anterior and preoptic hypothalamus (mainly to the medial preoptic nucleus), to the shell of the VMH and ventral lateral hypothalamic area in the tuberal hypothalamus, and to the PMv (Canteras *et al.*, 1992b). The pallial components of the stria terminalis are characteristically positive for zinc (Haug, 1973; Perez-Clausell *et al.*, 1989; Howell *et al.*, 1991), thus indicating their glutamatergic nature.

The pallial amygdala gives rise to additional projections to the hypothalamus. Thus, the anterior AB (ABa) and the COAa send a major projection to the lateral hypothalamic area (Price *et al.*, 1991; Canteras *et al.*, 1995; Petrovich *et al.*, 1996). This allows quite a direct influence of olfactory stimuli (received by the cortical amygdala) on physiology and behavior (probably reproductive and/or ingestive).

15.3.2.4 Projections from the extended amygdala to the hypothalamus The two main components of the subpallial amygdala are the sites of origin of projections directed to the BST that continue to the hypothalamus. Thus, the Me mainly projects to the posteromedial BST (a projection that delineates the so-called medial EA) and the whole medial EA projects to the preoptic hypothalamus, the anterior hypothalamic nucleus, the VMH, and the PMv (Kevetter and Winans, 1981; Price *et al.*, 1991; Canteras *et al.*, 1995; Dong and Swanson, 2004b). In contrast, the whole central EA projects to the posterior lateral hypothalamus (Price *et al.*, 1991; Bourgeais *et al.*, 2001) and the paraventricular nucleus (Gray *et al.*, 1989; Dong and Swanson, 2003, 2004a).

Projections from the amygdala to the hypothalamus may allow an influence of different stimuli (received directly and indirectly through the diverse pathways we have previously described), including pheromonal and olfactory ones (Me), in the expression of reproductive and agonistic behaviors (Canteras *et al.*, 1994, 1995). In addition, the direct and indirect projections from the amygdala (Ce) to the preoptic and paraventricular hypothalamus may be involved in the control of neuroendocrine responses associated with sexual and agonistic behaviors, as well as in responses associated with fear and stress (see below).

The central EA displays one of the densest populations of peptidergic cells of the cerebral hemispheres (Shimada *et al.*, 1989; Day *et al.*, 1999). Specifically, the Ce and the anterior and posterolateral BST contain cells immunoreactive for CRF (Figure 6f), NT, SP, and SS. Some of these peptides coexist in the same cells. Specifically, Shimada *et al.* (1989) described a population of cells co-expressing CRF and NT, and a second population in which SP and SS coexist. Peptidergic cells seem to be the projection neurons of the EA. For instance, the pathways from the Ce and Me to the BST, as well as the long-distance pathways directed to the lateral hypothalamus, are rich in SS/SP (Sakanaka *et al.*, 1981), NT (Allen and Cechetto, 1995), and CRF (Sakanaka *et al.*, 1986).

15.3.2.5 Amygdaloid projections to the brainstem Projections from the medial amygdala also reach portions of the periaqueductal gray, the VTA, and the midbrain raphe (Canteras *et al.*, 1995). In contrast the Ce displays much more abundant and long-distance projections that target centers in the midbrain and brainstem (Krettek and Price, 1978; Cassell *et al.*, 1986; Petrovich and Swanson, 1997; Bourgeais *et al.*, 2001). These include projections to most of the monoaminergic cell groups of the midbrain and brainstem, such as the dopaminergic cells in the VTA (A10), SN (A9), and retrorubral field (Gonzales and Chesselet, 1990; Vankova *et al.*, 1992; Dong and Swanson, 2003, 2004a), the adrenergic cells of the locus coeruleus (A6), as well as the noradrenergic and adrenergic cells in the nucleus of the solitary tract (C2/A2) (Wallace *et al.*, 1989). In addition, the Ce projects to the parabrachial nucleus and the NTS (Danielsen *et al.*, 1989; Petrovich and Swanson, 1997; Dong and Swanson, 2003, 2004a). Many of the neurons giving rise to these projections of the central EA are peptidergic (like the amygdalohypothalamic cells; see above). Thus amygdalonigral projections are rich in Met-enkephalin, dynorphin, and NT (Vankova *et al.*, 1992), whereas a low proportion of the Ce neurons projecting to the parabrachial region and nucleus of the solitary tract contain NT, SS, SP, or CRF (Veening *et al.*, 1984). In spite of the low proportion of projecting cells in the central EA containing this peptide, CRF-immunostaining depicts a nice amygdalofugal pathway, which is especially useful for comparative purposes. Thus, CRFergic fibers arising from the immunopositive cells in the CeL and CeM (Shimada *et al.*, 1989; Day *et al.*, 1999) and dorsolateral anterior BST course within the medial forebrain bundle and the periventricular system to innervate the substantia innominata, the medial and lateral preoptic areas, lateral hypothalamic area, central gray (Gray and Magnuson, 1992), laterodorsal tegmental nucleus, locus coeruleus (Van Bockstaele *et al.*, 2001), parabrachial nucleus, dorsal vagal complex, and regions containing the A1 and A5 catecholamine cell groups (Swanson *et al.*, 1983; Sakanaka *et al.*, 1987).

There is compelling evidence indicating that neuropeptide-rich descending projection systems arising from the central EA constitute the anatomical substrate for different fear reactions elicited by diverse stimuli under different experimental conditions (Ledoux *et al.*, 1988; Hitchcock and Davis, 1991; Rosen *et al.*, 1991; Walker and Davis, 1997; Kalin *et al.*, 2004; Sullivan *et al.*, 2004). In this context, CRF plays a double role in modulating fear, anxiety, and stress as both a neurohormone and a central neurotransmitter in the main descending projection of the central EA.

15.3.2.6 Amygdalocortical and amygdalothalamic projections Besides displaying projections to executive areas of the brain (striatum, hypothalamus, midbrain, and brainstem), the amygdala also originates ascending projections that target several areas of the cortex and projections to the thalamus that can influence cortical function.

As a rule, connections between the cortex and the pallial amygdala are reciprocal. Those areas of the pallial amygdala that receive the bulk of the cortical input, namely the L, B, and the anterior portion of the AB (Krettek and Price, 1978; Porrino *et al.*, 1981; Amaral and Price, 1984; Petrovich *et al.*, 1996), give rise to projections to the cortical fields that provide the most important inputs to the amygdala. Thus, amygdalocortical pathways mainly terminate in the prefrontal cortex (infralimbic, prelimbic, and anterior insular areas), the posterior insular and perirhinal cortices, as well as portions of the entorhinal cortex. Although there are several differences in the pattern of cortical projections of all three areas of the amygdala, probably the most striking one is the fact that the B (at least its anterior part) projects bilaterally to the cortex (Granato *et al.*, 1991). In addition to these projections, the AHA seems to be the interface area in the connections with the hippocampal formation (Canteras *et al.*, 1992a).

Amygdalocortical pathways constitute a likely substrate for modulation of memory storage within the cortex and of attention. Thus, stimuli related to emotional situations (such as fear, anxiety, or attraction and pleasure) are easily recalled. Beta-adrenergic transmission plays an important role in fear and anxiety enhancement of memory processes (Cahill and McGaugh, 1998). Therefore, it is likely that projections of the central EA (apparently mediating the emotional response of fear and anxiety; see above) to brainstem adrenergic cell groups (including locus coeruleus) are responsible for some of these effects. In addition, an action of corticosteroids on key brain structures such as the Hp also seems to account for fear and stress enhancement of memory acquisition (Blank *et al.*, 2003a, 2003b; Roozendaal *et al.*, 2003). Nevertheless, other emotional responses such as those elicited by rewarding stimuli, or by stimuli associated with reward, are probably mediated by amygdaloventral striatal pathways arising from the basolateral amygdala (Everitt and Robbins, 1992). Since the same areas that project to the ventral striatum are engaged in massive amygdalocortical projections (AB and B), it is tempting to suggest that corticosteroids may also play a role in memory enhancement by rewarding events. In addition, the amygdaloid input to the prefrontal (at least to the orbitofrontal) cortex has been shown to be necessary for maintaining in this structure an active representation of reward-predictive information (Schoenbaum *et al.*, 2003).

On the other hand, projections from the amygdala to the cortical areas providing sensory information can be regarded within the cortical circuitry as a feedback loop similar to the ones present within the cortex itself. Feedback pathways allow modulation of sensory processing in low-level sensory areas by higher-order ones, thus directing (for instance) attention to specific details of the sensory fields. This makes the amygdala a key structure within the circuitry of the isocortex.

Finally, the amygdala also projects to specific portions of the thalamus. Reardon and Mitrofanis (2000) have analyzed the amygdalothalamic pathways of the rat. Their results indicate that the amygdala displays reciprocal connections with the midline (e.g., paraventricular, parataenial) and intralaminar thalamus (including the medial division of the medial geniculate nucleus), to which all the divisions of the amygdala contribute (central, medial, olfactory, and basolateral). In addition, the basolateral amygdala (and, to a lesser extent, the medial and central amygdaloid divisions) projects to the mediodorsal nucleus and to the rostral zona incerta. Since the mediodorsal thalamus projects massively to portions of the prefrontal cortex (Porrino *et al.*, 1981), this constitutes an additional, indirect pathway for connections between the amygdala and prefrontal cortex. Nevertheless, both pathways to the prefrontal cortex may be functionally very different. McDonald (1987) demonstrated that the amygdaloid projection to the prefrontal cortex arises from class I cells, most of which are glutamatergic (McDonald *et al.*, 1989). In contrast, neurons within the basolateral amygdala projecting to the mediodorsal thalamus belong to class II cells (nonpyramidal), and the great majority of them did not exhibit glutamate or aspartate

immunoreactivity (McDonald, 1996). This is further supported by the lack of histochemically detectable zinc in the mediodorsal thalamus (Mengual *et al.*, 2001). The fact that the Ce and Me nuclei, which probably lack glutamatergic cells (Christensen and Geneser, 1995; Brown and Dyck, 2004) but are rich in GABAergic neurons (Swanson and Petrovich, 1998) also project to the mediodorsal thalamus supports the view that the amygdaloid projection to this thalamic nucleus is something more than a simple relay to the prefrontal cortex.

15.4 The Mammalian Amygdala: A Summary

The amygdala of mammals is composed of pallial and subpallial structures. The pallial amygdala (Table 1) is a mixture of lateropallial and ventropallial derivatives. The lateral pallium includes superficial (cortical) olfactory centers, including the COApl and maybe transitional areas such as the piriform (APir) and entorhinal cortices (TR). The only deep lateropallial structure of the amygdala is the B nucleus, which is also characterized by a dense cholinergic (AChase-positive) innervation and a less dense dopaminergic input from the midbrain tegmentum (mainly terminating in its caudal pole). Besides being involved in a complex intraamygdaloid circuitry, the B gives rise to a bilateral projection targeting both the dorsal and ventral striatum that constitutes the bulk of the amygdalostriatal pathway. Moreover, the B is bilaterally interconnected with the LOT and parts of the isocortex (mostly the prefrontal cortex), and projects to the mediodorsal thalamus.

The ventropallial amygdala also includes cortical superficial and deep nuclei. The cortical ventropallial amygdala is composed of olfactory areas, such as the COAa and LOT, and vomeronasal cortices, namely the BAOT and COApm. The COAa is especially rich in receptors to sexual steroids and, by means of its direct and indirect (through the anterior AB) projections to the lateral hypothalamus, constitutes a link for the transfer of olfactory information to the hypothalamus. The LOT is interconnected with the B, with which it shares a dense cholinergic and dopaminergic innervation as well as important contralateral connections through the anterior commissure. The COApm stands as a specialized vomeronasal cortex that projects to other secondary vomeronasal centers and back to the AOB, to olfactory centers (piriform and endopiriform), and to its contralateral counterpart.

The deep ventropallial amygdala is composed of the L, AB, and AHA nuclei. The former is usually considered as the sensory interface of the basolateral amygdala, since it is the target of most cortical and thalamic inputs, but shows projections virtually restricted to the remaining nuclei of this amygdaloid division. The AB projects to the ventral striatum and cortex. Moreover, it shows a double projection to the hypothalamus. Its anterior part (ABa), together with the overlying COAa (from which it receives a dense input), originates a projection to the lateral hypothalamus via the ansa lenticularis. The posterior AB, together with the AHA, gives rise to the pallial portion of the stria terminalis, and provides a glutamatergic and zinc-positive projection to the BST and medial hypothalamus (VMH). Finally, the AHA should be considered a deep pallial vomeronasal center since it is interconnected with the vomeronasal amygdala (mainly with the BAOT and medial EA), with which it shares a common pattern of afferents and efferents and the presence of receptors to sexual steroids.

The deep pallial amygdala gives rise to a massive projection to the central EA. This projection arises from all the nuclei of this amygdaloid division, with the exception of the anterior B and the AHA, the latter projecting to the medial EA instead.

The subpallial amygdala (Table 2) consists of two main divisions, namely the medial and the central EA. The medial EA is composed of the Me (with its different subdivisions that receive topographic names) and the posteromedial BST, which are deeply interconnected. It is dominated by cascade-like input from the AOB that includes direct and indirect projections through the COApm and AHA, and it is interconnected with the olfactory system. The whole vomeronasal amygdala (medial EA, AHA, COApm) is rich in receptors to steroid hormones. Its main outputs reach parts of the ventral striatum (mainly the olfactory tubercle), and different portions of the hypothalamus, including the preoptic, tuberal, and premammillary hypothalamus.

In addition, the medial EA shows projections to the basolateral amygdala, mainly targeting the ABp and L, as well as to the CeM. This interconnection allows the interplay between the medial and central EA, probably needed for the signaling of the attractive/aversive properties of conspecific chemical cues (such as pheromones) and associated stimuli.

The central EA (Table 2) is composed of the Ce (with medial, lateral, and capsular divisions) and the anterior and posterolateral BST, which are deeply interconnected. In contrast to the medial, the central EA does not express a high level of receptors to

Table 1 Summary of the characteristic features of the different nuclei of the mammalian pallial amygdala. In the columns describing the main inputs and outputs, the term 'intrinsic' has been used to refer to the existence of connections with other pallial amygdaloid nuclei. Most of these projections arise from the cortical amygdala and reach the basolateral (deep pallial) nuclei. In addition, there is an intricate set of interconnections among the nuclei in the basolateral amygdala

Pallial origin	*Topological position*	*Nucleus*	*Main afferents*	*Main efferents*	*Neurochemistry and other features*
Lateral pallium	Superficial	COApl	Main olfactory bulb	Intrinsic	Rich in receptors to sexual steroids
				Hippocampus (CA1, ventral subiculum)	Also called periamygdaloid cortex (see Majak and Pitkanen, 2003)
				Cortex	
	Deep	B	Intrinsic	Intrinsic	Cholinergic innervation
			NLOT (bilateral)	NLOT	Dopaminergic innervation
			Cortex	Cortex (bilateral)	Main output to the prefrontal cortex and striatum
				Ventral striatum (Acb, Tu; bilateral)	
				Dorsal striatum (CPu; bilateral)	
				Mediodorsal thalamus	
Ventral pallium	Superficial	NLOT	Main olfactory bulb	Main olfactory bulb	Dense cholinergic innervation
			B (bilateral)	B (bilateral)	
				Commissural	
				Ventral striatum	
		COAa	Main olfactory bulb	Lateral hypothalamus	Very rich in receptors to sexual steroids
					Olfactory link to the hypothalamus
		COApm	Accessory olfactory bulb	Accessory olfactory bulb	Vomeronasal cortex
			Commissural	Commissural	
			Intrinsic	Cortex	
			Piriform and endopiriform	Hippocampus (CA1)	
			Me	Intrinsic (especially to AB)	
				Medial EA	
	Deep	L	Intrinsic	Intrinsic (B, AB, and central EA)	Sensory interface of the basolateral division of the amygdala
			Thalamus		
			Cortex		
		AB	Intrinsic	Intrinsic (including central EA)	ABa: anterior part, deep and functionally related to COAa
			Cortex	Cortex	ABp: posterior part, functionally related to AHA
				Ipsilateral accumbens	
				Lateral hypothalamus (ABa)	
				Ventromedial hypothalamus (ABp)	
		AHA	Intrinsic (Me, COA)	Intrinsic (including medial EA and COApm)	Very rich in receptors to sexual steroids
			Hippocampus (CA1, ventral subiculum)	Hippocampus (CA1, ventral subiculum)	According to Canteras *et al.* (1992a), it is part of the posterior amygdala
			Ventral premammillary nucleus	Lateral septum (ventral)	Together with the ABp gives rise to the pallial portion of the stria terminalis
				Cortex	
				Ventral striatum	
				Ventromedial hypothalamus	
				Ventral premammillary nucleus	

Table 2 Summary of the characteristic features of the different nuclei of the mammalian subpallial amygdala (extended amygdala)

Division	*Nucleus*	*Main afferents*	*Main efferents*	*Neurochemistry and other features*
Medial EA	MeAD, MeAV, MePD, and MePV	Accessory olfactory bulb (AOB) Posteromedial BST COApm Secondary olfactory centers Subiculum Ventral premammillary hypothalamus AHA? Main olfactory bulb (only partially)	AOB COApm AB, L Lateral entorhinal cortex Posteromedial BST Ventral striatum (olfactory tubercle) CeM Lateral septum (ventral) Hypothalamus: medial preoptic, ventromedial, and ventral premammillary nuclei	• Vomeronasal subpallial amygdala • Very rich in receptors to sexual steroids • Sexually dimorphic population of vasopressinergic cells (projecting to septum and habenula)
	Posteromedial BST	Accessory olfactory bulb (only partially) Me COApm COApl Amygdalopiriform transition (APir) AHA Ventral premammillary hypothalamus	Me ABp and AHA CeM (only the Tr) Lateral septum (ventral) Hypothalamus: medial preoptic, ventromedial, and ventral premammillary Periaqueductal gray (PAG) Midbrain tegmentum (VTA, RR) Parabrachial/pericoerulear area (including Barrington nucleus)	• Very rich in receptors to sexual steroids • Sexually dimorphic population of vasopressinergic cells (projecting to septum and habenula)
Central EA	CeM, CeL, and CeC	Infralimbic and insular cortices CA1 Basolateral amygdala (L, ABa, ABp, Bp) Amygdalopiriform transition (APir) Postpiriform transition area (TR) Anterior and posterolateral BST MeAV Paraventricular, ventromedial, and subparafascicular thalamus Parabrachial pons	Anterior and posterolateral BST Substantia innominata Lateral hypothalamus Paraventricular and mediodorsal thalamus Midbrain tegmentum (VTA, SN, RR) PAG Parabrachial and pericoerulear pons NTS dorsal vagal complex	• Receptors for corticosteroids • NT/CRF and SP/SS projecting cells in the CeL and CeM (not in the CeC) • CGRP innervation of CeC and CeL (parabrachial and thalamic origin)
	Anterior and posterolateral BST	Basolateral amygdala (ABa, ABp, Bp) Ce Amygdalopiriform transition (APir) Postpiriform transition area (TR) Parabrachial pons	Ce Substantia innominata Lateral hypothalamus Paraventricular and mediodorsal thalamus Midbrain tegmentum (VTA, SN, RR) PAG Parabrachial and pericoerulear pons NTS dorsal vagal complex	• NT/CRF and SP/ SS projecting cells (lateral aspect). At least the NT/CRF population extends rostrally into the nucleus accumbens • CGRP innervation that extends rostrally into the nucleus accumbens

sexual steroids but it does to corticosteroids. It receives a cascade of sensory inputs (viscerocceptive, nociceptive, gustatory, somatosensory, auditory, and maybe visual) directly and indirectly from the brainstem (mainly the parabrachial area and nucleus of the solitary tract), thalamus and cortex (perirhinal, insular, infralimbic), and basolateral amygdala (mainly L, AB, and posterior B), the latter constituting its main input. Many of the projection cells of the central EA are GABAergic and express neuropeptides (CRF, NT, SS, SP, enkephalin). The distribution of peptides may be of interest for comparative purposes. Most of the peptidergic cells are located in the CeL (plus CeM) division, whereas the CGRPergic input (visceroceptive nociceptive) from the parabrachial pons and thalamus terminates in the CeC and, to a lesser extent, in the CeL, and extends to the amygdalostriatal transition. Therefore, the Ce displays a medial aspect rich in peptidergic cells and a lateral one defined by CGRP innervation that partially overlaps with the former.

The projections of the central EA reach a huge variety of structures, including the paraventricular and lateral hypothalamus, periaqueductal gray, midbrain reticular formation, including dopaminergic cell groups (VTA, SN, retrorubral field), locus coeruleus and pericoerulear area, parabrachial area, nucleus of the solitary tract, and dorsal vagal complex. This allows a coordinated control of the somatomotor, vegetative, and endocrine components of fear/anxiety reactions.

This summary reveals the existence of two parallel systems in the amygdala of mammals. One is a multimodal system, composed of the basolateral amygdala (B, L, and AB) and the central EA and seems involved in the generation of two kinds of basic emotional reactions. The fear–anxiety reactions are produced through the long descending outputs of the central EA. Moreover, the basolateral nuclei (mainly the AB and B) massively project to the ventral striatum, and this may mediate the generation of reactions of attraction/reward elicited by incoming stimuli.

The second system of the mammalian amygdala is mainly composed of the secondary vomeronasal centers, since it includes the vomeronasal cortex (COApm) and the medial EA, plus a deep pallial nucleus, the AHA. Their pattern of connections with the preoptic, tuberal, and premammillary hypothalamus and septohippocampal system, as well as the presence of receptors to sexual steroids in most of the centers of this circuit, suggests that this system is involved in the control of reproductive and agonistic behaviors elicited by conspecific chemical signals (mostly pheromones). Both systems are interconnected. Most of these connections arise from the medial EA or COApm and terminate in the basolateral amygdala and central EA. These connections are seemingly providing a substrate for eliciting reactions to conspecific vomeronasal-detected chemicals (e.g., pheromones) such as fear/anxiety against a competitor (territorial behaviors) or attraction/reward, induced by probable mates.

15.5 The Amygdala of Reptiles

Identifying the amygdala of nonmammals constitutes a true challenge. One of the first attempts was due to Johnston (1923), who assumed that the amygdala was found in the caudal and basal cerebral hemispheres, in close association with the LOT. In fact, in a ventral view of the brain of most reptiles (Figure 8 shows the brain of the Old World lizard *Podarcis hispanica*), the *lot* is seen to arise from the olfactory bulbs, very developed when compared with mammals, and apparently terminating halfway within the cerebral hemispheres. The structures caudal to the *lot* are good candidates for the reptilian amygdala.

However, to identify the reptilian amygdala with certainty, we will apply to the reptilian brain the same criteria that define the different parts of the mammalian amygdala. Thus, we will explore the reptilian pallium and subpallium to try to delineate the reptilian pallial and subpallial (extended) amygdala.

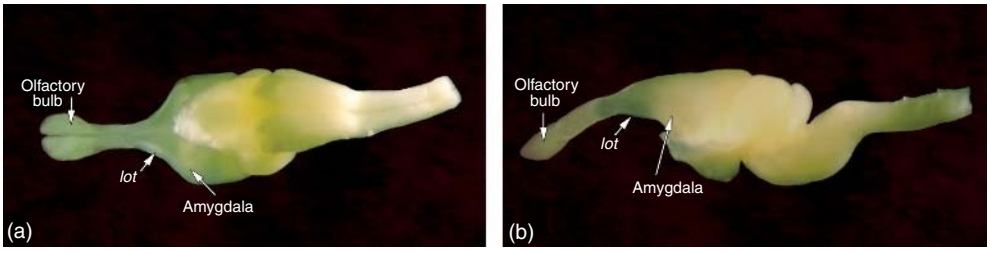

Figure 8 The amygdala in the brain of reptiles. In a ventral (a) and a lateral (b) view of the brain of the lizard *Podarcis hispanica*, the lateral olfactory tract (*lot*) is seen to leave the huge olfactory bulbs to terminate in the caudobasal cerebral hemispheres, where the presumed amygdala is located.

15.5.1 A Topological View of the Reptilian Pallial Amygdala

In order to delineate the pallial amygdala of reptiles we will use three main criteria. First, the afferents from the olfactory bulbs will be used to identify the different areas of the presumptive (superficial) cortical amygdala. Second, structures topologically deep to the cortical, olfacto-recipient amygdala would constitute the basolateral division of the amygdala. Finally, we will use topological data together with data on the expression of homeotic genes during development to delineate the lateral versus the ventral pallial derivatives. All of these data allow us to make a proposal of homologies between the reptilian and mammalian amygdalae, which will be further explored using connectional and histochemical data (Figure 9).

15.5.1.1 The olfacto-recipient pallial amygdala of reptiles There are several studies of the projections of the main and accessory olfactory bulbs in different reptiles, including squamate reptiles (lizards and snakes: Ulinski and Peterson, 1981; Martinez-Garcia *et al.*, 1991; Lohman and Smeets, 1993; Lanuza and Halpern, 1998) and turtles (Reiner and Karten, 1985). In all reptiles, the main olfactory bulbs project to the outer half of the molecular layer of what is usually called the LC, throughout its rostrocaudal axis. They also project to more ventral cell groups associated with the *lot*, named nucleus of the *lot* (NLOT) (Martinez-Garcia *et al.*, 1991; Figures 9a and 10a) or external amygdala (Lanuza and Halpern, 1998). At caudal levels, the LC

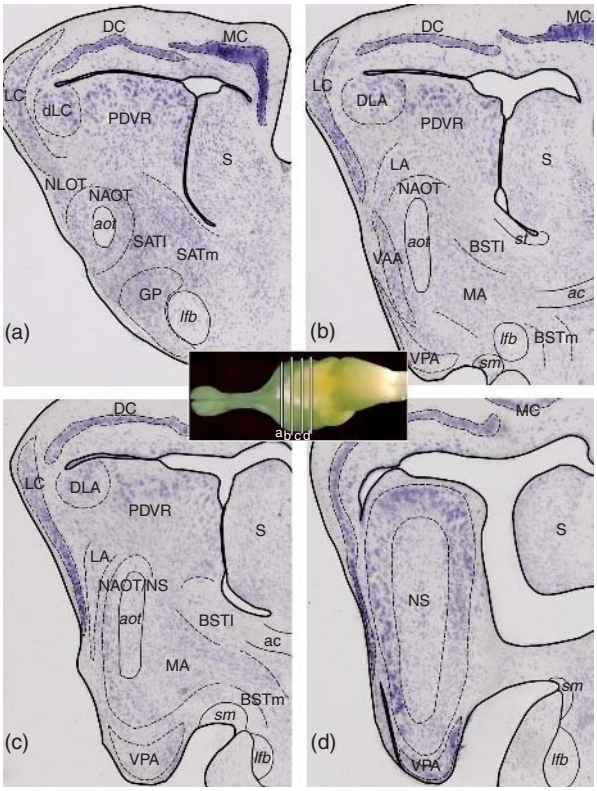

Figure 9 Cytoarchitecture of the amygdala of the lizard *Podarcis hispanica*. Nissl-stained frontal sections through the left cerebral hemisphere of a lizard at slightly precommissural (a); commissural (b); anterior postcommissural (c); and caudal levels of the telencephalon (d). The central inset shows the approximate location of the sections on a ventral view of the brain. The different nuclei and cortical areas of the amygdala and adjoining telencephalic structures are delineated using thin discontinuous lines, whereas thin solid lines indicate the main fiber tracts.

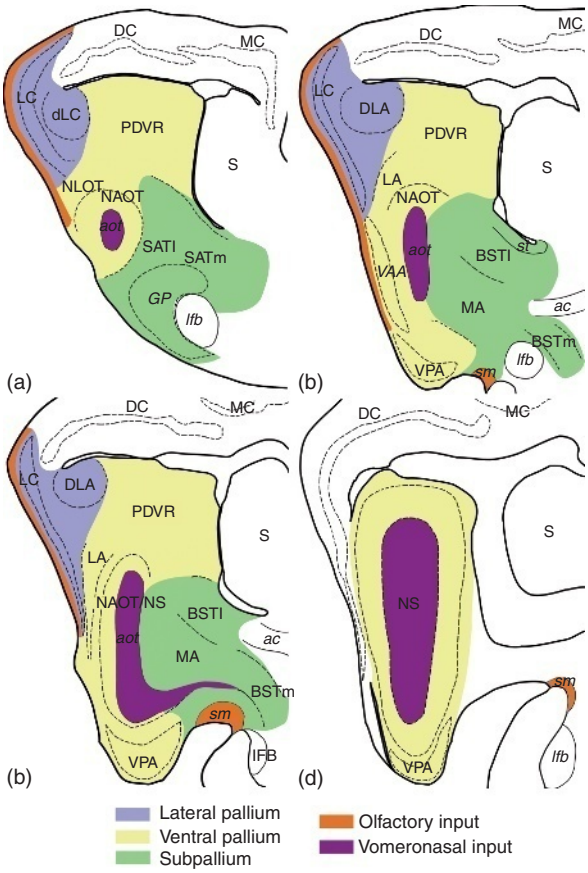

Figure 10 Pallial and subpallial territories within the reptilian amygdala. This diagram, which is based on the Nissl-stained sections of Figure 9, shows the palliosubpallial boundary as well as the lateral and ventral pallial territories in the amygdala of reptiles. The termination areas of the projections from the main (orange) and accessory olfactory bulbs (violet) are also indicated. This reveals that, as in mammals, the olfactory projection reaches mainly superficial lateral and ventropallial regions, whereas the vomeronasal one reaches ventropallial (NS) and subpallial regions (MA). In the brain of squamate reptiles the accessory olfactory tract is internalized (not superficial) and this results in an invagination of the vomeronasal cortex, the NS, which therefore shows an inverted lamination. On the other hand, the olfactory projection courses through the stria medullaris, just superficial to the MA, and reaches the contralateral hemisphere via the habenular commissure.

extends further ventrally (LCc; Figures 9b and 10b). At these levels, MOB fibers extend beneath the surface of the ventrolateral cerebral hemispheres to enter the stria medullaris. In their way, they innervate a layered cell group, caudal to the NLOT, known as the VAA (Figures 9b and 10b; Martinez-Garcia *et al.*, 1991; Lanuza and Halpern, 1998).

In the rostral telencephalon, the fibers arising from the accessory olfactory bulb deepen into the cerebral hemispheres making up the accessory olfactory tract. The accessory olfactory tract is surrounded by a group of cells called bed nucleus of the accessory olfactory tract (NAOT), which is just deep to the boundary between the LCc and the VAA. The NAOT is rostral to and continuous with the main secondary vomeronasal center of squamate reptiles, the NS, which at caudal levels occupies the whole subventricular telencephalon (Figures 9c and 9d). In spite of its subventricular position, the NS displays a neat laminar organization (Ulinski and Kanarek, 1973), with most cells arranged in a single layer (mural layer) sandwiched between two molecular strata called marginal (juxtaependymal) layer and the hilus, where the accessory olfactory tract terminates (Figures 10c and 10d). This is suggestive of a cortical nature. In agreement with this interpretation, like other cortical structures the NS shows most of the GABAergic cells in the molecular layers and only a few of them in the mural layer (Martinez-Garcia *et al.*, 2002a). Perez-Clausell (1988) compared the distribution of zinc between the LC and NS and concluded that the NS is a cortical field whose lamination has become inverted. In fact, in a series of frontal sections through the caudal telencephalon of a squamate reptile, it becomes clear that, as the accessory olfactory tract deepens into the cerebral hemispheres, there is an invagination of the cortical amygdala to which it innervates. Consequently, the hilus constitutes the outer molecular layer, whereas the marginal layer is, in fact, the inner molecular layer. Therefore, in reptiles the vomeronasal cortical amygdala is typically invaginated in the form of an NS. The AOB also projects to a region of the striatopallidal forebrain, just medial to the rostral NS (Figures 10b and 10c) that is usually named as medial amygdala and/or BST (see below).

Considering together all these data, the olfactory cortical amygdala of reptiles seems to be composed of the NLOT, the VAA, and, presumably, parts of the LC. Although the LCc occupies a position compatible with its consideration as part of the caudalmost olfactory cortical amygdala, this is not clearly supported by hodological data (Hoogland and Vermeulen-Vanderzee, 1995; see below). In addition, reptiles possess two vomeronasal cortical structures, the BAOT and the NS.

15.5.1.2 The deep pallial amygdala: The reptilian basolateral amygdala Like its mammalian counterpart, the basolateral division of the reptilian amygdala should occupy a topological position deep to the cortical (olfacto-recipient) amygdala. This probably includes the PDVR plus two adjoining cell groups named lateral amygdala (LA) and DLA. Thus, dextranamine injections in the ependymal layer of the medial PDVR in *Podarcis* result in extensive labeling of the glial processes that could be followed up through the deep PDVR and rostral LA, to their contact with the pial surface at the level of the NLOT (Martinez-Garcia *et al.*, 2002a). On the other hand, the rostral LA looks deep to the VAA (Figure 9b). This allows us to consider the PDVR, LA, VAA, and NLOT as neurogenetically related structures. On the other hand, the distribution of radial glia in lizards (Monzon-Mayor *et al.*, 1990; Yanes *et al.*, 1990; Guirado *et al.*, 2000) indicates that the DLA, a cell group just below the lateral sulcus of the lateral ventricle at these caudal levels (Figures 9b and 9c), is topologically deep to the LCc.

In addition, there is a small cell group ventral to the rostral-to-intermediate NS, which, because of the inverted lamination of the NS, should be interpreted as deep to it. This is called VPA. The VPA is contiguous to the LA (Figure 9c) and caudal to the VAA. Some authors consider the VPA a caudal portion of the external amygdaloid nucleus, but this does not seem appropriate for a deep nucleus.

15.5.1.3 Lateropallial and ventropallial territories in the amygdala of reptiles From a comparative viewpoint, it is interesting to know the territory of the pallium to which each one of these structures belongs. Since the projections from the olfactory bulb terminate in the superficial layers of the lateral and ventral pallia, we will specifically analyze the ventropallial or lateropallial nature of the abovementioned structures (Figure 10). In this respect, it is sensible to consider the DLA and the LCc, apparently superficial to the former, as lateropallial structures. On the other hand, in their original definition of the ventral pallium, Puelles *et al.* (2000) indicated that it was composed of structures deep to the LOT. Therefore, it is reasonable to suggest that the LOT and its caudal continuation, namely the VAA and NS, are ventropallial derivatives. Finally, the structures deep to these cortical areas, the PDVR and LA and VPA, are also very likely ventropallial. This proposal is supported by data on the expression

of homeotic genes during the development of turtles. Indeed, Smith-Fernandez *et al.* (1998) used *Emx-1* and *Dlx-1* as markers of early telencephalic regionalization and concluded that the dorsal ventricular ridge (probably including its posterior part) is part of the intermediate territory (situated between the pallium and subpallium) renamed by Puelles *et al.* (2000) as ventral pallium.

15.5.1.4 A proposal of homologies for the reptilian and mammalian pallial amygdala This allows us to make a proposal of homologies between the reptilian and mammalian amygdala based mainly on topology (superficial-to-deep; ventral or lateral pallium) and on the projections from the olfactory bulb, which is presented in Table 3. According to this proposal, the LC would include the homologues of the COApl plus maybe the transitional areas with the piriform (APir) and entorhinal areas (TR). In turn, the deep lateropallial DLA is the best candidate for the homologue of the B. In the ventral pallium, the LOT and BAOT would be homologues to their mammalian homonyms; the olfactory cortical areas of mammals (COAa) and reptiles (VAA) would be homologous, as would be the vomeronasal cortices of mammals (COApm) and reptiles, the NS. In addition, the reptilian deep ventropallial nuclei, PDVR, and LA are the most likely candidates for the homologues of the mammalian L and AB.

Finally, being a caudal cell group deep to the vomeronasal cortex (NS), the VPA occupies a topological position comparable to that of the mammalian AHA (which indeed is just deep to the COApm). This is further supported by the continuity shown between the reptilian LA and VPA that recalls the relationship between the posterior AB and the AHA of mammals.

This proposal of homologies would be further explored and detailed by analyzing the available literature on the connections and histochemistry of the reptilian caudal cerebral hemispheres.

15.5.2 Connections and Histochemical Properties of the Reptilian Pallial Amygdala: Comparison with Mammals

15.5.2.1 Cortical amygdala Besides their afferents from the main or accessory olfactory bulb, the cortical amygdaloid areas of reptiles also share many connectional features with their mammalian counterparts, which we analyze in detail below.

15.5.2.1.(i) The olfactory cortical amygdala In their study of the connections of the LC of the gecko, Hoogland and Vermeulen-Vanderzee (1995) made restricted injections of lectins in different parts of the LC. According to their results, the dorsal rostral LC and the LCc display exclusively efferent projections to the hippocampal and parahippocampal cortices (medial and dorsal cortical areas). This had been previously reported in *Podarcis* by Martinez-Garcia *et al.* (1986) and confirmed in the snake *Thamnophis sirtalis* (Martinez-Marcos *et al.*, 1999). In contrast, the rostral ventral LC is closely related to the amygdaloid complex, since it projects to the external amygdala (presumably the VAA; from which it receives afferents; Martinez-Garcia *et al.*, 1986), PDVR, and LA (Lanuza *et al.*, 1998; Martinez-Marcos *et al.*, 1999). This was further supported by the results of deep injections in the ventral rostral LC (deep LC according to Novejarque *et al.*, 2004). This suggests that in reptiles and mammals the olfactory areas of the lateral pallium are differently compartmentalized. Whereas in mammals there is a number of cortical areas that project to the Hp and amygdala (COApl, APir, TR, entorhinal cortex), in reptiles the areas of the LC giving rise to these two projection systems are anatomically segregated.

The connections of the VAA (anterior part of the external amygdala, depending on the nomenclature) have been specifically studied in the context of amygdalohypothalamic pathways in lizards (Bruce and Neary, 1995a, 1995b; Lanuza *et al.*, 1997) and snakes (Martinez-Marcos *et al.*, 1999). The results of these studies indicate that the VAA projects mainly to the lateral hypothalamus and, apparently, to a cell population lateral and caudal to the ventromedial hypothalamus, called by Lanuza *et al.* (1997) lateral tuberomammillary nucleus (LTM), that might include ventral premamillary nuclei. A similar situation is observed in *T. sirtalis* (Martinez-Marcos *et al.*, 1999). Since the injections on which this projection was defined in lizards also included the VPA, it is difficult to ascertain whether the VAA and VPA display differential projections to the hypothalamus. New evidence suggests, however, that the projection to the lateral hypothalamus arises from the VAA, whereas the one directed to the LTM/ventral premammillary hypothalamus arises from the VPA (see below).

In conclusion, the available data on the connections of the olfactory cortical amygdala in reptiles suggest that the lateropallial olfactory cortices are differently compartmentalized in reptiles and mammals, so that specific homologues to the COApl and the transitional cortices of mammals are not found in reptiles. In contrast, within the ventral pallium the VAA seems the most likely reptilian homologue for the mammalian COAa (Table 3).

Table 3 Proposal of homologies between the mammalian, reptilian, and avian amygdaloid nuclei and areas

Embryological origin	*Topological position*	*Mammalian nucleus/area*	*Reptilian nucleus/area*	*Avian nucleus/area*	*Properties*
Lateral pallium	Superficial	COApl (APir, TR)	Parts of the LC?	Parts of the CPi?	Mainly intra-amygdaloid and to hippocampal formation
	Deep	B	DLA	Lateral NC TPO AD Lateral PoA	• Bilateral projections to striatum and NLOT • Dopaminergic+cholinergic innervation
Ventral pallium	Superficial	LOT	NLOT	AA	• Connected to the deep lateropallial amygdala • Cholinergic innervation
		COAa	VAA	Rostral olfacto-recipient area of the arcopallium, ventral to CPi	• Projections to striatum • Projections to lateral hypothalamus
		COApm	NS		Vomeronasal cortex
	Deep	L	PDVRdm (ADVR?)	Medial NC (field L, intermediate and frontal N, E, Bas?)	• Sensory interface • Intra-amygdaloid projections • Few extra-amygdaloid projections (to the striatum)
		ABa	Deep VAA	Rostrolateral AV deep to the olfacto-recipient arcopallium	• Projections to striatum • Projections to lateral hypothalamus
		ABp	PDVRv+LA	Posterior AV, nonolfacto-recipient	• Projections to VMH • Minor projections to striatum
		AHA	VPA	Ventromedial PoA / Caudal edge of the AV	• Projection to ventrolateral septum • Projections to preoptic hypothalamus • Interconnected with the ventral premammillary hypothalamus • Receptors to steroid hormones[a]
Subpallium		Central EA	SAT, BSTl	SpA, BSTL (subpallial AM?)	• Long-distance descending projections (e.g., lateral hypothalamus, parabrachial area, dorsal vagal complex) • CRF, NT cells (medial aspect)[b] • CGRP innervation (lateral aspect)
		Medial EA	MA, BSTm	TnA, BSTM (subpallial AM?)	• Massive projections to preoptic, lateral tuberal (ventromedial), and premammillary hypothalamus • High levels of receptors to sexual steroids • Vasopressin/vasotocin-containing projecting cells

[a]A cell group expressing receptors to sexual steroid is lacking in birds.
[b]The distribution of CRF and NT has not been properly studied in reptiles.

15.5.2.1.(ii) The vomeronasal cortical amygdala The connections of the NS have been studied in detail in the snake *T. sirtalis* (Lanuza and Halpern, 1997) and the lizard *P. hispanica* (Lanuza *et al.*, 1997; Novejarque *et al.*, 2004). The NS of squamate reptiles shows projections back to the AOB and to the remaining vomeronasal centers (putative medial amygdala and BST), as well as its contralateral counterpart through the anterior commissure. It also projects to olfactory structures such as the LC and displays intrinsic amygdaloid projections (mainly to deep nuclei; PDVR, DLA). Additionally, it displays important projections to the ventral striatum (olfactostriatum; Martinez-Marcos *et al.*, 2005) and projects to parts of the dorsal (hippocampal) cortex. This perfectly fits the pattern of projections from the mammalian COApm (Table 1), thus strongly supporting the homology between both structures.

15.5.2.2 The reptilian basolateral amygdala The basolateral amygdala of mammals receives sensory inputs from different sources (brainstem, thalamus, and cortex) and neurochemically identified modulatory afferents. In turn, its components show differential projections to the hypothalamus, to the dorsal and ventral striatum, and to portions of the cortex. As we discuss in detail, most of these same features are met by the proposed homologues in the reptilian brain.

15.5.2.2.(i) The deep lateropallial amygdala: The DLA as the reptilian homologue to the B nucleus of mammals The projections from the caudal cerebral hemispheres to the striatal territories in reptiles were neglected until recently (Gonzalez *et al.*, 1990), although there was some evidence suggesting that they existed in lizards (Voneida and Sligar, 1979; Martinez-Garcia *et al.*, 1993), turtles (Siemen and Kunzle, 1994), and snakes (Perez-Santana *et al.*, 1997). We reinvestigated this issue in the lizard *P. hispanica* (Novejarque *et al.*, 2004) using a combination of retrograde and anterograde tracing. Our results demonstrate that the DLA provides a massive and bilateral projection to the dorsal striatum (Figure 11). Moreover, the pallial amygdala gives rise to a massive projection to a continuum of

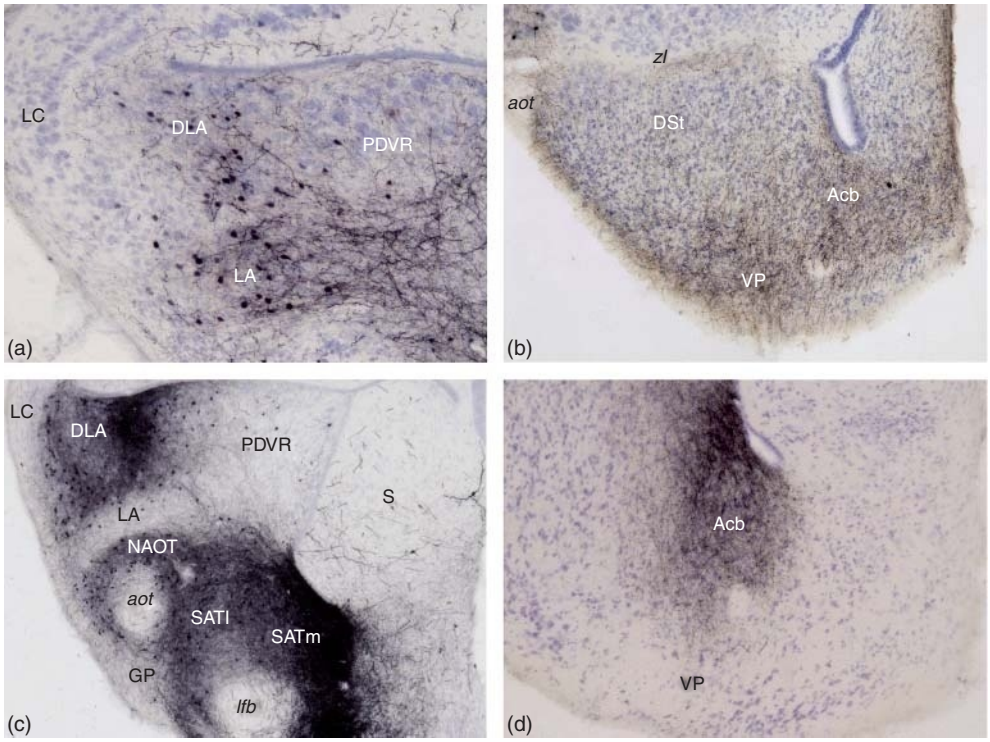

Figure 11 Amygdalostriatal connections in lizards. a and b, The DLA of lizards projects bilaterally to both the dorsal and ventral striata. Thus, retrograde transport is observed in the DLA (and part of the LA) in a lizard that received a tracer (dextranamine) injection encompassing the ipsilateral dorsal striatum (a). In addition, tracer injections into the DLA anterogradely label the dorsal and ventral striatum in the ipsilateral (b) and contralateral (not shown) telencephalon. This amygdaloid projection also reaches parts of the pallidum (VP). c and d, The PDVRdl of lizards projects exclusively to the ipsilateral Acb. Retrograde transport after injections in the Acb (c) indicates that the bulk of this projection arises from the DLA and the PDVRdl, whereas the PDVRvm and LA do not contribute substantially to it. Tracer injections restricted to the PDVR (d) result in anterograde labeling of the Acb but not in the dorsal striatum.

structures in the ventral striatum connecting the striatoamygdaloid transition area (SAT) and the Acb (rostrally). This projection arises mainly from the DLA, the dorsolateral aspect of the PDVR (PDVRdl), the VAA, VPA, and NS, and the deep LC (dLC). Among all these nuclei, only the DLA projects substantially to the contralatateral Acb.

Therefore, the DLA stands as the main source of amygdalostriatal pathways since it projects massively and bilaterally to the dorsal and ventral striatum. This strongly supports our proposal that the DLA is the reptilian homologue of the basal nucleus of the mammalian amygdala. In addition, like the mammalian B, the DLA projects bilaterally to the region of the NLOT (Novejarque *et al.*, 2004).

In the mammalian brain, the pallial amygdala is the target for two well-characterized ascending projections, namely the cholinergic input from the basal forebrain and the dopaminergic input from the dorsocaudal group A10. Both projections converge at the level of the B nucleus. Using appropriate (immuno)-histochemical techniques these projections can also be delineated in reptiles, and the results fully agree with the proposed homologies. Thus, the distribution of both ChAT (Medina *et al.*, 1993) and acetylcholinesterase (Lanuza *et al.*, 1997) reveals a dense cholinergic innervation of portions of the NLOT (Figure 12a) and of the DLA (Figure 12b). In addition, immunohistochemical detection of markers of dopaminergic fibers reveals a dense innervation of the DLA in the lizards *Psammodromus* (Andreu *et al.*, 1994) and *Podarcis* (Figure 12c).

15.5.2.2.(ii) Deep ventropallial nuclei: The PDVR and LA In squamate reptiles, the PDVR is composed of a juxtaventricular zone rich in cell groupings called glomeruli, plus a central core that shows no clear boundaries with the LA. In *Podarcis* the PDVR does not seem homogeneous, but is composed of cytoarchitectonically different zones: the ventromedial PDVR (PDVRvm) shows small glomeruli, the dorsomedial PDVR (PDVRdm) shows larger glomeruli, and the dorsolateral PDVR (PDVRdl), next to the DLA, display giant glomeruli. As we will see, the three areas display different connections that allow us to refine our proposal of homologies.

15.5.2.2.(ii).(a) The PDVRvm and LA constitute the reptilian homologue to the ABp of mammals The ABp of the mammalian amygdala gives rise to a part of the pallial component of the stria terminalis that terminates in the core of the VMH (Petrovich *et al.*, 1996). The amygdalohypothalamic projections have been fully characterized in different reptiles using retrograde and anterograde tracing techniques (Bruce and Neary, 1995a, 1995b; Lanuza *et al.*, 1997; Martinez-Marcos *et al.*, 1999). In all the species studied, the PDVRvm and LA (Figures 13a and 13b) project to the ventral and medial anterior tuberal hypothalamus (including the retrochiasmatic area and VMH core; Figure 13c). This pathway courses through a compact bundle that leaves the amygdala dorsal and caudal to the anterior commissure, and is called stria terminalis. From a comparative viewpoint, this seems quite a correct name in terms of both its origin (deep pallial amygdala) and its termination site (ventromedial hypothalamus). This tract contains zinc-rich fibers (Perez-Clausell, 1988; Smeets *et al.*, 1989), thus

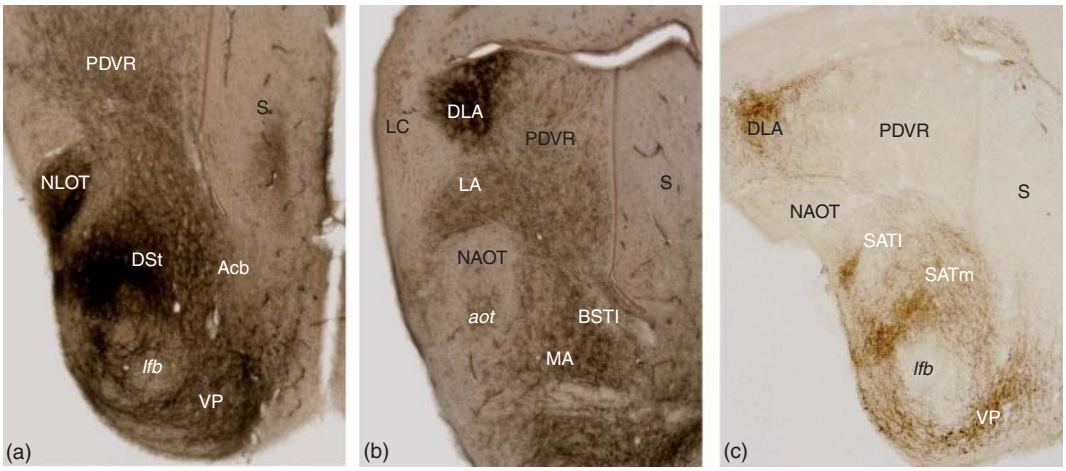

Figure 12 Chemoarchitecture of the amygdala of *Podarcis*. The (immuno)histochemistry for AChase (a and b) and tyrosine hydroxylase (TH, c) reveals a convergent cholinergic/dopaminergic innervation of the DLA. The NLOT also shows a dense AChase reactivity. Compared to the dorsal striatum proper (DSt), the subpallial amygdala shows a scarce AChase reactivity and a low density of TH-immunoreactive fibers.

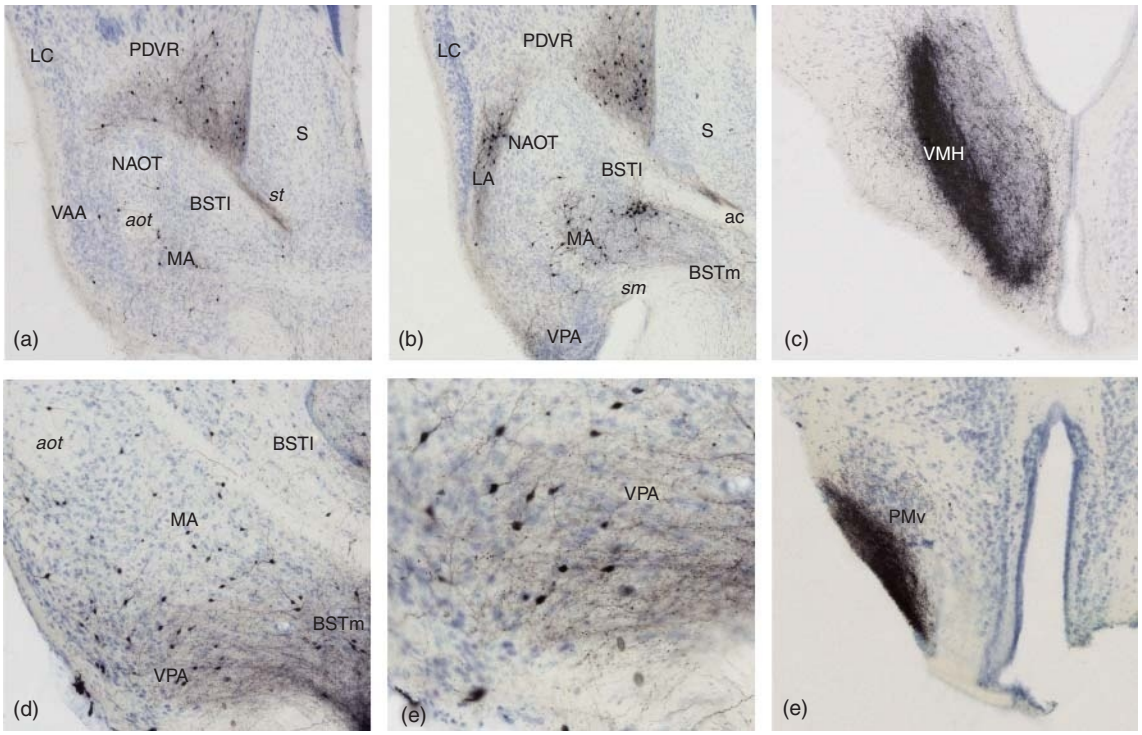

Figure 13 Amygdaloid projections to the hypothalamus in lizards. a and b, Rostral (a) and caudal (b) sections through the amygdala of a lizard that received an injection of dextranamine in the core of the ventromedial hypothalamic nucleus. Cells projecting to the VMH core are seen in the PDVRvm and LA as well as in most of the MA and portions of the BSTm. The pallial and subpallial bundles of the stria terminalis join at preoptic levels. c, The same projection is revealed by anterograde labeling after a tracer injection restricted to the PDVR. Labeling is seen in the VMH core. d and e, Labeling found in the amygdala of a lizard that received an injection of dextranamine in the premammillary hypothalamus, which did not involve the VMH core. Although the labeling in the subpallial amygdala is also located in the MA and BSTm, in the pallial amygdala, labeling is mainly observed in the VPA. At higher magnification (e), both retrograde and anterograde labeling is visible in the VPA, thus indicating that this nucleus is reciprocally connected with the premammillary hypothalamus. f, In a lizard that received an injection involving the VAA and VP, the presumed ventral premammillary hypothalamus shows a dense anterogradely labeled terminal field. More rostrally, labeled fibers avoid the VMH core but innervate the lateral shell (see Lanuza *et al.*, 1997).

confirming the glutamatergic nature (pallial origin) of some of its fibers. This strongly suggests that the ABp finds its homologue in the PDVRvm/LA of the reptilian ventral pallium (Table 3).

15.5.2.2.(ii).(b) The dorsomedial PDVR as the sensory interface of the reptilian amygdala As we have already seen, the PDVRdl projects to the ventral striatum (Figure 11c) and the PDVRvm contains the cells of origin of the pathway to the ventromedial hypothalamus (Figures 13a and 13b). In contrast, the PDVRdm seems only involved in intra-amygdaloid connections. Thus, tracer injections in the PDVR result in massive anterograde and retrograde labeling in the LA and DLA (Figure 14a). Moreover, in our material the PDVRdm only shows retrogradely labeled neurons after tracer injections involving most of the SAT, which, as we will discuss below, is the most likely candidate for the reptilian central amygdala.

The afferents to the PDVR were traced for the first time by Lanuza *et al.* (1998) in the lizard *P. hispanica*. Their findings suggest that the dorsal ventricular ridge of reptiles is anatomically and functionally heterogeneous. Its anterior aspect (anterior dorsal ventricular ridge: ADVR) receives discrete nonoverlapping auditory, somatosensory, and visual thalamic inputs. In contrast, the PDVR is the target of the medial posterior and posterocentral thalamus, which receives a convergent set of afferents from auditory, visual, and somatosensory midbrain and brainstem structures. The PDVR also receives direct afferents from the parabrachial region, probably conveying nociceptive, visceroceptive, and/or gustatory information, and from the nucleus of the lateral lemniscus (auditory). In addition, highly processed sensory inputs arising from the dorsal (hippocampal) cortex (multimodal contextual), the ventral rostral LC (olfactory), and the three sensory areas of the ADVR (auditory, somatosensory, and visual) also reach the PDVR. Finally,

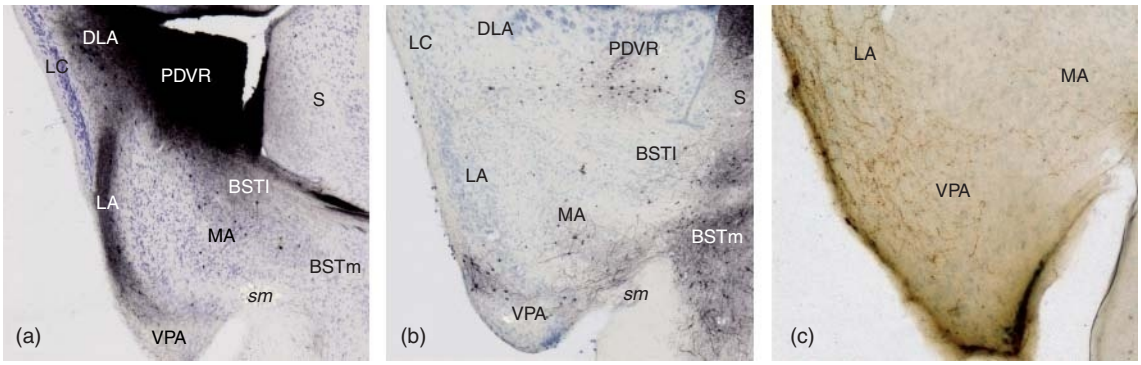

Figure 14 Intrinsic, preoptic, and hippocampal connections of the reptilian amygdala. a, Injections of dextranamine in the PDVR reveal an intricate set of interconnections within the basolateral amygdala of lizards. Thus, both the DLA and the LA show retrograde and anterograde labeling. In addition, the BSTI and SAT (Figure 15c), which constitute the central extended amygdala of lizards (Table 3), show anterograde labeling indicative of abundant basolateral-to-central intra-amygdaloid projections. The MA also seems to project to the basolateral amygdala (see retrogradely labeled cells). b, Retrograde labeling in the amygdala after a tracer injection involving the BSTm and the medial preoptic area. The presence of labeled cells in the PDVRvm should be attributed to the involvement of a part of the pallial stria terminalis in the injection site. In addition, strong retrograde labeling in the MA and VPA indicates a projection of both nuclei to the BSTm/preoptic area. c, Tracer injections (*Phaseolus vulgaris* leucoagglutinin) in the caudal dorsal (hippocampal) cortex of lizards reveal a projection to the basolateral amygdala, including the DLA (not shown), and LA as well as to the VPA and MA.

the PDVR is engaged in intra-amygdaloid afferents (with the DLA, LA, and MA; Figure 14a). Additional afferents to the PDVR arising from the caudal VTA, several hypothalamic nuclei (including the VMH), and the basal forebrain depict a complex pattern of sensory and modulatory afferents that fits with its homology with the basolateral amygdala of mammals.

In summary, the PDVRdm is the ventropallial amygdaloid center that receives massive sensory inputs and is only involved in projections to regions of the basolateral amygdala and to the central EA. In other words, it is the sensory interface of the basolateral amygdala of reptiles, thus being connectionally comparable to the mammalian L. However, when comparing reptiles and mammals, it becomes evident that the mammalian amygdala displays a more elaborated amygdaloid circuitry in which sensory interface and output centers are clearly separated (and interconnected), whereas in the reptilian amygdala both centers are somewhat mixed up.

After their studies of the projections to the hypothalamus in the gecko, Bruce and Neary (1995a, 1995b, 1995c) proposed that the anterior DVR, which receives direct sensory afferents from the thalamus (Ulinski, 1983) and projects to the PDVR (Lanuza *et al.*, 1998) but not to the hypothalamus, is the reptilian homologue of the mammalian L. This hypothesis is also supported by the ventropallial nature of the ADVR (Smith-Fernandez *et al.*, 1998). The amygdaloid nature of the ADVR was questioned by Lanuza *et al.* (1998) on the basis of its unimodal thalamic afferents, which contrasts with the polimodal and limbic thalamic afferents attributed to amygdaloid centers. However, this view needs to be revised in the light of new evidence suggesting that the principal sensory nuclei of the dorsal thalamus of reptiles (medial, posteromedial, posterocentral), including the nucleus rotundus (Guirado *et al.*, 2000), might indeed be homologues to the posterior/intralaminar thalamus of mammals (Davila *et al.*, 2000).

Another important difference between the mammalian L and the ADVR of reptiles that is very significant from a comparative viewpoint is the pattern of projections to the striatum. Whereas the ADVR projects massively and topographically to the dorsal (but not to the ventral) striatum, the mammalian L shows projections to the ventral (and scarce projections to the dorsal) striatum (our unpublished results; Pitkanen, 2000). Nevertheless, the comparative significance of the reptilian dorsal striatum (e.g., its homology with the mammalian CPu) is also a debatable issue (Lanuza *et al.*, 2002). Therefore, the possible homology of the ADVR of reptiles (and birds; see below) with part of the mammalian LA cannot be ruled out, although alternative views should be carefully considered (Striedter, 1997; Puelles, 2001).

15.5.2.2.(ii).(c) Amygdalocortical projections Our proposal of homologies between the reptilian and mammalian amygdala predicts that the DLA, PDVR, and LA should project to the dorsal pallium, thus reciprocating the projection from the dorsal pallium to the amygdala (Hoogland and

Vermeulen-Vanderzee, 1989; Lanuza *et al.*, 1998). This would be the reptilian counterpart of the corticoamygdalocortical loops that in the mammalian brain involve the B and AB on the one hand, and the frontotemporal cortex on the other. However, in contrast to mammals, the amygdala of reptiles apparently display few projections directed to the dorsal pallium. This fact probably indicates that the presence of projections to the dorsal pallium is an acquired trait of the mammalian amygdala, related to the important changes that the dorsal pallium underwent during the early evolutionary history of mammals.

In contrast, all the studied reptiles display important interconnections with the ventropallial areas that provide nonchemosensory information to the amygdala. Thus, the LA projects back to the ADVR, thus reciprocating the strong input from the ADVR to the PDVR/LA (Bruce and Butler, 1984). The comparative significance of this pathway awaits further data on the nature of the ADVR.

15.5.2.2.(iii) The VPA shows many similarities with its putative mammalian homologue, the AHA The study of the amygdalohypothalamic pathways of reptiles has revealed the presence of a second projection within the pallial component of the stria terminalis, besides the one arising in the PDVRvm/LA. This projection arises in the ventral amygdala (VPA and VAA), leaves the cerebral hemispheres through the so-called lateral amygdalofugal tract (Lanuza *et al.*, 1997; Figure 13d), and terminates in the VMH shell, the LTM, and premammillary hypothalamus (Figure 13f). Injections of tracers in the premammillary and mammillary hypothalamus, which did not involve the VMH at all (and, as a consequence, show no labeled cells in the PDVR and LA), resulted in the presence of abundant, intensely labeled neurons in the VPA (Figures 13d and 13e). This strongly suggests that the VPA is the origin of the projection to the LTM and premammillary hypothalamus whereas the VAA originates a projection directed at the dorsal lateral hypothalamus.

In mammals, the amygdala also projects to the region of the posteromedial BST and the medial preoptic hypothalamus. This projection arises from both the EA (namely, the medial amygdala; Canteras *et al.*, 1995) and the AHA in the pallial amygdala (Canteras *et al.*, 1992a). The forebrain of reptiles displays a similar projection that arises from the VPA (Figures 14b and 14c). As in mammals, in lizards this connection with the preoptic hypothalamus is reciprocal.

Therefore, the VPA is connected with medial preoptic hypothalamus, the shell of the VMH, and the premammillary hypothalamus. These hodological properties strongly support its homology with the mammalian AHA, based on a similar topological position within the posterior ventral pallium and deep to the cortical vomeronasal amygdala.

Other hodological and histochemical properties of the VPA further support this homology. Thus, like the AHA of mammals (Canteras *et al.*, 1992a), the VPA projects to the ventral lateral septum (Font *et al.*, 1998) and receives afferents from the portion of the Hp (CA1 in mammals) that projects to the ventral lateral septum (Figure 14c). In addition, like the AHA of mammals (Simerly *et al.*, 1990), the VPA is the most prominent pallial cell group of the reptilian telencephalon, expressing high levels of receptors to sexual steroids. The distribution of receptors to sexual steroids has been studied with different techniques in different reptiles, such as the whiptail lizard (Young *et al.*, 1994), the geckonids *Gecko* (Tang *et al.*, 2001) and *Eublepharis* (Rhen and Crews, 2001), in *Sceloporus* (Moga *et al.*, 2000), in the green anole (Rosen *et al.*, 2002), and in the garter snake (Halpern *et al.*, 1982). In lizards steroid-sensitive cells are observed in a cell group called external amygdala that seems to correspond to the VPA, as defined here. In snakes, Halpern *et al.* (1982) described a group of steroid-concentrating neurons located in the ventral telencephalon just rostral to the ventral NS, named ventral amygdaloid nucleus, that seems also to correspond to the VPA (Lanuza and Halpern, 1998).

All these data indicate that the VPA, like its mammalian homologue (AHA), is engaged in a circuit composed of vomeronasal centers (medial EA) and steroid-sensitive structures in the forebrain (ventrolateral septum, medial EA, premammillary hypothalamus), and expresses itself as receptors to sexual steroids. This strongly suggests that the VPA is a nucleus of the pallial amygdala specialized in the control of agonistic and reproductive behavior, as has been suggested for its mammalian counterpart.

15.5.3 The Reptilian Subpallial Amygdala

The caudal pole of the striatopallidal telencephalon of reptiles, adjacent to the above-defined pallial amygdaloid centers, includes a number of nuclei that are generally considered as the reptilian subpallial amygdala. There, nuclei have been named using a mixture of topographical and comparative

terminology, such as the medial amygdala, the BST, central amygdala, or striatoamygdaloid transition area. Nevertheless, the delineation and identification of these nuclei are not clear in the literature due to both the use of inappropriate criteria to define them and to the presence of interspecies differences. In addition, the use of topographical terminology might cause confusion since it is suggestive of homologies with the mammalian amygdala that might be erroneous (Box 1).

Box 1 The vomeronasal system and the variability in the organization of the reptilian pallial amygdala

Our proposal of homologies for the reptilian pallial amygdala is mainly based on data from Lacertidae lizards and fit some of the published literature in teiidae (*Varanus* and *Tupinambis*; Hoogland, 1977; Voneida and Sligar, 1979). However, some of the published data in other squamate reptiles apparently do not fit well with the view proposed here. Thus, for instance, in geckonid lizards the massive projections from the amygdala to the striatum have not been described. This is probably due to slight differences in the organization of the pallial amygdala rather than to the absence of these projections in geckonids. For instance, the main source of amygdalostriatal projections in *Podarcis*, the DLA, can be identified in other reptiles by means of the histochemical markers referred to above. Thus, the caudal cerebral hemispheres of geckonids displays an area innervated by cholinergic/AChase fibers (*Gecko gekko*: Hoogland and Vermeulen-Vanderzee, 1990; *Tarentola mauritanica*: unpublished observations) that also shows a rich dopaminergic innervation (Smeets *et al.*, 1986b). Therefore, the DLA of geckonid lizards seems to show a relatively ventral location within the cerebral hemispheres compared with the lizards of the family Lacertidae (such as *Podarcis* and *Gallotia*).

These differences in the organization of the caudal cerebral hemispheres are probably related to the different development of the vomeronasal system that is revealed by the size of the NS, which is much smaller in Geckonidae than in Lacertidae. This is supported by the finding of a more ventral situation of the AChase/tyrosine hydroxylase-innervated amygdaloid area (probably the DLA) in anoles, where the vomeronasal system is nearly absent (Figures B1a and B1b).

On the other hand, comparison of the data in Lacertidae with those of snakes, where the vomeronasal system probably reaches its highest degree of development, also reveals some apparent differences. These pertain to the presence of the projections from the DLA to the hypothalamus in snakes (Martinez-Marcos *et al.*, 1999). Although this may reflect real differences between lizards and snakes, it may also be due to a misdefinition of the DLA of snakes. In fact, the structure named as DLA by Martinez-Marcos *et al.* (1999, see figure 1 in this article) does not seem deep to the lateral cortex (thus lateropallial) but a cell group interposed between the PDVR and the LA, located next to the NS (thus very likely ventropallial). In this respect, it is interesting to note that the DLA cells identified as projecting to striatal territories (olfactostriatum) in *Thamnophis* (see figures 7c and 8c in Martinez-Marcos *et al.*, 2005) occupy a position that is clearly more rostral than those projecting to the hypothalamus. Dealing with this, the study of the distribution of AChase and tyrosine hydroxylase (TH) reveals convergent catecholaminergic and cholinergic innervations (Figures B1c and B1d) of a cell group apparently displaying the same location as the cells projecting to the olfactostriatum (Martinez-Marcos *et al.*, 2005) but clearly rostral to the ones projecting to the hypothalamus (Martinez-Marcos *et al.*, 1999). Therefore, it seems that, when properly defined using reliable histochemical markers, the DLA of snakes turns out to be a cell group projecting bilaterally to the striatum, but not to the hypothalamus.

Although data in crocodiles and turtles are scarcer, in general they agree with the view we propose. For instance, in turtles the region of the posterior DVR shows a similar pattern of multimodal thalamic inputs (Belekhova and Chkheidze, 1992) and intratelencephalic (ADVR-PDVR) sensory afferents (Belekhova and Chkheidze, 1991; Chkheidze and Belekhova, 1992). In addition, the posterior DVR region of turtles also displays abundant projections to the striatum (Siemen and Kunzle, 1994) comparable to the ones described in lizards by our group (Novejarque *et al.*, 2004). Moreover, there is evidence of the existence of massive projections to the hypothalamus arising from the caudal cerebral hemispheres (PDVR and the so-called basal DVR) in turtles (see figure 9 in Cordery and Molnar, 1999).

In a similar way, Pritz and Stritzel (1992) described a projection to the posterior DVR of crocodiles from a thalamic cell group in the neighborhood of the auditory relay, the nucleus reuniens pars diffusa. As discussed in detail by Lanuza *et al.* (1998), this cell group seems part of the multimodal posterior

thalamus, thus suggesting an amygdaloid nature of the PDVR and adjacent areas also in crocodiles. Moreover, in the caudal cerebral hemispheres of the caiman (Brauth and Kitt, 1980), there are two patches intensely reactive for AChase in the NLOT and the lateral edge of the caudal DVR, which projects to the striatum, a situation that recalls the DLA. Data on the anatomy of the cerebral hemispheres of crocodilians are urgently needed, especially since they represent the closest living relatives of the stem reptiles that gave rise to birds (Whetstone and Martin, 1979). In fact, the study of the distribution of glial fibrillary acidic protein reveals a similar organization of the cerebral hemispheres of crocodiles (Kalman and Pritz, 2001) and chicken (Kalman *et al.*, 1998) that may be very useful to understanding the evolution of the amygdala.

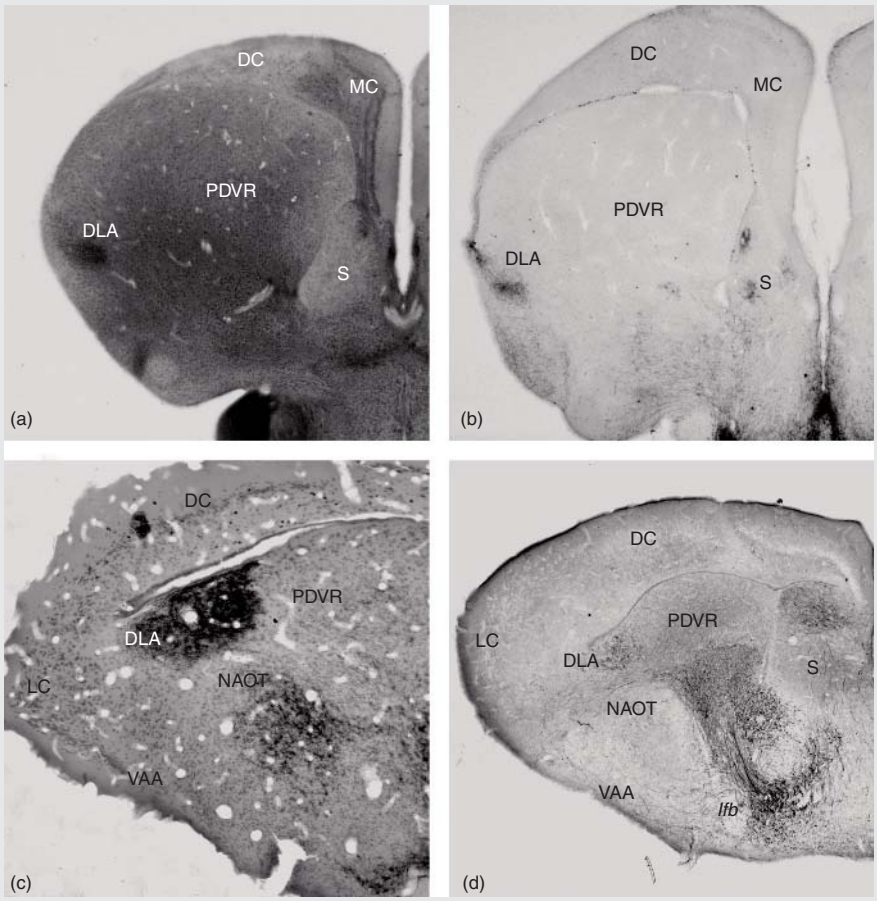

Figure B1 The amygdala of microsmatic and macrosmatic squamate reptiles. a and b, The strong reduction of the olfactory and vomeronasal system in the lizard *Anolis carolinensis* has introduced important changes in the organization of its brain. Nevertheless, the DLA can be identified by the convergent innervation of AChase (a) and TH (immuno)reactivity (b). The so-defined DLA seems displaced ventrally relative to the position it occupies in *Podarcis* (Figure 12), probably due to the lack of a nucleus sphericus in *Anolis*. The DLA of *Anolis* recalls the dorsal arcopallium of birds (Figure 19). c and d, This contrasts with the situation in the snake *Thamnopis sirtalis,* where the presence of a huge nucleus sphericus occupying the whole caudal half of the cerebral hemispheres has displaced the DLA rostralwards, as indicated by the AChase (c) and TH (immuno)reactivity (d). The pictures of *Thamnophis* have been kindly donated by Drs. Alino Martinez-Marcos and Mimi Halpern.

In this section, we try to clarify the architecture of the subpallial amygdala of reptiles, mainly using data of squamate reptiles. In this respect, we will identify the medial and central EA of reptiles by studying the features that define them in mammals.

15.5.3.1 The reptilian extended amygdala: The identity and divisions of the bed nucleus of the stria terminalis As we have seen above, in mammals the internal capsule separates the amygdala proper from the extra-amygdaloid BST (with the supracapsular BST and the sublenticular EA

connecting both structures). In reptiles, and in general in nonmammals, an internal capsule is absent (the lateral forebrain bundle is reduced to a compact tract that runs through the basal telencephalon; Figure 9). Therefore, in the subpallial amygdala of reptiles (and this is also valid for birds), the centromedial amygdala and the BST are not separated, but conform a single cell mass in the basal caudal cerebral hemispheres. This has generated a confusing and contradictory nomenclature of this area of the caudobasal cerebral hemispheres, which we are trying to clarify here.

The name 'bed nucleus of the stria terminalis' designates cells that are intermingled among the fibers of the stria terminalis and are targets for them. Our material reveals that, in lizards, the stria terminalis is composed of two main tracts (Figures 13a and 13b) that join at preoptic levels. The dorsal stria terminalis is a compact tract that leaves the PDVR medially, just ventral to the ventral sulcus of the lateral ventricle, and enters the hypothalamus caudal to the anterior commissure. The ventral stria terminalis (which arises from the VPA and MA; see below) is a less compact fiber tract that enters the preoptic area ventral to the anterior commissure. Both components of the stria terminalis have very short intratelencephalic trajectories, and the cell groups with which they are associated should be considered the two main parts of the reptilian BST, namely the lateral (accompanying the dorsal stria terminalis, BSTl) and the medial BST (BSTm).

Comparing this view with previous literature on the architecture of the reptilian forebrain, we conclude that the BSTl as defined here is equivalent to the structures of the brain of the gecko, named as nucleus of the anterior commissure by Smeets *et al.* (1986a), and to the caudomedial SAT according to Bruce and Neary (1995b). In turn, the BSTm is comparable to the BST of the gecko according to the terminology of Smeets *et al.* (1986a), the interstitial amygdala of the gecko according to Bruce and Neary (1995b), and the nucleus interstitialis of the whiptail lizard (Young *et al.*, 1994). The latter terminology was also adopted by our group in some papers on the anatomy of the brain of *Podarcis* (Font *et al.*, 1997; Lanuza *et al.*, 1997).

15.5.3.2 The medial extended amygdala of reptiles In most studies of the projections from the AOB, a more or less prominent terminal field has been observed just medial and ventral to the transition between the rostral NS and the BAOT. This structure was named ventromedial amygdala (Martinez-Garcia *et al.*, 1991), medial amygdala (MA; Lanuza and Halpern, 1998; this name was used in the gecko by Bruce and Neary (1995a), and has finally been adopted by us) or central amygdaloid nucleus (Lohman and Smeets, 1993) depending on the authors and/or species under study. In all of these studies, the projection from the AOB is seen to extend further medially into the ventrolateral aspect of the BSTm (Figure 10c). At these levels, the fibers from the main olfactory bulb running within the stria medullaris are just superficial to the MA. This situation recalls the TnA of birds (see below).

There are no studies devoted specifically to the study of the connections of the MA and BSTm. (In a series of experiments designed to unravel the neural basis of tongue-flick (a behavior that delivers chemicals into the vomeronasal organ), Martinez-Marcos *et al.* (2001) analyzed in detail the amygdaloid projections to the hypoglossal nucleus of the snake *T. sirtalis*. Their results indicate that the medial amygdala projects indirectly to the hypoglossal nucleus using a relay in the LHN, but directly to other centers of the dorsal medulla. However, if one compares the retrograde labeling they find after injections into the LHN (see figure 4g in Martinez-Marcos *et al.*, 2002) or their injections into the medial amygdala (see their figure 4a), with the projections from the AOB (see figures 1e and 1f in Lanuza and Halpern, 1998), it becomes evident that most of the cells projecting to the LHN are out of the chemosensory subpallial amygdala (MA), but in a location dorsal to the anterior commissure that probably corresponds to the SAT of snakes.) However, data derived from tracer injections in other areas of the brain of lizards and snakes indicate that, from a comparative point of view, the names MA and BSTm seem appropriate for these structures. As we have described above, the BSTm is crossed by axons from MA cells on their way to the hypothalamus and therefore, very likely, receives projections from the MA. In addition, tracer injections in the preoptic hypothalamus (Lanuza *et al.*, 1997; Martinez-Marcos *et al.*, 1999) suggest that the MA and the BSTm are reciprocally connected with the medial preoptic hypothalamus (Figure 14b). Moreover, both the MA and the BSTm project to the VMH (Figures 13a and 13b) and premammillary hypothalamus (Figures 13d and 13e; Bruce and Neary, 1995b; Lanuza *et al.*, 1997; Martinez-Marcos *et al.*, 1999). This clearly recalls the situation in mammals and suggests that the MA and BSTm conform to the reptilian medial EA. In fact, as

we will see, they share most of their connections and histochemical properties.

Like the mammalian medial EA, the reptilian MA and BSTm provide a feedback projection to the AOB (Martinez-Garcia *et al.*, 1991; Lanuza and Halpern, 1998) and receive a direct projection from the vomeronasal cortex (NS; Lanuza and Halpern, 1997; Lanuza *et al.*, 1997; Novejarque *et al.*, 2004). Moreover, like the medial EA of mammals, the BSTm of lizards projects to the ventral aspect of the lateral septum (Font *et al.*, 1997). Although there is no direct evidence using double-labeling experiments, immunohistochemical data in several reptiles suggest that this projection is rich in one of the forms of the reptilian vasopressin (vasotocin), like its mammalian counterpart (Wang *et al.*, 1993). Thus, the BST (very likely the BSTm plus portions of the MA) displays cells immunoreactive for vasotocin in lizards (Stoll and Voorn, 1985; Thepen *et al.*, 1987), snakes, and turtles (Smeets *et al.*, 1990), and in all the reptiles studied the ventral lateral septum is innervated by vasotocin-immunoreactive fibers. In agreement with the situation in mammals (Wang *et al.*, 1993), this projection system displays sexual dimorphism in reptiles (Stoll and Voorn, 1985; Smeets *et al.*, 1990).

This proposal of homology is strongly supported by the fact that the MA and BSTm of lizards (together with the ventral lateral septum, with which they are connected), display the most remarkable population of sexual steroid-sensitive cells in the whole subpallial telencephalon (Young *et al.*, 1994; Moga *et al.*, 2000; Rhen and Crews, 2001; Rosen *et al.*, 2002). A similar situation is present in snakes (Halpern *et al.*, 1982). This constitutes another of the defining features of the medial EA of mammals (Tables 2 and 3).

15.5.3.3 Central extended amygdala of reptiles

The name 'central' amygdala was first applied to designate a cell group in the brain of the gecko (Smeets *et al.*, 1986a) that occupies a central location within the putative amygdaloid region. However, since this nucleus is the target for a projection from the AOB (Lohman and Smeets, 1993), it is not comparable to the central but to the medial amygdala of mammals (see above). The reptilian homologue of the mammalian Ce was identified a few years later. In research primarily devoted to the study of the efferent connections of the dorsal and ventral striatum, Russchen and Jonker (1988) realized that injection of tracers in the caudal basal ganglia, what they called striato-amygdaloid transition area (SAT), resulted in a distinct pattern of anterograde labeling. The SAT seemed to project to the lateral hypothalamus (lateral posterior hypothalamic nucleus, LHN; Lanuza *et al.*, 1997), to the midbrain tegmentum (like the rest of the striatopallidal system) and to more distant targets in the central gray, parabrachial area (Figure 15a) and medulla (nucleus of the solitary tract and dorsal motor vagal complex; Figure 15b). This is a pattern of connections identical to the one displayed by the mammalian central EA. It is important to note that the caudal SAT fuses with the BSTl, so that it is tempting to suggest that, together, both structures conform the central EA of reptiles.

Several lines of evidence are in agreement with this proposal of homology. Like the mammalian Ce and the dorsal and posterolateral BST, the SAT of lizards receives afferents from the parabrachial nucleus (Figure 15a), as well as from several nuclei in the posterior thalamus, including the nucleus rotundus (Guirado *et al.*, 2000) and the posteromedial and posterocentral nuclei (Lanuza *et al.*, 1998). In addition, it receives projections from the basolateral amygdala (PDVR and DLA; Novejarque *et al.*, 2004; Figure 15c) and from the rostral deep lateral pallium (dLC). Moreover, the SAT displays at least some of the remaining defining features of the mammalian central EA. Thus, in *Podarcis* the lateral aspect of the SAT and the region of the striatum adjacent to it, just medial to the NAOT, display a dense CGRPergic innervation (Martinez-Garcia *et al.*, 2002b; Figure 15d), which recalls the projection to the CeC and amygdalostriatal transition of the mammalian brain. The origin of these CGRPergic fibers is not clear but the most likely candidates are the two cell groups projecting to the amygdala that display CGRP-immunoreactive cells, namely the parabrachial nucleus and the ventral aspect of the posterior thalamic nuclei (Lanuza *et al.*, 1998). This is also a feature shared with the mammalian central EA (Schwaber *et al.*, 1988; Yasui *et al.*, 1991). Unfortunately, there are no detailed descriptions of the distribution of CRF and NT in the brain of reptiles. These data are needed to prove the hypothesis of homology between the mammalian central EA and the SAT of reptiles.

15.5.4 The Reptilian Amygdala: A Summary

All the data reviewed above convincingly demonstrate that the reptilian amygdala contains pallial and subpallial components comparable to those of the amygdala of mammals. The pallial amygdala of

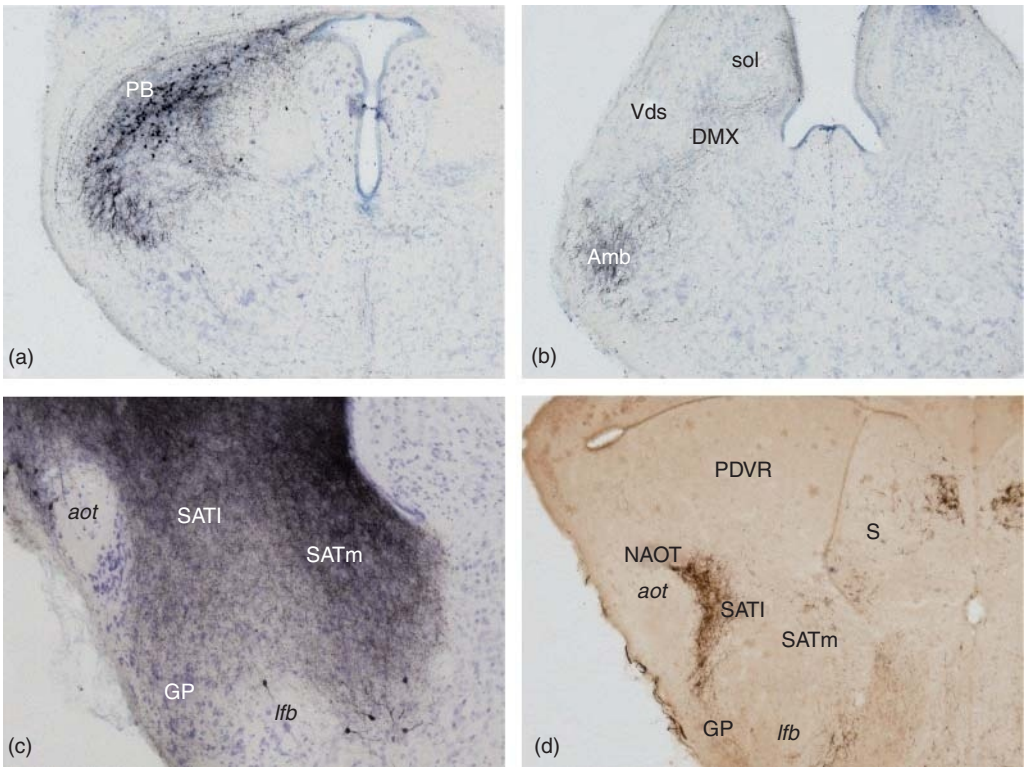

Figure 15 Connections and histochemical properties of the SAT. a and b, Tracer injections encompassing the SAT of *Podarcis* give rise to anterograde labeling in the lateral hypothalamus (Lanuza *et al.*, 1997), but also in the parabrachial area (a), where retrograde labeling is also visible, and in the medulla (b), including the nucleus of the solitary tract (sol), the dorsal motor vagal nucleus (DMX), and the ambiguus (Amb). c, Anterograde labeling in the SAT following a tracer injection in the PDVR and LA in *P. hispanica*. d, The lateral SAT (SATl) of the lizard *P. hispanica* shows a dense innervation by CGRP-immunoreactive fibers. The medial SAT (SATm) shows a much weaker innervation that is mainly composed of nests around the cell bodies.

reptiles is composed of cortical olfactory (VAA, portions of the LC) and vomeronasal centers (NS) plus deep, unlaminated regions (PDVR, LA, and DLA) that receive multimodal and unimodal afferents from different levels of the neuroaxis. An additional nuclear pallial nucleus that (in spite of its superficial location) is topologically deep to the vomeronasal cortex, the VPA, is part of a forebrain circuit closely related to the vomeronasal system which is rich in cells expressing receptors for sexual steroids.

The vomeronasal amygdala includes not only the NS (and VPA) but also a portion of the subpallium, the MA, and BSTm (medial EA), which is also very rich in receptors to sexual steroids. The pallial and subpallial centers of the vomeronasal amygdala are interconnected and project massively to centers in the limbic forebrain (septum, preoptic, and tuberal and premmamillary hypothalamus), probably involved in the control of reproductive and agonistic behaviors.

In contrast, the multimodal centers of the pallial amygdala (PDVR, LA, and DLA) project massively to the ventral striatum, including the Acb and the SAT. The latter is a part of the subpallial amygdala that shows long-distance projections reaching hypothalamic, tegmental, periaqueductal, pontine, and medullary targets. This pathway seems involved in the control of somatomotor, endocrine, and vegetative reactions. In addition, the deep lateropallial nucleus, the DLA, projects bilaterally to the dorsal striatum.

This picture is virtually identical to the one observed in mammals, thus suggesting that the amygdala as a whole has undergone quite a conservative evolution in the phylogeny of amniote vertebrates. As we will see, data from birds further support this view.

15.6 The Amygdala of Birds

Identifying the avian amygdala becomes a fundamental issue after the recent joint effort of an international forum of comparative neuroscientists to rename and reinterpret the telencephalon of

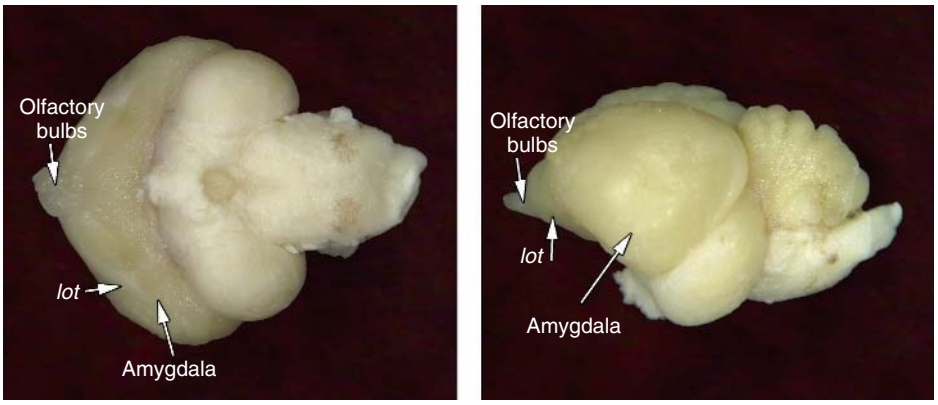

Figure 16 Ventral and lateral views of the brain of the quail (*Coturnix coturnix*). The regression of the olfactory system is evidenced by the small size of the olfactory bulbs (where accessory olfactory bulbs are absent) and the inconspicuous lateral olfactory tract (*lot*). An arrow points to the putative amygdala.

birds (Reiner *et al.*, 2004; Jarvis *et al.*, 2005). However, two features of the avian brain make this task especially difficult. First, as compared to mammals, birds possess very peculiar cerebral hemispheres, since they have a huge dorsal ventricular ridge (occupying an apparent subventricular position), but lack anything recalling the mammalian isocortex. This has resulted in important changes in the topographical relationships between the different areas of the pallium and subpallium of avian cerebral hemispheres, as compared to mammals. In addition, birds have undergone a regression of the olfactory and a virtual atrophy of the vomeronasal system, which, as we have seen, provide important inputs to the mammalian and reptilian amygdalae, where they constitute the defining features of their cortical and medial portions. This is clearly observed in a macroscopic view of the brain of birds (Figure 16), in which the olfactory bulbs are very small, the lateral olfactory tract is hardly visible, and no rhinal fissure is observed.

Nevertheless, once the reptilian amygdala and its components have been identified with a high degree of certainty, the similarities between the avian and reptilian brains (Ulinski, 1983) may be very helpful for our goal. In this section, we are using this strategy to identify the amygdala in the caudolateral telencephalon of birds and to delineate its main pallial and subpallial components by using the features that define them in both mammals and reptiles.

15.6.1 On the Nomenclature and Architecture of the Telencephalon of Birds

In this article, we will follow the revised nomenclature of the avian forebrain (Reiner *et al.*, 2004; Jarvis *et al.*, 2005). However some issues concerning this nomenclature need to be discussed prior to analyzing the identity of the amygdala of birds.

The caudal cerebral hemispheres of birds (Figure 17) includes several structures that conform a regular (superficial and layered) cortex, composed of several areas, from medial to lateral, the Hp, the APH, the area corticoidea dorsolateralis (CDL), and the CPi, that needed no renaming since their cortical nature was always recognized. Inner structures of the dorsal telencephalon include those pallial structures whose name contained the suffix striatum, which have been renamed to recognize their pallial nature. Thus, the deep pallium of the posterior telencephalon of birds is composed of the caudal edge of the mesopallium (M, formerly ventral hyperstriatum) and the caudal nidopallium (formerly caudal neostriatum), in which lateral and medial divisions are usually recognized (NCL and NCM respectively). The NCM includes the auditory field L. In addition, there is a region just deep to the CPi that extends dorsally up to the CDL, which is called the area temporoparieto-occipitalis (TPO).

More ventrally, the caudal poles of the lateral striatum (LSt; formerly paleostriatum augmentatum) and GP (formerly paleostriatum primitivum) occupy the medial aspect of the hemispheres. Just ventral to them (thus justifying the old name of ventral paleostriatum) there is a region now renamed as SpA, topologically superficial to the so-called lateral BST (occupying a juxtaventricular position).

Finally, ventral and lateral to the caudal subpallium there is a region traditionally called archistriatum and the nucleus teniae of the amygdala (TnA). The archistriatum was considered to be composed of several divisions that were named

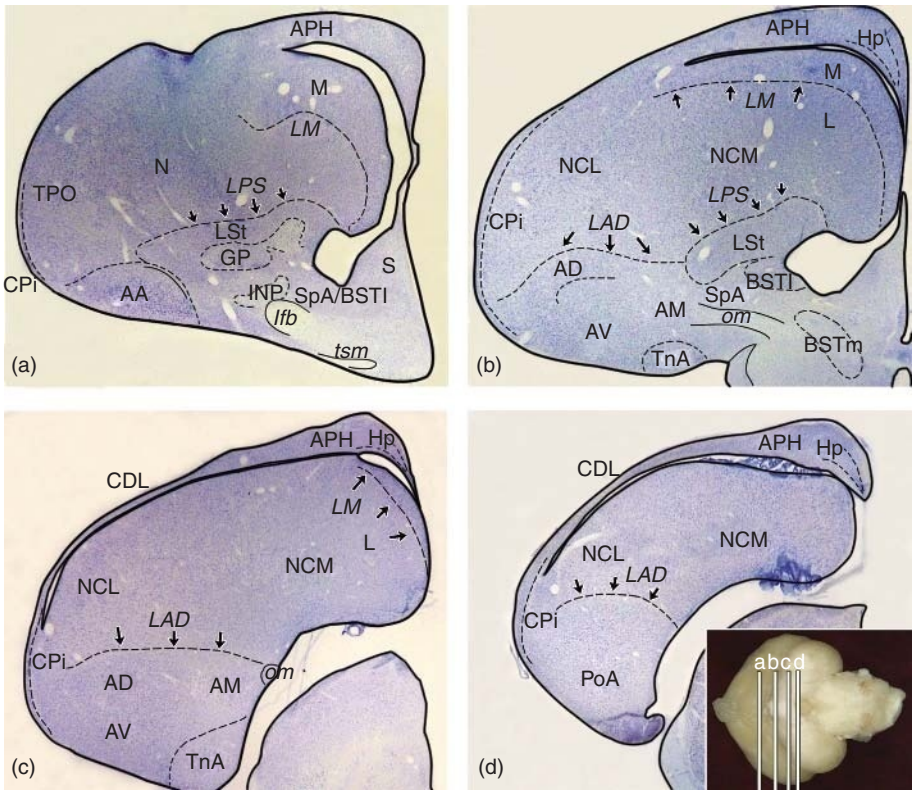

Figure 17 Cytoarchitecture of the caudal cerebral hemispheres of the chicken. Nissl-stained frontal sections through the left cerebral hemisphere of a newborn chicken at slightly precommissural (a); commissural (b); postcommissural (c); and caudal (d) levels of the telencephalon. The inset shows the approximate location of the sections on a ventral view of the avian brain. The different nuclei and cortical areas of the telencephalic structures are delineated using thin discontinuous lines, whereas thin solid lines indicate the main fiber tracts. Small arrows point to the laminae that separate the main divisions of the avian forebrain (also delineated in discontinuous lines), whose abbreviations are written in italics.

topographically as anterior, medial, intermediate (with dorsal and ventral subdivisions), and posterior. The new nomenclature reflects the putative pallial nature of the whole archistriatum by naming its parts as anterior arcopallium (AA), medial arcopallium (AM), dorsal arcopallium (AD; corresponding to the dorsal intermediate archistriatum), ventral arcopallium (AV; corresponding to the ventral intermediate archistriatum), and the posterior nucleus of the pallial amygdala (PoA, corresponding to the former posterior archistriatum).

Two problematic issues directly related to our purposes need to be discussed. First, the boundaries between the AA and the AD/AV (formerly intermediate archistriatum) have never been properly defined and the same happens with the border separating the AD/AV from the PoA. In this respect, the identity of the PoA is not clear and, consequently, different authors have labeled different structures as PoA (or posterior archistriatum), thus generating a confusing panorama (see, for instance, the extent of the PoA in Reiner and Karten, 1985, as compared to Kroner and Gunturkun, 1999). Most authors simply label as PoA (or posterior archistriatum) the caudal edge of the former archistriatum, thus including the caudal tip of the AD (like the latter, deep to the caudal-most CPi), plus the posterior aspect of the AV. Dealing with this, it is important to recall that the nowadays-accepted classification of the arcopallial-amygdaloid centers of birds is based on a topographical compartmentalization of this area introduced by Zeier and Karten (1971), who reported to have identified some four to eight cytoarchitecturally discrete nuclei in each of the major subdivisions of the former archistriatum. The poor understanding of the actual organization of the arcopallium/PoA is probably hindering our knowledge of its comparative and functional significance.

Second, the use of the term 'arcopallium' instead of the old name 'archistriatum' (Reiner *et al.*, 2004) is somewhat problematic since it excludes the possibility that a part of the former archistriatum may be subpallial. This is suggested by the distribution of

radial glia in developing chicken (Striedter and Beydler, 1997), indicating that the AM (formerly medial archistriatum) may be partially subpallial in nature and, therefore, part of the SpA. This issue requires a thorough analysis using modern techniques to identify the palliosubpallial boundaries in the avian brain.

15.6.2 Topological Identification of the Avian Pallial Amygdala

The mammalian and reptilian amygdalae contain lateropallial and ventropallial territories that, in turn, are composed of superficial laminar structures receiving subpial projections from the olfactory bulbs (the cortical amygdala) and deep nonlaminated centers (mainly multimodal), which constitute their basolateral amygdala. In this section, we are reviewing the literature available to identify and delineate these two pallial components of the avian amygdala (Figures 17 and 18). In addition, we will review the data on connections and histochemistry that may help to refine the comparison of the pallial amygdala of birds with that of reptiles and mammals.

15.6.2.1 Olfactory areas in the avian pallium: The avian cortical amygdala The small size of the olfactory bulbs of birds is probably the reason why their projections have been poorly investigated. Fortunately, Reiner and Karten (1985) traced the olfactory projections in pigeons by means of injections of tritiated amino acids. Their results demonstrate olfactory projections to the olfactory tubercle and the presence of abundant labeled fibers in the frontoarcopallialis (FA; frontoarchistriatal tract in the old nomenclature) through which they reach several parts of the pallium, including the whole CPi and more ventral regions in the caudal cerebral hemispheres. There, the olfactory projection reaches cortical areas

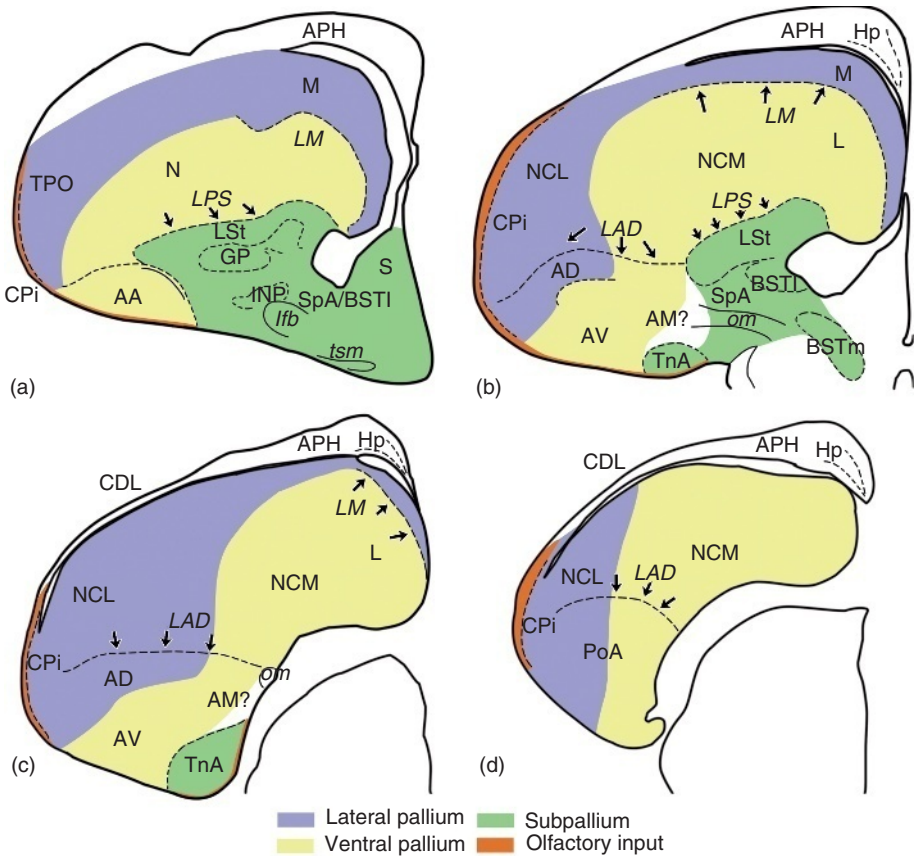

Figure 18 Pallial and subpallial territories of the avian amygdala. A schematic diagram of the amygdala of birds, based on the Nissl-stained sections of Figure 17, which shows the palliosubpallial boundary and the extent of the latero- and ventropallial territories within the pallial amygdala (based on Puelles *et al.*, 2000). The projection fields of the olfactory bulbs (based on the description by Reiner and Karten, 1985) are also indicated. The actual boundary of the ventral pallium with the subpallium is difficult to trace at the level of the AM (white region in the AM).

superficial to the AA, AD, and anterior part of the AV, up to the level of TnA. From there, olfactory fibers seem to enter the stria medullaris to reach the contralateral cerebral hemisphere through the habenular commissure. More caudally, a gap appears between the olfactory projection to the CPi and that to the TnA, so that apparently the structures superficial to the caudal portion of the AV are not olfacto-recipient. These results have been replicated in chick embryos using lipophilic tracers (Striedter *et al.*, 1998).

Reiner and Karten (1985) interpreted their results as demonstrative of the absence of olfactory projections to the amygdala in birds, with the exception of the olfactory pathway to the TnA. However, in the light of the results in reptiles and mammals, and of the interpretation of the nature and organization of the amygdala derived from them, the structures superficial to the AA and to the rostral AV, plus the caudal CPi, are to be interpreted as the cortical amygdala of birds. Reiner and Karten (1985) also discuss the lack of vomeronasal organ and of accessory olfactory bulbs and its possible consequences on the organization of the projections from the olfactory bulbs. In our view, this may have resulted in the disappearance of the projections to the superficial caudal aspect of the caudal AV that would have been the natural target for the projections from the AOB. This will be further discussed in relation to the existence or absence of a subpallial chemosensory amygdala (equivalent to mammalian Me).

15.6.2.2 The deep pallial amygdala in birds According to the general scheme of the amygdala of mammals and reptiles, those pallial structures deep to the olfacto-recipient cortical areas described above would constitute the pallial nuclear amygdala, e.g., the basolateral amygdala plus the AHA of birds. The distribution of radial glia in the cerebral hemispheres of chicken embryos (Kalman *et al.*, 1993; Striedter and Beydler, 1997) strongly suggests that the dorsolateral aspect of the arcopallium (pallial archistriatum, in the words of Striedter and Beydler, 1997) plus the caudal M, the NC, and TPO, are topologically deep to the olfacto-recipient cortical structures mentioned above (Figure 18). This was subsequently confirmed by combining an innovative method of tracing the fate of cells generated in specific zones of the telencephalic ventricles with labeling of the projections from the olfactory bulb in chicken embryos (Striedter *et al.*, 1998). In addition, as discussed above, the PoA contains the structures deep to the posterior edge of the CPi and fuses without clear boundaries with the caudal edge of the AD/AV. Consequently, its new name seems especially appropriate to designate a pallial amygdaloid structure.

These data indicate that the deep pallial amygdala of birds is conformed by, at least, the NC and the TPO plus the pallial arcopallium and the PoA.

15.6.2.3 Lateral and ventral pallial derivatives in the caudal avian cerebral hemispheres Although the olfactory projection is often taken as a marker of the lateral pallium (see, for instance, the title of the paper by Striedter *et al.*, 1998), data from the mammalian neuroanatomy reviewed above indicate that the olfacto-recipient cortical areas include lateropallial and ventropallial derivatives. This is fully supported by the pattern of expression of homeotic genes in the cerebral hemispheres of birds (Smith-Fernandez *et al.*, 1998), which indicates the lateropallial origin of the M and the ventropallial origin of the N. Using a similar approach and a detailed anatomical analysis, Puelles *et al.* (2000) mapped the ventropallial and lateropallial territories in the caudal cerebral hemispheres of chicken embryos. According to their results and their interpretation of the cytoarchitecture of the cerebral hemispheres of birds, the embryonic ventral pallial territories are composed of the rostral N, including the entopallium (E, formerly ectostriatum) and the basorostral pallial nucleus (Bas; formerly basal nucleus). In addition, the AV (classically called ventral intermediate archistriatum) displays a profile of gene expression typical of the ventral pallium that extends caudally into what most authors consider the PoA.

In turn, the lateral pallium includes the CPi, and the structures topologically deep to it, namely the whole M and the AD. Moreover, the lateral pallium seems to impinge into the PoA. As noted previously (Martinez-Garcia *et al.*, 2002a), topology and cytoarchitecture strongly suggest the existence of a lateropallial bridge connecting the rostral (M) with the caudal (AD) territories of the lateral pallium. This bridge is probably composed of the lateral aspect of the NC, including the TPO, part of which is indeed immediately deep to the CPi. This was fully confirmed by the pattern of expression of cadherins in the cerebral hemispheres of embryonic chicken (Redies *et al.*, 2001).

The position of the AA within this map requires further discussion. According to Puelles *et al.* (2000) and Redies *et al.* (2001), the AA (anterior archistriatum in the classical terminology) is part of the

subpallium. We consider, however, that the structure that they label as AA is a cytoarchitectonically distinct portion of the LSt of the embryonic chicken brain. In contrast, most of the avian neuroanatomists call AA to the anterior pole of what Puelles *et al.* (2000) and Redies *et al.* (2001) label as intermediate archistriatum, plus their nucleus of the LOT (see figures 3 and 4 in Redies *et al.*, 2001). If this interpretation is correct, the AA turns out to be a rostral ventropallial region (Figure 18) closely associated with the LOT (which in birds is named as frontal arcopallial tract, FA; Striedter *et al.*, 1998). As we will see, connectional data clearly support this view.

The palliosubpallial boundary is delineated by the lamina palliosubpallialis. However, at commissural and postcommissural levels, the AM is interposed between the SpA and the TnA, and crossed by the fibers of the occipitomesencephalic tract (OM) as they leave the cerebral hemispheres. Since the SpA and AM are subpallial, a transition between ventropallial and subpallial territories is expected at this level. Tracing this boundary becomes an unresolved and potentially important issue.

15.6.2.4 The avian pallial amygdala: A proposal of homologies with mammals The map of the pallial amygdala of birds depicted in Figure 18 leads to a proposal of homologies of the caudolateral cerebral hemispheres of birds with the mammalian pallial amygdala that is topologically consistent and shows congruence with the proposed map of the reptilian amygdala, with which the comparison is quite straightforward (Table 3). According to this proposal, within the lateral pallium, the CPi would contain the homologues of the mammalian lateropallial olfactory cortices, including the COApl and the transition areas with the piriform and entorhinal cortices (APi and TR, respectively). The structures of the avian brain deep to the CPi, including the lateral NC, TPO, AD, and the lateropallial PoA, would be the putative homologues for the mammalian deep lateropallial amygdala, the B nucleus.

The superficial ventropallial regions of the telencephalon of birds should be homologous to the olfactory and vomeronasal cortical areas of the mammalian amygdala. The AA is a rostral ventropallial region associated with the *lot* (FA), and consequently constitutes the most likely candidate for the avian homologue to the LOT of mammals. On the other hand, the superficial anterior part of the AV, which receives direct projections from the olfactory bulbs, occupies a position in the avian brain comparable that of the COAa in the mammalian amygdala. The deep anterior AV/AM seems topologically equivalent to the ABa of mammals, and therefore we conceive both structures as homologous.

In contrast, at the level of the caudal AV, a superficial olfacto-recipient cortex is lacking (this region is labeled as posterior archistriatum by Reiner and Karten, 1985). This occupies the topological position where the projections from the accessory olfactory bulbs should terminate (if they existed). Therefore, according to our view, birds have an only-deep caudal ventropallial region, where a vomeronasal cortex equivalent to the mammalian COApm is lacking, but the structures equivalent to the nuclei deep to it (ABp and AHA) are presumably located. In this respect, the location of the PoA in the caudal edge of the arcopallium (thus its name) suggests that its ventropallial portions are equivalent to the mammalian AHA (named posterior nucleus of the amygdala by Canteras *et al.*, 1992a) and the reptilian VPA. Finally, the NCM, a deeper ventropallial structure located in the dorsomedial PDVR of birds, is a good candidate for the mammalian L, thus equivalent to the reptilian PDVRdm (Table 3).

15.6.3 Connections and Histochemistry of the Avian Pallial Amygdala: Comparative Implications

There are few studies of the connections of the centers here proposed as the avian homologues of the pallial amygdala, and this is especially true for the putative cortical amygdala. Nevertheless, since the pioneer work by Zeier and Karten (1971) on the archistriatum of pigeons, all the published reports of projections of the arcopallium, PoA, and adjoining areas (Bingman *et al.*, 1994; Davies *et al.*, 1997; Dubbeldam *et al.*, 1997; Kroner and Gunturkun, 1999) coincide in indicating that the caudolateral pallial telencephalon originates a long descending projection known as occipitomesencephalic pathway (OM). This projection reaches not only parts of the hypothalamus (OM pars hypothalamii, OMH), as expected from a pallial amygdaloid structure, but also the posterior thalamus and deep midbrain (thus its name). This projection has been reported to reach also pontine regions, the reticular formation, and dorsal medulla (Zeier and Karten, 1971). No region of the caudal pallium of mammals or reptiles gives rise to such a projection. Thus, the OM seems exclusive of the avian brain. Consequently, we will not use this trait to discuss the identity of the avian pallial amygdala, but we devote a section to analyzing its possible origin, as well as its comparative and functional significance.

15.6.3.1 The connections of the cortical amygdala of birds The connections of the CPi have been studied in pigeons using large injections of anterograde and retrograde tracers (Bingman *et al.*, 1994). The results indicate that the CPi displays the characteristic projections of the piriform cortex, including associative and commissural projections and connections with other secondary olfactory centers, plus other connections that are difficult to interpret from a comparative viewpoint (e.g., connections with the dorsal mesopallium). In addition, the CPi is connected with the parahippocampal cortex and presumed deep pallial amygdaloid centers such as the AD, PoA, and TPO/N. It is also reciprocally connected with the bulbo-recipient subpallial amygdala (TnA). Moreover, it displays some descending projections to striatal territories, including the Acb and MSt (formerly lobus paraolfactorius), parts of the lateral septum, the BSTl, and the SpA. Finally, tracer injections in the CPi give rise to fiber labeling in the OM.

This pattern of intratelencephalic connections of the avian CPi is much more extensive than expected for a mere piriform cortex. Although the authors (Bingman *et al.*, 1994) accept that some of this labeling is seemingly due to the involvement of deep tissue in the injection site, these data can also be interpreted as suggestive of a field homology among the CPi of birds with the whole lateropallial olfactory cortex of reptiles and mammals. As discussed above, the olfacto-recipient lateral pallium seems to have undergone a differential compartmentalization in each group of amniotes.

Data on the ventropallial olfactory-related areas derive from the report by Davies *et al.* (1997) on the connections of the chicken archistriatum. Their results confirm the view put forward by Zeier and Karten (1971) that the AA is the region of the arcopallium giving rise to most projections through the anterior commissure. These contralateral projections mirror the intratelencephalic ipsilateral efferents of the AA, thus resulting in a pattern of bilateral intratelencephalic projections to extensive areas of the pallium (Hp, Wulst, M, and N). Bilateral efferents from the AA also target the olfactory bulbs and retrobulbar formation, the CPi, and portions of the AD and AV. Morever, the AA also shows bilateral projections to the subpallium, which specifically innervate the MSt (just lateral to the Acb) and olfactory tubercle. Finally, the AA projects massively to its contralateral counterpart.

This is demonstrative of the pallial nature of the AA (thus contradicting the view proposed by Puelles *et al.*, 2000), since only cortical areas are involved in commissural and ipsilateral projections to cortical areas (corticocortical pathways; see above). Moreover, this recalls the massive homotopic commissural projections of the mammalian LOT (Johnston, 1923) and its bilateral pathways to the olfactory system (olfactory bulbs, anterior olfactory nucleus, piriform and endopiriform cortex, olfactory tubercle), to atypical striatal structures (fundus striatum, IPAC), to parts of the pallial amygdala and to limbic and transitional cortical areas (Santiago and Shammah-Lagnado, 2004). Therefore, connectional data fully confirm our proposal of a homology of the avian AA with the LOT of mammals and reptiles (Table 3).

15.6.3.2 Connections of the avian basolateral amygdala The basolateral amygdala of mammals (B, L, and AB) receives sensory inputs from many different sources. In addition, its nuclei show differential projections to the dorsal and ventral striatum, to the hypothalamus, and to the cortical fields from which they receive their main inputs. As we describe below, most of these features are met by the proposed homologues to the basolateral amygdala in the brain of birds.

15.6.3.2.(i) Identity of the basal nucleus of the avian amygdala in the deep lateral pallium The B of the mammalian amygdala is the main source of projections to the dorsal and ventral striatum. The projections to both striatal compartments within the cerebral hemispheres have been thoroughly studied in pigeons twice by means of anterograde and retrograde tracing techniques (Veenman *et al.*, 1995; Kroner and Gunturkun, 1999). These studies reveal projections to dorsal striatal territories arising from some of the proposed pallial amygdaloid centers. Specifically, the deep lateropallial derivatives (deep CPi/TPO, NCL, and AD, plus maybe part of the PoA) project massively to the LSt. At least part of this projection, arising from rostral levels of the arcopallium, is bilateral. The deep lateropallial amygdala also projects to the ventral striatum, namely the Acb (formerly, the medial aspect of the lobus paraolfactorius). In many cases, this projection extends caudally to reach also the lateral BST and the SpA. Thus, tracer injections involving the posterior NCL and/or PoA (Veenman *et al.*, 1995; Kroner and Gunturkun, 1999) reveal a projection to the continuum Acb/BSTl-SpA. A similar pattern of anterograde labeling is found after large tracer injections into the CPi that also encompass deep structures (Bingman *et al.*, 1994; Veenman *et al.*, 1995).

This supports our hypothesis that the TPO/NCL, the AD, and its caudal continuation within the PoA

constitute the B nucleus of the amygdala of birds (Table 3). If so, these structures should display a convergent dopaminergic and cholinergic innervation, which constitutes the most remarkable defining histochemical feature of the mammalian B. In fact, birds show a dense dopaminergic innervation of the striatum plus a field of dopaminergic fibers in the caudolateral cerebral hemispheres that comprises the NCL, AD, and extends into the PoA (Figures 19a and 19b; Durstewitz *et al.*, 1999). These same pallial areas show a dense innervation by cholinergic fibers, as revealed by either ChAT immunostaining (Medina and Reiner, 1994) or AChase histochemistry (Figures 19c and 19d).

Therefore, the AD plus its neighboring areas within the deep caudal lateral pallium make up the most likely homologue of the mammalian B and the reptilian DLA. In fact, if one compares the images of the caudolateral cerebral hemispheres of birds (Figure 19) with similar images from the brains of

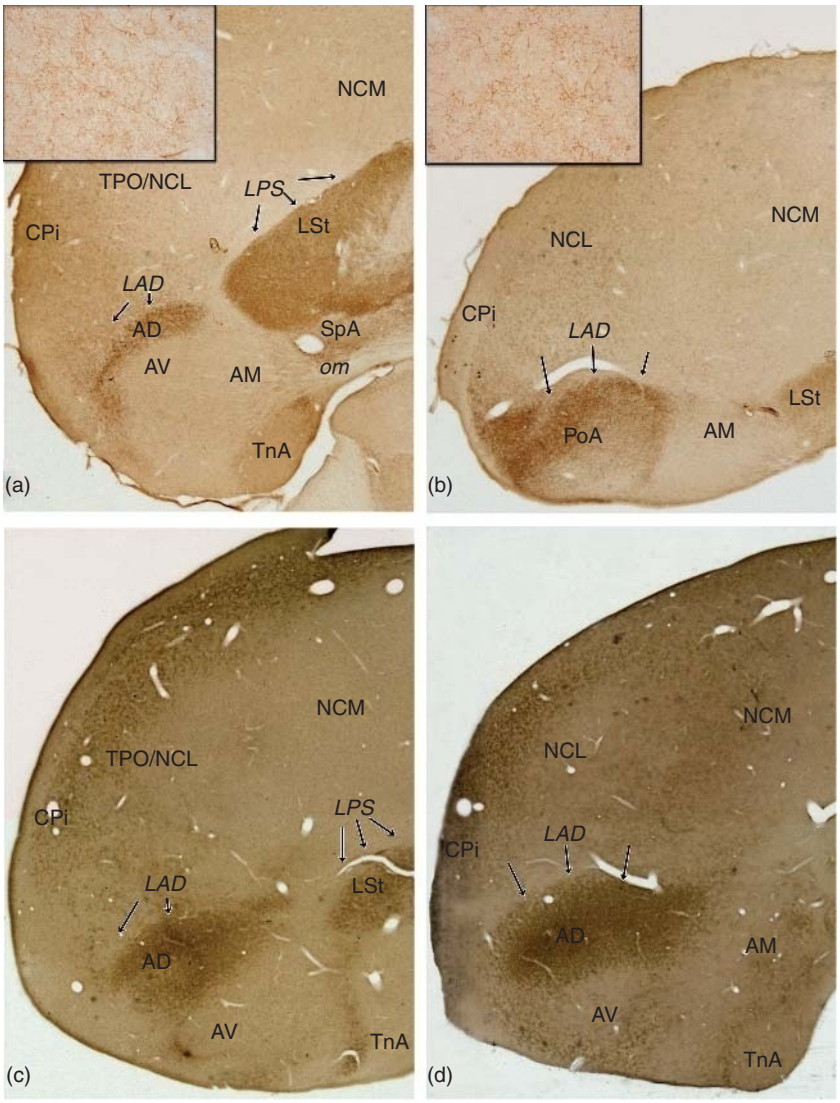

Figure 19 Histochemistry of the pallial amygdala of birds. a and b, The distribution of fibers immunoreactive for tyrosine hydroxylase (TH) in the caudal telencephalon of the quail (*Coturnix coturnix*) is shown in two frontal sections of the left hemisphere (slightly postcommissural (a) and caudal section (b)). Dopaminergic fibers innervate the striatum and parts of the amygdaloid complex. This includes a dense innervation of the AD and parts of the PoA and a scarcer innervation of the TPO and NCL (insets). In addition, the SpA also shows a remarkable TH-immunopositive innervation (a). c and d, Distribution of AChase histochemistry in two frontal sections of the left cerebral hemisphere of the chicken (*Gallus domesticus*) at slightly postcommissural (c) and caudal levels (d). The AD shows a remarkable AChase-positive innervation that extends into the TPO and NCL. Therefore, all three structures are targeted by convergent dopaminergic and cholinergic innervations, which supports their homology with the basal nucleus of the mammalian amygdala.

microsmatic lizards (such as *Anolis*; Box 1), the resemblance is astonishing. The lack or strong reduction in the vomeronasal system in birds and anole lizards has resulted in a similar displacement of the deep lateropallial amygdala. Their histochemical features, however, remain the same and clearly indicate that they are the sauropsidian homologues of the basal nucleus of the amygdala of mammals.

15.6.3.2.(ii) Deep ventropallial nuclei: The AV/AM and NCM The ventropallial basolateral amygdala of mammals contains a deep sensory interface, the L nucleus, and another more superficial nucleus projecting to the ventral striatum and hypothalamus, the AB. According to our hypothesis (Table 3), the avian AV/AM should display projections to the ventral striatum and hypothalamus (like the mammalian AB), whereas the deepest ventropallial amygdala, the NCM, would constitute the sensory interface of the pallial amygdala, like the mammalian L.

15.6.3.2.(ii).(a) The homology between the AV/AM and the mammalian AB The projections from the AV/AM to the ventral striatum have indeed been described. Tracer injections into the Acb of the pigeon (formerly medial lobus paraolfactorius; Veenman *et al.*, 1995; Kroner and Gunturkun, 1999) retrogradely label neurons in the lateropallial structures referred to above (NCL/TPO, AD, and, to a lesser degree, PoA) but also in the caudal AV (named archistriatum centrale by Veenman *et al.*, 1995). Anterograde tracing of this projection (Veenman *et al.*, 1995; Kroner and Gunturkun, 1999) indicates that it extends into the BSTl/SpA. The arcopallial projection to the Acb/BSTl/SpA also arises in part from the AM, as indicated by both retrograde (Veenman *et al.*, 1995; Kroner and Gunturkun, 1999) and anterograde tracing experiments (Davies *et al.*, 1997). This suggests that the AV/pallial AM are the avian counterpart of the mammalian AB, in terms of both topology and hodology.

Concerning the projections to the hypothalamus, our hypothesis predicts two projections arising from different areas of the AV/AM. Like the ABa, the rostral portions of the AV/AM should project to the lateral hypothalamus, whereas the caudal AV/AM, likely homologous to the mammalian ABp (and the reptilian PDVR and LA), would therefore project to the ventromedial tuberal hypothalamus. The existence of such a pathway in the brain of birds is evidenced by the presence of a zinc-rich terminal field in a nucleus of the caudal ventromedial hypothalamus of the chick (Faber *et al.*, 1989), clearly misidentified by the authors as the nucleus papillioformis, which seems to correspond to medial nucleus of the posterior hypothalamus (PMH). In addition, Montagnese *et al.* (1993) described a dense, zinc-containing terminal field in the PMH and an additional one in the nucleus tuberis of the premammillary/mammillary hypothalamus.

In this respect, the seminal paper by Zeier and Karten (1971) already described a tractus occipitomesencephalicus pars hypothalami (OMH) terminating mainly in the medial PMH (posterolateral and posteromedial nuclei) but also in the lateral hypothalamus and the so-called stratum cellulare internum and stratum cellulare externum. According to their description, this termination field extends into the infundibular hypothalamus at premammillary–mammillary levels. The site of origin of this zinc-rich arcopallial/amygdaloid projection (or projections) to the hypothamus is unclear. Zeier and Karten (1971) observed degeneration of the OMH after lesions of the posterior archistriatum and/or superficial parts of the caudal AV, plus the subpallial TnA (the latter projection is discussed below). However, they described the OMH as a single pathway, and did not contemplate the possibility of it being composed of several projections with different sites of origin in the archistriatum and termination fields in the hypothalamus, as apparently happens in mammals and reptiles.

Nevertheless, modern neuroanatomical techniques based on intra-axonic transport of tracers suggest that this is indeed the case. Thus, Davies *et al.* (1997) report anterograde labeling in the lateral hypothalamus after tracer injections centered in the anterior AM of the chicken. In contrast, injections in the caudal AM, just dorsal to the TnA in ring doves (Cheng *et al.*, 1999), specifically label fibers of the OMH directed to the preoptic anterior hypothalamus and to the PMH, which do not reach the mammillary–premammillary levels. This suggests that the AM includes a rostral portion displaying projections similar to those of the ABa and a caudal one, the hypothalamic projections of which recall those of the ABp. It is tempting to suggest that the anterior AM (projecting to the lateral hypothalamus) is topologically deep to the anterior olfacto-recipient archistriatum illustrated by Reiner and Karten (1985) in the pigeon, whereas the caudal AM, which projects to more medial areas of the preoptic to tuberal hypothalamus, is deep to the caudal, nonolfactory-recipient AV. However, studies on the histogenetic gradients and topological relationships of the arcopallium are lacking.

As a conclusion, like its putative mammalian homologue (the AB), the AV/AM of birds projects to the ventral but not the dorsal striatum. Apparently, part of this arcopallial region gives rise to a projection to the lateral hypothalamus. In addition, birds, like mammals and reptiles, display a zinc-rich projection directed to a cell group in the ventromedial posterior (tuberal) hypothalamus. This projection seems to arise, at least in part, from the caudal aspect of the AM, just dorsal to the TnA, which is therefore the most likely homologue of the mammalian ABp, in terms of both topology and connections. A systematic study of the projections to the hypothalamus from the caudal cerebral hemispheres using both retrograde and anterograde tracing techniques, and a deep analysis of the architecture of the arcopallium, PoA, and hypothalamus of birds, are urgently needed to clarify this issue further.

15.6.3.2.(ii).(b) Sensory afferents: The caudomedial nidopallium as the sensory interface of the avian amygdala In view of the organization of the reptilian amygdala we have described above, we expect to find a sensory interface of the pallial amygdala within the dorsomedial PDVR, which in birds is represented by the caudomedial nidopallium. In this respect, the NC is the target for important afferents from the different sensory centers of the cerebral hemispheres. This was reviewed in detail by Metzger *et al.* (1998) and Kroner and Gunturkun (1999), who realized that the NC is the target for thalamic and intratelencephalic sensory afferents. Thus, it receives projections from the secondary sensory areas of the neostriatum (fields L1 and L3, entopallial belt, frontal nidopallium, nucleus basalis, and intermediate nidopallium). The thalamic input to the NC arises mainly from the shell of nucleus ovoidalis and the dorsolateral posterior thalamic nucleus (DLP), which, according to Korzeniewska and Gunturkun (1990), constitute multimodal thalamic relays. Moreover, studies in chicken and quails strongly suggest that this thalamonidopallial pathway contains CGRP, as is the case for the projections to the L in mammals (see Lanuza *et al.*, 2000). In contrast to the NCL, the NCM seems mainly involved in intra-amygdaloid projections, since it does not display substantial projections to either the dorsal or the ventral striatum. Thus, NC projects to the AV and AD (Metzger *et al.*, 1998) in a topographic manner. These projections are reciprocated by arcopallial–nidopallial pathways (Kroner and Gunturkun, 1999).

These results indicate that the deep caudal cerebral hemispheres are organized very alike in birds and reptiles. In both groups, the caudal DVR receives afferents from different telencephalic and thalamic centers, and it is divided in two main areas with different embryological origin and connectional properties. The medial area (avian NCM and reptilian PDVRdm) is a ventropallial derivative mainly engaged in intra-amygdaloid projections within the ventral pallium (intra-amygdaloid) directed to the projecting areas of the pallial amygdala (AV and AD in birds, PDVRvm, LA, and DLA in reptiles). The lateral area is a lateropallial derivative (the avian NCL/TPO plus the AD; the reptilian DLA) that projects to the dorsal and ventral striatum. In this scheme, the NCM should be considered the sensory interface of the avian pallial amygdala, thus becoming functionally equivalent to its most likely homologue in the mammalian brain, the lateral nucleus of the amygdala. As in reptiles, in birds the possibility that the anterior sensory DVR (e.g., the sensory centers of the nidopallium, including the entopallial nucleus) constitutes an enlarged and highly specialized LA cannot be ruled out (Table 3). The presence of a primary auditory pallium immersed in the avian lateral amygdala, the field L, is a specific feature of the avian brain that probably had a strong influence in the organization of other parts of the amygdala of birds.

15.6.3.2.(ii).(c) Amygdalocortical projections Another attribute of the basolateral amygdala is the presence of projections to the cortical areas from which it receives projections. In mammals, this is represented by the projections arising in the basolateral amygdala (B and AB) directed to the prefrontal, insular, and perirhinal cortices. In reptiles, equivalent projections arise from the LA and terminate in the ADVR (Bruce and Butler, 1984; Martinez-Garcia *et al.*, 1993), thus constituting a corticocortical projection within the ventral pallium that reciprocates the ADVR–PDVR/LA projection. In contrast to mammals, in reptiles the amygdala does not project back to the dorsal pallial region (dorsal cortex; Hoogland and Vermeulen-Vanderzee, 1989; Lanuza *et al.*, 1998).

Birds apparently display a reptile-like organization of the amygdalocortical interconnections. Thus, the AV is interconnected with those portions of the anterior nidopallium that project to the pallial amygdala, namely the medial intermediate N, and the frontal N (Kroner and Gunturkun, 1999). This supports the proposed homology between the AV and the reptilian LA. However, pigeons also display projections from portions of the AV to dorsal pallial sensory areas such as the visual Wulst (part of the hyperpallium apicale), which provides a scarce

projection back to the AV (Shimizu *et al.*, 1995). This seemingly constitutes an apomorphic feature of the avian brain.

15.6.3.2.(iii) The posterior amygdala of birds and its homology with the mammalian AHA Several lines of evidence strongly suggest that the PoA constitutes the avian homologue for the AHA of the mammalian amygdala. From a topological point of view, the PoA and AHA occupy the caudal edge of the area bounding the ventral and lateral pallial territories. This is also consistent with the proposed scheme of the reptilian amygdala, since the position of the VPA (the reptilian homologue of the AHA; Table 3) in the reptilian cerebral hemispheres is clearly reminiscent of the avian PoA.

Moreover, most of the defining features of the mammalian AHA are accomplished by the PoA of birds. Thus, the PoA not only projects to the posterior medial hypothalamus, including the infundibular mammillary/premammillary levels (Zeier and Karten, 1971; Davies *et al.*, 1997; Kroner and Gunturkun, 1999), but also receives a projection from supramammillary–premammillary hypothalamus (Berk and Hawkin, 1985). Although there are no studies on the comparative significance of the mammillary region of the hypothalamus of birds, its interconnection of the PoA is reminiscent of the reciprocal connections displayed between the AHA and the PMv of mammals. This is supported by the fact that PoA, like the AHA, is the only structure of the pallial caudolateral cerebral hemispheres also projecting to the medial preoptic hypothalamus (Absil *et al.*, 2002).

The PoA is also connected to the septohippocampal system. Thus, reciprocal connections of the APH with the caudal PoA were reported in the pigeon by Casini *et al.* (1986), and recently confirmed by Atoji and collaborators (Atoji *et al.*, 2002; Atoji and Wild, 2004). In addition, the PoA projects unidirectionally to the portion of the ventral lateral septum that receives projection from the APH (Atoji and Wild, 2004). Therefore, the ventral lateral septum receives a projection from two pallial centers, one in the hippocampal formation (APH) and the other in the amygdala (the PoA), which are interconnected. This circuit recalls the one established by the mammalian AHA with the CA1-subiculum and the ventral lateral septum, thus giving strong support to our hypothesis of homologies (Table 3).

It is important to stress the similarity in the pattern of connections between the caudal AV/PoA and the whole medial EA, including the TnA (see below), concerning its connections with the hypothalamus and septohippocampal system. Thus, like the mammalian AHA, the caudal PoA is functionally related to the medial EA.

In this respect, the only feature expected for the homologue of the mammalian AHA (Tables 1 and 3) that seems not accomplished by the PoA, is the expression of receptors to sexual steroids (Simerly *et al.*, 1990). Data on this issue are, however, somewhat contradictory. Thus, whereas Watson and Adkins-Regan (1989) reported the presence in the quail of a few cells accumulating steroids (mainly estrogens) "in the basal archistriatum dorsal and lateral to the borders of the large-celled nucleus teniae," these results were not replicated using immunohistochemical detection of receptors (Balthazart *et al.*, 1989, 1992). On the other hand Metzdorf *et al.* (1999) described the presence of scattered cells expressing aromatase throughout the archistriatum (arcopallium plus PoA), not only in songbirds but also in nonsongbird species. It is interesting to note that songbirds display cells expressing receptors for sexual steroids in all the vocal centers of the forebrain, including the high vocal center in the mesopallium–NC interface and the nucleus robustus arcopallialis (Gahr *et al.*, 1993; Metzdorf *et al.*, 1999; Gahr, 2000). In addition, areas of the posterior AD, AV, and PoA next to the robustus also express androgen receptors in canaries (Balthazart *et al.*, 1992; Gahr and Wild, 1997) and zebra finches (Gahr and Wild, 1997; not detected by Balthazart *et al.*, 1992). The presence of cells expressing aromatase in the arcopallium/PoA of some nonsongbirds and of cells expressing receptors to steroids in arcopallial/PoA nonvocal centers in songbirds is suggestive of a minor expression of steroid receptors of this particular region.

As a conclusion, on topological and hodological grounds the PoA seems to be the avian homologue of the AHA of the mammalian amygdala. The lack or very low expression of steroid receptors by its cells seems, however, an apomorphic feature of birds, the significance of which is not yet understood.

15.6.3.2.(iv) The occipitomesencephalic tract, the somatomotor arcopallium, and the significance of birdsong According to our proposal, the amygdala of birds includes the whole arcopallium, the TnA, and the PoA (plus the posterior DVR: NCM, NCL, and TPO). This contrasts with the view, put forward by Zeier and Karten (1971), that the former archistriatum is composed of a limbic (amygdaloid) part that projects to the hypothalamus (TnA, AM, and PoA), and a

somatomotor one (AA, AD, and the dorsal part of the AV, sometimes called jointly central archistriatum) that is the origin of the OM. The rationale for this view comes from the fact that the OM, as traced in pigeon by Zeier and Karten (1971), innervates parts of the thalamus (mainly the dorsal posterior thalamus), tectal and tegmental structures in the midbrain (thus its name), the lateral reticular formation, lateral pontine nuclei, sensory nuclei in the dorsal medulla, and even the cervical spinal cord. The resemblance of the course of the OM, as defined by Zeier and Karten (1971) in pigeons, with a fascicle of the pyramidal tract of goats (Bagley's bundle), led the authors to propose that the central archistriatal regions originating in the OM should be considered equivalent to the sensorimotor cortex of mammals. Since then, this view has been further refined. In brief, the projections of parts of the arcopallium to the dorsal striatum have also been argued in favor of the comparative meaning of the arcopallium (or part of it) as a cortical sensorimotor area comparable to the infragranular layers of the mammalian isocortex (Veenman *et al.*, 1995). In line with this view, Veenman *et al.* (1995) propose that the thalamo-recipient parts of the nidopallium would be equivalent to isocortical layer 4 and the rest of the nidopallium would represent the supragranular layers of the avian isocortex. In this framework, the arcopallium is interpreted as the main motor output region of the cerebral hemispheres (Figure 20a).

However, several anatomical and functional observations seriously challenge this view. First, as discussed above, the OM has no counterpart in the reptilian brain, so that it probably represents an apomorphic character of the avian forebrain. In addition, the Wulst is a much more likely candidate for the avian homologue of the sensorimotor cortex (Medina and Reiner, 2000), a view that is topologically consistent since, like the mammalian isocortex, the Wulst is a dorsopallial derivative (but see Martinez-Garcia, 2003). Second, lesions of the AM encompassing the OM make animals apparently tame (thus recalling the effects of amygdaloid lesions; see Weiskrantz, 1956) but do not impair their movement (Phillips, 1964). In contrast, temporary inactivation of the LSt of pigeons results in a complete halt of movements so that animals appear paralyzed and unresponsive when handled or moved (Kalenscher *et al.*, 2003). These data suggest that telencephalic motor control in birds is executed by efferent projections from the LSt and, maybe, the somatomotor Wulst, instead of the OM.

Therefore, it is time to analyze which is the function of the OM on the control of behavior. The results of intra-axonic transport of tracers in pigeons (Wild *et al.*, 1993; Kroner and Gunturkun, 1999) and chicken (Davies *et al.*, 1997) have not revealed any target of the OM caudal to the nucleus of the lateral lemniscus. The caudal terminal fields reported by Zeier and Karten (1971) to show degeneration after transections of the OM are likely due to involvement of the BSTl/SpA in the lesion (Berk, 1987). In their study of the auditory forebrain of the pigeon, Wild *et al.* (1993) realized that the OM is a link within the auditory circuitry of the avian forebrain. Thus, the OM originates in the portion of the arcopallium that receives projections from the auditory N and terminates mainly in auditory thalamic (ovoidalis shell, nucleus semilunaris paraovoidalis, nucleus paramedianus internus) and midbrain/pontine centers such as the dorsomedial part of nucleus intercollicularis (ICo/DM) and the nucleus of the lateral lemniscus. On the other hand, songbirds display a very developed OM, which arises from a distinct arcopallial cell group called nucleus robustus arcopallialis (RA; Wild, 1993; Wild *et al.*, 1993). The RA directly innervates the tracheosyringeal hypoglossal motoneurons that control the syringe and premotor neurons for the respiratory system in the nucleus retroambigualis (Wild, 1997), thus having quite a direct control of the motor patterns leading to song generation.

Although this has led to the suggestion that the RA constitutes a portion of the motor cortex (Bottjer *et al.*, 2000) devoted to song control, in this kind of analysis the behavioral/physiological meaning of birdsong has largely been ignored (Cheng and Durand, 2004). Darwin already recognized vocalization as a form of emotional expression. Nonsongbirds, including pigeons, doves, chicken, and quails (which have often been used in anatomical studies), emit a repertoire of vocalizations for all kind of social interactions, including agonistic–territorial, parental, and reproductive ones. It is generally recognized that alarm calls or peeps emitted by newborn chicken when separated from the hen (distress peeps) are emotional behaviors. This has led Cheng (2003) to propose that song and vocalizations are amygdala-dependent behaviors. The pathway she suggests to mediate vocalization includes projections from the TnA to the PMH and then to midbrain song centers, namely the ICo/DM, whose electrical stimulation elicits singing. However, kainic acid lesions of the archistriatum of young chicken result in a decrease of distress calls (loud peeps; Phillips and Youngren,

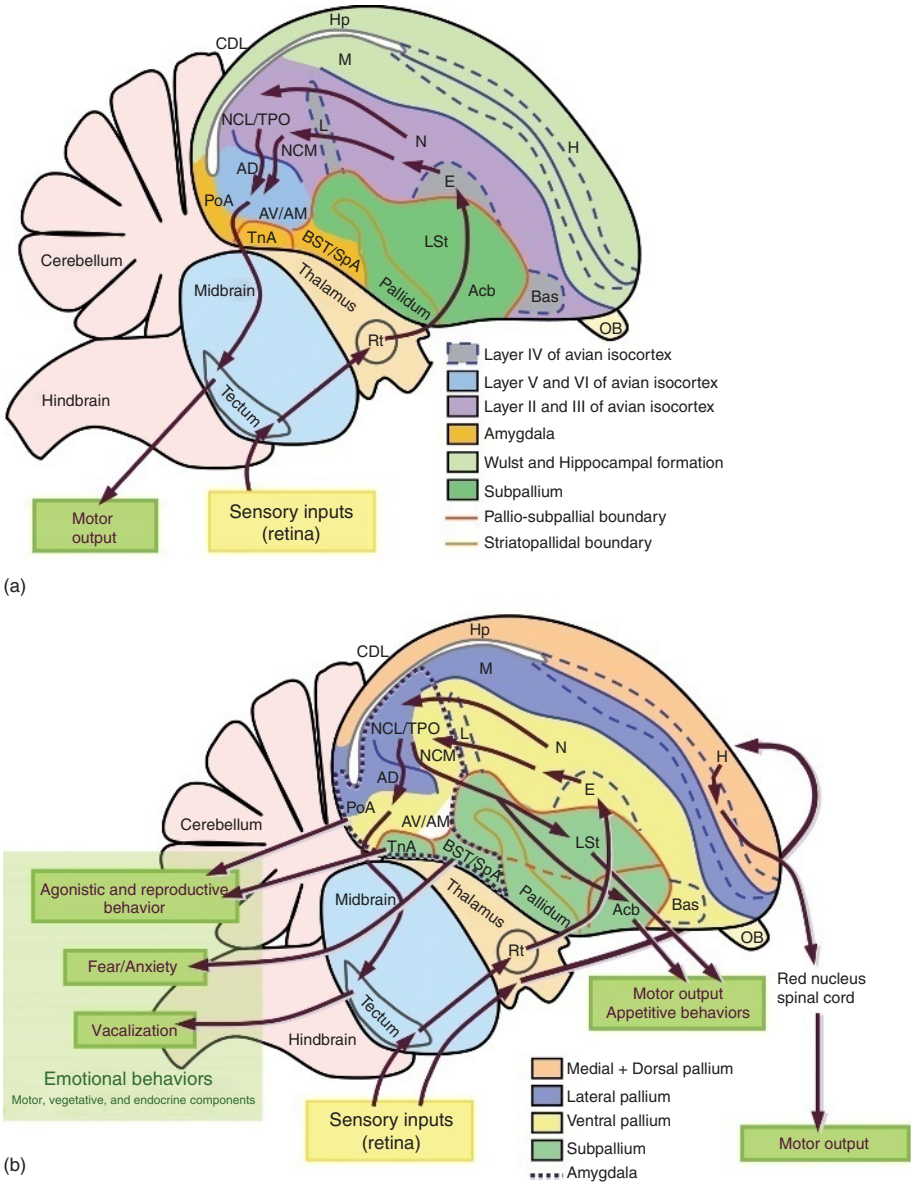

Figure 20 Alternative views on the comparative and functional neuroanatomy of the avian cerebral hemispheres. a, Diagram summarizing the classical view of the comparative neuroanatomy of the subventricular cerebral hemispheres of birds. According to this view, they include pallial regions equivalent to the granular (sensory nidopallium, including field L, basorostral and entopallial nuclei), supragranular (rest of the nidopallium, including the NCL and NCM), and infragranular layers of the mammalian isocortex (arcopallium). The arcopallium thus constitutes the main motor output region of the whole system, whereas the OM appears comparable to the mammalian pyramidal tract. In this view, the pallial amygdala of birds is restricted to the PoA. Arrows indicate the flow of sensory information (exemplified in the visual system) and the main intratelencephalic pathways for sensorimotor integration. b, Schematic diagram of the view proposed in this article. The pallial territories are labeled and the boundaries of the avian amygdala are delineated by a discontinuous line. Three main motor outputs, used for coordinating different kinds of response, arise from different output regions of the cerebral hemispheres. A motor cortex, engaged in coordinating pure motor actions through direct projections to the spinal cord and red nucleus, is present in the Wulst (hyperpallium). In addition, the lateral and ventral pallia are involved in processing sensory information (again, the visual system is drawn as an example) to elaborate different kinds of emotional response. Appetitive ones (involving delayed reward acquisition) are executed through palliostriatal pathways, whereas direct amygdaloid projections to the hypothalamus, midbrain, and brainstem are used to coordinate the motor, vegetative, and endocrine components of innate and learned emotional responses such as agonistic and reproductive behaviors, fear and anxiety, or vocalizations (song emission) used in both contexts. a, Modified from Jarvis, E. D., Gunturkun, O., Bruce, L., *et al.* 2005. Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* 6, 151 159.

1986), but no other visible motor impairments. Involvement of the OM in the control or generation of vocalizations is also suggested by the fact that in both songbirds (Wild, 1993) and nonsongbirds (Wild *et al.*, 1993) the OM reaches directly the midbrain song centers (ICo/DM). In this context, the OM can be envisaged as a projection of the avian amygdala accomplishing a specialized emotional behavior that includes generating or modulating the motor patterns leading to vocalization but also, very likely, the accompanying endocrine and vegetative responses (Figure 20b). The courtship and/or territorial singing of songbirds constitutes a sophisticated behavior with a strong emotional component that, in many species, includes a process of motor learning, for which intra-amygdaloid circuits are especially appropriate (Cheng and Durand, 2004).

15.6.4 The Subpallial Amygdala of Birds

The subcortical components of the avian cerebral hemispheres extend caudally into the amygdaloid complex. There, a subpallial striatopallidal amygdala is expected, which probably contains homologues of both the medial and central EA. As we discuss in detail below, the lack of a vomeronasal system in birds has not been accompanied by a disappearance of the medial EA.

15.6.4.1 The medial extended amygdala of birds The TnA has traditionally been considered the avian counterpart of the medial amygdala of mammals, based on three lines of evidence. First, it is one of the targets for the projections from the olfactory bulbs (Reiner and Karten, 1985). In birds, where the accessory olfactory bulb does not exist, the counterpart of the mammalian Me may still receive a projection from the main olfactory bulb, as occurs at least in the MeA of mammals and the region superficial to the MA associated with the stria medullaris in reptiles (Figures 10b and 10c). Second, the TnA also accomplishes another of the defining features of the medial EA of mammals (Table 2), since its cells express receptors to sexual steroids, especially to estradiol, in both nonsongbirds (Balthazart *et al.*, 1989, 1992; Watson and Adkins-Regan, 1989; Aste *et al.*, 1998; Foidart *et al.*, 1999) and songbirds (Metzdorf *et al.*, 1999). Third, the TnA contributes to the OMH (Zeier and Karten, 1971; Cheng *et al.*, 1999), through which it projects specifically to the medial preoptic region, the lateral hypothalamus, and medial nucleus of the posterior hypothalamus (which, as discussed above, seems to be the avian counterpart of the VMH). This projection continues further caudally to reach regions of the premammillary hypothalamus (Cheng *et al.*, 1999).

The remaining connections of the TnA, as described by Cheng *et al.* (1999) in doves and starlings, are remarkably similar to those described for the different portions of the Me of mammals (Canteras *et al.*, 1995). Thus, the TnA projects to a ventral striatal territory comprising the caudal Acb and the BSTl/SpA, which mimics the projections from the MeA to the Acb and central EA of mammals (the identity of the avian central amygdala is discussed below). In addition, the TnA projects to parts of the intermediate to caudal arcopallium, which would represent projections from the medial to the basolateral amygdala similar to those described in mammals. In addition the TnA is connected to the septohippocampal system consisting of projections to the ventral lateral septum and reciprocal connections with the parahippocampal cortex (AHP; Cheng *et al.*, 1999), for which comparable projections are found in the connections of the Me of mammals with fields CA1 and subiculum of the hippocampal formation.

The OMH, which at least partially arises from the TnA (Cheng *et al.*, 1999), courses through a region located just caudal and mostly ventral to the anterior commissure that links the cerebral hemispheres with the preoptic region. Along its course through this area, the OMH fibers show varicosities. This is suggestive of this area being the avian counterpart of the posteromedial BST of mammals. Aste *et al.* (1998) studied in detail the cytoarchitecture and the distribution of vasotocinergic and aromatase-expressing cells in this area and reported a sexual dimorphism in the vasotocinergic cell population (Panzica *et al.*, 2001). Therefore, they concluded that this area constitutes the medial BST of birds (BSTm) and named it accordingly. In fact, like the mammalian posteromedial BST, the avian BSTm is also rich in receptors to sexual steroids (see references cited above) and displays a similar set of connections with the preoptic hypothalamus (Absil *et al.*, 2002), the premammillary hypothalamus (Berk and Hawkin, 1985), and the PoA (Zeier and Karten, 1971; Kroner and Gunturkun, 1999).

Therefore, birds seem to possess a medial EA composed of, at least, the TnA (plus maybe parts of the AM just dorsal to it; Cheng *et al.*, 1999) and the BSTm. Unlike its mammalian and reptilian counterparts, the medial EA of birds does not receive vomeronasal inputs but instead does receive a scarce olfactory one. However, it shares

with the medial EA of the remaining amniotes the main defining features of this part of the subpallial amygdala. It is rich in receptors to sexual steroids and shows sexual dimorphism, including sexually dimorphic projections containing vasotocin. It is connected to the preoptic, lateral, ventromedial (medial posterior), and premammillary divisions of the hypothalamus, and receives ascending projections at least from the latter. This strong resemblance suggests a similar role of the medial EA in the control of behavior in all amniotes.

15.6.4.2 The central extended amygdala The identity of the central amygdala of birds is an unresolved issue. However, our understanding of the organization of the central EA of mammals and reptiles helps us to delineate its counterpart in the avian forebrain using appropriate histochemical and connectional data.

As in reptiles, in birds the first clues to the identity of the central EA came from studies on descending projections of the cerebral hemispheres. Thus, Berk (1987) demonstrated a projection from the basal telencephalon to the lateral hypothalamus, SN, parabrachial, pericoerulear, and subcoerulear areas and to the dorsal vagal complex and nucleus of the solitary tract in the medulla. When traced retrogradely, this projection was seen to arise from the former BST, now renamed BSTl, and the former ventral paleostriatum, now called SpA. In mammals, the only area of the cerebral hemispheres giving rise to a similar set of projection is the central EA.

This proposal of homology fits the remaining available data on the connections and neurochemistry of the avian brain. Thus, the projection of the BSTl/SpA to the parabrachial region was further analyzed by Wild *et al.* (1990), who also reported an ascending projection of the parabrachial region back to the BSTl/SpA. Therefore, like the central EA of mammals, the BSTl/SpA of birds is reciprocally connected to the parabrachial region. As in mammals, the ascending projection of the parabrachial area reaches not only the subpallial amygdala (BSTl/SpA) but continues to the pallial one to reach at least parts of the arcopallium, PoA, and TPO/NCL, thus giving additional support to our proposal of homologies for the pallial amygdala (see Box 2).

The intratelencephalic connections of the BSTl/SpA also agree with their homology with the mammalian EA. Thus, as reviewed above, the NCL/TPO and AD as well as most of the AV (Veenman *et al.*, 1997; Kroner and Gunturkun, 1999), display substantial projections to the BSTl and/or SpA. This recalls the important projections from the basolateral amygdala to the central EA in the mammalian brain. Additional data on the connections of the BSTl/SpA, which also fit our proposal of homologies, are described in the literature. Thus, the BSTl and SpA receive an important projection from the thalamus, including the periovoidal region (Durand *et al.*, 1992) and the adjoining dorsolateral and dorsointermediate cell groups of the posterior thalamus (Wild, 1987a, 1987b). These cell groups apparently constitute the posterior intralaminar thalamus of birds on the basis of its location, afferents, multimodal physiological response (Gamlin and Cohen, 1986; Wild, 1987a; Korzeniewska and Gunturkun, 1990; Durand *et al.*, 1992), and the presence of abundant CGRP immunoreactive neurons (Lanuza *et al.*, 2000; Durand *et al.*, 2001). Therefore, the BSTl/SpA, like the mammalian central EA, receives a dense afferent projection from the posterior intralaminar thalamus. These thalamic cell groups are the same ones that project sparsely to the NC (Metzger *et al.*, 1998; Kroner and Gunturkun, 1999), to the TnA, and to parts of the arcopallium (Durand *et al.*, 1992; Cheng *et al.*, 1999). This can be interpreted as the presence in birds of a massive projection of the posterior intralaminar thalamus to the central EA (and medial EA) and a sparse one to the basolateral amygdala. This mimics the situation in mammals and, therefore, supports our proposal on the identity of the avian amygdala. Therefore, the available data on the anatomical relationships of the BSTl/SpA support its homology with the central EA of mammals, but a detailed analysis of the connections of the central EA is still needed to improve our understanding of the organization of the avian forebrain.

Finally, neurochemical data fully support the view of the BSTl/SpA as the avian central EA. Thus, like the central EA of mammals, the BSTl/SpA displays populations of CRFergic (Richard *et al.*, 2004) and NTergic cells (Atoji *et al.*, 1996; see figure 5f in Reiner *et al.*, 2004) and a dense innervation by CGRP-immunoreactive fibers (Lanuza *et al.*, 2000). Comparison of the location of CGRP fibers and CRF-immunoreactive cells in the BSTl/SpA reveals a differential distribution. Cells expressing CRF (Figure 21a), like those expressing NT, are mainly located in the BSTl and extend into the SpA, whereas the CGRPergic innervation is very dense in the anterior SpA (Figure 21b) and much scarcer in the BSTl. This also recalls the situation in mammals (Figures 6e and 6f), where CRFergic cells are mainly distributed in the CeM

Box 2 On the identity of the prefrontal cortex of birds

The efforts of comparative neuroanatomists to understand the evolution of the brain have led to a continuous search for counterparts of areas of the mammalian brain with important and/or well-defined functions, in the brain of nonmammals. Although the prefrontal cortex (PFC) may not be present in all mammals, it is nevertheless a paradigmatic example of these attempts. Thus, Mogensen and Divac found that the TPO/NCL of the avian cerebral hemispheres displays two of the defining features of the PFC. First, the TPO/NCL shows a high content of dopamine (Divac and Mogensen, 1985; Divac *et al.*, 1985), due to a dense meshwork of dopaminergic fibers (see, for instance, Waldmann and Gunturkun, 1993; Wynne and Gunturkun, 1995), arising from the ventral tegmental cell groups (mainly A10) (Metzger *et al.*, 1996), which recalls the dopaminergic innervation of the PFC of mammals. The presence of a dense dopaminergic innervation arising from the A9–A10 tegmental cell groups, however, does not constitute by itself a defining feature of the prefrontal cortex since in the mammalian brain the B nucleus of the amygdala is also targeted by a similar dopaminergic pathway (see main text).

In addition, Mogensen and Divac (1982, 1993) reported that lesions of the avian TPO/NCL resulted in deficits in behavioral tasks of delayed alternation. This seems indicative that the NCL/TPO is involved in working memory, a kind of memory that in mammals is dependent on prefrontal cortex function (Goldman-Rakic, 1996). Since then, the homology of the TPO/NCL with the mammalian PFC has gained a wide acceptance (Reiner, 1986). This view and the idea that the N and M are part of the isocortex of birds were mutually reinforcing.

Functional experiments demonstrate that the amygdala and the orbitofrontal cortex are part of the neural machinery for the control of those forms of associative learning (both Pavlovian and instrumental) that involve delayed reinforcement and reward expectancy (Cardinal *et al.*, 2002; Holland and Gallagher, 2004). In other words, in the face of a given stimulus (cue) the animal learns to behave (action) so that it gets a rewarding item (usually food, sucrose, or water). During learning the stimulus becomes a reward-predicting cue. Execution of those behaviors demands that reward expectancy during the delay phase (between the detection of the reward-predicting cue and reward consumption) is encoded by the activity of cells responding to the cue during the delay phase, usually just prior to the action leading to reward acquisition. In a go/no-go task that uses an odor as the reward-predicting cue and sucrose (vs. quinine) consumption as the reward, these working memory cells have been observed in mammals in both the basolateral amygdala and orbitofrontal PFC (Schoenbaum *et al.*, 1999).

Similar working memory cells, including reward expectancy-encoding cells, are found in the NCL of pigeons (Kalt *et al.*, 1999; Diekamp *et al.*, 2002), executing comparable (but more instrumental) go/no-go tasks, in which the cue was auditory or visual. These results led the authors to conclude that the NCL contains the analogue of the mammalian PFC. As they further discuss, differences in the topological position of both structures (revealed by the different patterns of expression of homeotic genes during development) make their homology very unlikely.

As reviewed above, the mammalian B and parts of the orbitary and medial and insular PFC are extensively and massively interconnected (McDonald, 1998). Consequently, in their functional classification of the mammalian amgydaloid nuclei, Swanson and Petrovich (1998) considered the B nucleus as part of the frontotemporal cortical system. Therefore, it is not surprising that lesions of the avian counterpart of the B nucleus lead to behavioral impairments reminiscent of those resulting from lesions of the PFC in mammals. On the other hand, in a forebrain lacking a true isocortex, like the avian one, it is likely that the amygdaloid portion of the frontotemporal system has assumed the functions of the whole system. In other words, it is sensible that the TPO/NCL and other deep lateropallial derivatives of the avian cerebral hemispheres (such as the AD and lateropallial PoA) are not just the homologues of the B nucleus of the mammalian amygdala but also the analogues of the PFC, including its dorsolateral portion.

and CeL, whereas the CGRPergic innervation is concentrated in the lateral-most capsular division of the central amygdala (CeC).

The name BSTl is suggestive of a homology of this juxtaventricular cell group with parts of the BST of mammals and, as a consequence, of the SpA with the mammalian Ce. The histochemical data discussed above clearly indicate that this is not the case. In mammals, the central EA is composed of two centers separated by the internal capsule, namely the Ce and anterior posterolateral BST. However, in the forebrain of birds (as in

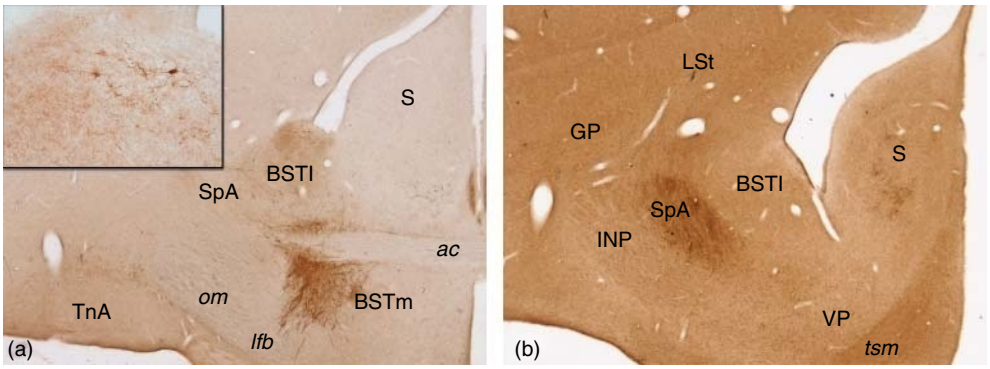

Figure 21 Histochemical characterization of the avian central extended amygdala. a, CRF-immunoreactivity in the BSTl of the left cerebral hemisphere of a quail (the inset shows a detail of the labeling). This is the main CRFergic cell group in the telencephalon of birds that supports the homology of the BSTl with parts of the central EA of mammals. CRF-immunoreactive fibers (probably arising from the reactive cells in the BSTl; Richard *et al.*, 2004) are observed in the lateral aspect of the BSTm. b, Frontal section of the left telencephalic hemisphere of a chicken through the anterior aspect of the BSTl, which has been processed for the immunohistochemical detection of CGRP. A dense CGRPergic innervation is observed in the former ventral paleostriatum, now renamed SpA. Taken together, these data (and additional data reported in other species) suggest that the central EA of birds is composed of the BSTl and SpA.

reptiles), an internal capsule is lacking and both groups are fused together: the SpA is adjacent and topologically superficial to the BSTl. We envisage both structures as the avian counterpart of the entire central EA of mammals in a case of field homology.

15.6.5 The Extent of the Avian Amygdala

The renewed avian neuroanatomical terminology (Reiner *et al.*, 2004) recognizes the homology of parts of the avian cerebral hemispheres with the mammalian amygdala. These include the TnA and PoA, as well as the so-called SpA. Our analysis confirms that these cell groups display histochemical, hodological, and topological features comparable to parts of the mammalian amygdala. As indicated in Table 3, the PoA seems comparable to the AHA of mammals, the TnA to part of the medial EA, and the SpA to part of the central EA.

Thus, the new nomenclature of the avian forebrain leaves it without a recognized homologue for the basolateral amygdala of mammals (L, B, and AB; Figure 20a). The search for this homologue has been hindered by the lack, in turn, of a clear idea of the identity of the central EA of birds. Now, when a wide consensus has been reached (in the Nomenclature Forum) to name SpA and BSTl to the structures composing the central EA of birds, it is time to look seriously for the avian basolateral amygdala. Any candidate for this should be ventro- and lateropallial, and provide a strong input to a continuum in the ventral striatopallidal telencephalon linking the central EA (SpA and BSTl) with the Acb. In addition, it should be adjacent to the known components of the avian amygdala (TnA, SpA, and PoA).

This simple reasoning, the quite straightforward comparison of the avian telencephalon with the cerebral hemispheres of reptiles, together with a cladistic analysis, leads us to reinterpret the comparative meaning of the arcopallium and the NC (Table 3; Figure 20b). They are the pallial derivatives of the avian brain that are best positioned using topological, embryological, and hodological data, to constitute the avian basolateral amygdala. In addition, their histochemical properties are comparable to those of the mammalian and reptilian components of the basolateral amygdala.

This solid hypothesis requires serious consideration. This would not only contribute to our better understanding of the organization and evolution of the brain of amniotes, but would give an extraordinary impulse to avian neurobiology. If we are right, birds are the amniote vertebrates that display a bigger and more accessible basolateral amygdala. Moreover, birds display a complex behavior that includes remarkable learning capacities such as auditory and visual imprinting, passive avoidance learning, song and instrumental conditioning (Jarvis *et al.*, 2005). Therefore, birds might constitute the ideal animal model for the study of the amygdaloid function (Box 2).

15.7 The Evolutionary Origins of the Amniote Amygdala

The common pattern of organization of the amygdala in reptiles, birds, and mammals described

above suggests that the amygdaloid complex of the ancestral amniote already possessed a pallial amygdala with lateropallial and ventropallial components and a subpallial EA with medial and central components. In this section, we discuss whether this pattern of organization of the amygdala was already present in anamniotes. To do so we review the available data on the structure and function of the telencephalon of amphibians, and some functional data available in fishes.

15.7.1 Functional Data in Teleostean Fishes

The telencephalon of ray-finned (actinopterygian) fishes is everted (Nieuwenhuys, 1963; see Evolution of the Nervous System in Fishes), instead of evaginated like the telencephalon of tetrapod vertebrates. Therefore, the structures located medially in the pallium of ray-finned fishes correspond to those with a lateral location in the telencephalon of tetrapods, and vice versa. This has led to the hypothesis that the amygdala should be located medially in the telencephalon of fishes, whereas the Hp should be positioned laterally. Anatomical and functional data strongly support this view as it concerns the hippocampal pallium (Northcutt and Braford, 1980; Nieuwenhuys and Meek, 1990; Braford, 1995; Northcutt, 1995; Butler, 2000; Rodriguez *et al.*, 2002; Salas *et al.*, 2003). In contrast, very few anatomical data are available either to support or discard the putative homology between the medial pallium of fishes and the amygdaloid complex of tetrapod vertebrates (but see Braford, 1995; Butler, 2000). There are, however, some functional studies (Portavella *et al.*, 2002, 2004) showing that the medial pallium of teleost fishes is involved in avoidance conditioning (a case of emotional learning). Therefore, topological and functional data suggest that ray-finned fishes and land vertebrates probably share an ancestor that already possessed an amygdala (medially located in everted brains, laterally located in evaginated brains) involved in fear/aversion acquisition and expression. Whether this structure includes pallial and/or subpallial derivatives is, at present, unclear.

15.7.2 Anatomical Data in Amphibians

The existence of an amygdala in the telencephalon of amphibians was already suggested in early anatomical studies by Herrick (1921). After that, the amphibian amygdala was defined as an area receiving direct projections from the accessory olfactory bulb (Scalia, 1972; Northcutt and Royce, 1975). The classical understanding on the organization of the amphibian amygdala divides it, on topographical grounds, into a lateral and a medial part (pars lateralis and pars medialis; Northcutt and Kicliter, 1980; Neary, 1990). However, recent data on neurochemistry (Marin *et al.*, 1998), hodology (Bruce and Neary, 1995c; Marin *et al.*, 1997a; Moreno and Gonzalez, 2003, 2004; Roth *et al.*, 2004; Laberge and Roth, 2005), and developmental gene expression (Brox *et al.*, 2003, 2004; Moreno *et al.*, 2004) have led to a complete redefinition of the amphibian amygdaloid complex (Figure 22). Although, at present, there are some discrepancies on the nomenclature of the amphibian amygdaloid complex, the terminology by Marin *et al.* (1998) and Moreno and Gonzalez (2003, 2004) will be used in the present section, since it is the most useful one for comparative purposes.

15.7.2.1 Vomeronasal and olfactory projections to the amygdala in amphibians The telencephalic target of the accessory olfactory bulb projections corresponds to the classical amygdala pars lateralis (Northcutt and Kicliter, 1980; Neary, 1990) but, given its comparative meaning, it was recently renamed as medial amygdala by Marin *et al.* (1998) (see also Moreno and Gonzalez, 2003). However, other authors keep using a purely topographical nomenclature, and name the structure receiving the bulk of the projection from the accessory olfactory bulb as lateral amygdala in anurans (Roth *et al.*, 2004) and caudal amygdala in urodeles (Laberge and Roth, 2005). Therefore, the same structure is named medial or lateral amygdala by different authors studying different species. However, from a comparative point of view, there is a certain consensus in considering the vomeronasal target within the caudal ventrolateral telencephalon homologous to the medial amygdala of mammals (the possible existence of a vomeronasal pallial amygdala in amphibians is discussed below).

Regarding the olfactory projections, the main structure receiving direct input from the main olfactory bulb is the lateral pallium (Northcutt and Kicliter, 1980; Neary, 1990). Within the lateral pallium, a dorsal and a ventral division have classically been recognized (Neary, 1990). Using neurochemical data, Marin *et al.* (1998) suggested that the ventral division of the lateral pallium was an amygdaloid structure, which in turn has been divided into an anterior amygdala (Aa, previously called striatopallial transition area by the same authors; Marin *et al.*, 1997a, 1997b) and a lateral amygdala (La, Figure 22) (Marin *et al.*, 1998; Moreno and Gonzalez, 2004).

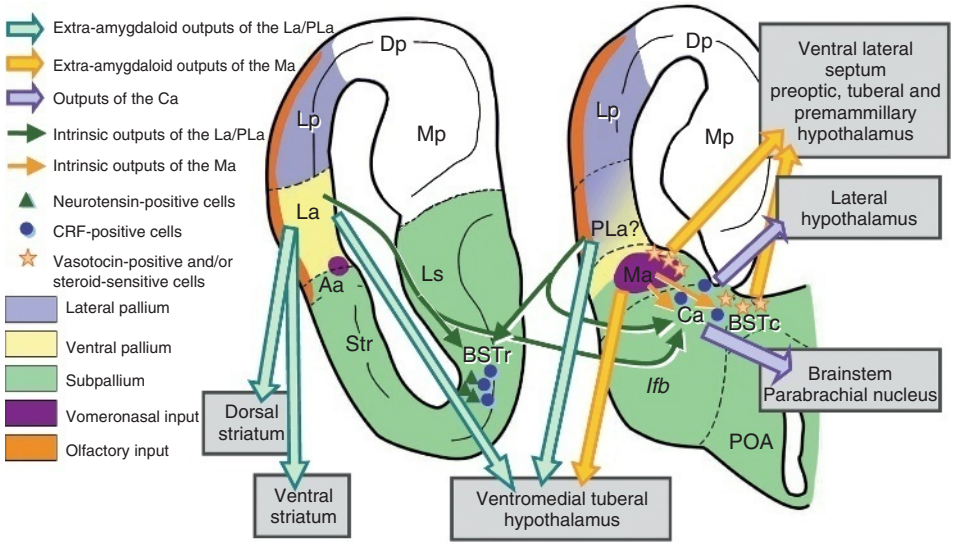

Figure 22 The amphibian amygdala. Schematic view of the amygdala of amphibians, based on two frontal sections (rostral (a); caudal (b)) of the left cerebral hemisphere of an anuran. The map of pallial territories (based on Brox *et al.*, 2003, 2004 and Moreno *et al.*, 2004), the bulbar afferents, the connections with the hypothalamus and striatum, and the intrinsic connections are represented. The PLa is depicted in a color mixture to represent that its ventropallial or lateropallial nature is uncertain. The Ma is depicted as composed of ventropallial (dorsal) and subpallial (ventral) regions. In addition, the location of cells expressing receptors for sexual steroids and/or immunoreactive for vasotocin, as well as the distribution of cells immunopositive for corticotropin-releasing factor and neurotensin (see section 15.7.2.5 of the text for the appropriate references), are depicted. These data suggest the existence of a medial and central EA, as well as a pallial amygdala in which cortical and deep nuclei are not separated (due to the low degree of cell migration).

15.7.2.2 New data on the divisions of the amphibian pallium: The pallial amygdala As discussed above, the pattern of expression of developmental genes in vertebrate embryos has revealed the existence of a pallial region interposed between the lateral pallium and the striatum, the ventral pallium. Similar studies in amphibians (Smith-Fernandez *et al.*, 1998; Bachy *et al.*, 2001, 2002; Brox *et al.*, 2003, 2004; Moreno *et al.*, 2004) indicate that a ventral pallium is also present in ampibians. In anurans, this is mainly represented by the La (formerly ventral division of the lateral pallium), which should therefore be homologous to the ventropallial derivatives of the amygdala of amniotes.

In addition, these studies indicate that, although the medial amygdala (as defined by Moreno and Gonzalez, 2004) (Ma, see Figure 22) is mainly subpallial (Brox *et al.*, 2003; Moreno and Gonzalez, 2004), a part of it (very likely its dorsal aspect adjacent to the La) might be ventropallial (Brox *et al.*, 2004). This suggests that the amphibian brain displays homologues for both the medial amygdala of amniotes (subpallial Ma; see below) and the ventropallial vomeronasal cortex (COApm of mammals, NS of reptiles), as previously suggested (Northcutt and Kicliter, 1980; Scalia *et al.*, 1991). The homology and boundaries of this putative ventropallial portion of the Ma with the vomeronasal cortex of amniotes should be further explored by analyzing in detail the connections and neurochemistry of the amphibian Ma.

The anterior amygdala is a relatively small structure located in a rostral position close to the accessory olfactory tract (Marin *et al.*, 1998). Available data on developmental gene expression and the presence of GABAergic cells suggest that it is a subpallial structure (Gonzalez *et al.*, 2002b; Brox *et al.*, 2003; Moreno and Gonzalez, 2004), but its amniote homologue is unknown.

One of the main conclusions of the study of the developmental gene expression patterns in the amphibian telencephalon is that there appears to be no amygdaloid division originated in the embryonic lateral pallium, in contrast to the situation in mammals (B and COApl), reptiles (DLA and LCc), and birds (NCL/TPO, AD, and lateropallial PoA). Therefore, the lateropallial amygdala would be an evolutionary acquisition of the amniote lineage. The opposite interpretation derives from recent data on the expression of the homeobox gene *xEmx1* (Brox *et al.*, 2004), which suggest that the posterior La (PLa) of anurans is a lateropallial derivative. However, in contrast to the lateropallial derivatives of the amniote amygdala (see above), the PLa of amphibians projects to the ventromedial hypothalamus (Bruce and Neary, 1995b; Moreno and

Gonzalez, 2004; see below). Thus, the possibility that the La of amphibians contains a lateropallial portion equivalent to the B and COApl of mammals requires further analysis (Figure 22).

15.7.2.3 Projections to the hypothalamus: The pallial stria terminalis of amphibians Besides its afferents from the olfactory bulbs, the other widely accepted defining feature of the amygdala of amniotes is the presence of important projections to the hypothalamus, mainly to its medial tuberal division. The telencephalic projections to the hypothalamus have been studied in anurans (Neary, 1995; Moreno and Gonzalez, 2004, 2005) and the results indicate that the amphibian La gives rise to important projections to the core of the ventromedial hypothalamus. Therefore, the La of amphibians is the ventropallial, olfactorecipient structure that originates a major projection to the ventromedial hypothalamus through the stria terminalis. These features suggest that the LA includes the amphibian homologues for the mammalian AB and of the overlying COAa, as well as the PDVR and ventropallial olfactory amygdala of sauropsids.

The pallial component of the stria terminalis of mammals also includes fibers arising from the AHA that reach mainly the preoptic and tubero-premammillary hypothalamus (VMH shell and PMv). A homologue for the AHA of mammals, equivalent to the VPA of reptiles and the avian ventropallial PoA, is still to be found in amphibians. A useful clue to explore this possibility would be the study of the expression of receptors for sexual steroids in the La of amphibians, since they are present in the AHA of mammals and its reptilian homologue (VPA), although this trait has apparently been lost in birds (Table 3). Dealing with this, the few available data (Davis and Moore, 1996; Perez *et al.*, 1996) suggest that steroid-sensitive cells are lacking in the La of amphibians, but detailed studies are needed to clarify this issue.

15.7.2.4 The amygdalostriatal pathways and the lateropallial amygdala of amphibians The telencephalon of amniotes displays a set of amygdalostriatal projections arising from the pallial amygdala. First, the deep lateropallial amygdala (B in mammals, DLA in reptiles, AD and TPO/NCL) projects massively to the ipsilateral (and also to the contralateral) dorsal and ventral striatum, including its caudal extension, the central EA. In addition, parts of the ventropallial basolateral amygdala (mammals, AB; reptiles, PDVR and LA; birds, AV and AM) project to the ipsilateral Acb and to the central EA.

Anatomical studies in anurans and urodeles indicate the existence of similar projections in amphibians. Thus, the rostral La projects to the ipsilateral Acb (Marin *et al.*, 1997a, 1998; Moreno and Gonzalez, 2004), as well as to more caudal striatopallidal territories, including the central amygdala, the bed nucleus of the stria terminalis, the VP, and the nucleus of the diagonal band. This projection system recalls the connections of the pallial amygdala to the central EA (see next section), and further supports the homology of the La of amphibians with the basolateral division of the amygdala of amniotes.

Both anurans and urodeles display a bilateral projection to the dorsal striatum that arises from the La (Marin *et al.*, 1997a). However, since the amphibian La seems a ventropallial derivative, it is not clear whether this projection is equivalent to the amygdaloid pathway to the dorsal striatum of amniotes, which arises from the deep lateropallial nuclei (mammals, B; reptiles, DLA; birds, TPO/NCL, AD, and lateropallial PoA). In fact, the portion of the La for which a lateropallial nature has been suggested, the PLa (Brox *et al.*, 2004), apparently does not project to the dorsal striatum (Moreno and Gonzalez, 2004). In addition, no portion of the amphibian La shows remarkable immunoreactivity for either choline acetyltransferase (Marin *et al.*, 1997c) or for tyrosine hydroxylase (Marin *et al.*, 1998), which constitute the most outstanding histochemical features of the deep lateropallial amygdala of amniotes. The absence of a cholinergic innervation of the pallial amygdala of amphibians could be explained in view of the scarce population of ChAT-positive cells in the basal telencephalon of anurans and urodeles (Marin *et al.*, 1997c; Gonzalez *et al.*, 2002a). In contrast, the lack of dopaminergic input to the lateropallial amygdala is surprising, given that other dopaminergic inputs to the telencephalon are notably conserved (Marin *et al.*, 1998).

As a conclusion, at present it is unclear whether a homologue for the lateropallial amygdala of amniotes is present in amphibians. The lateropallial nature of the PLa (Brox *et al.*, 2004) is not consistent with its massive projections to the hypothalamus and the lack of projections to the dorsal striatum. Detailed analysis of the chemoarchitecture, development, and connections of the LA of amphibians is needed to clarify this issue.

15.7.2.5 The subpallial amygdala of amphibians The available data on the connections and neurochemistry of the telencephalon of amphibians strongly suggest that the caudal subpallium

includes structures comparable to the EA of amniotes. Moreover, there is evidence supporting the view that amphibians possess a central and a medial EA comparable to their amniote homonyms.

15.7.2.5.(i) The medial extended amygdala of amphibians As we have already discussed, the caudal cerebral hemispheres of amphibians include a subpallial structure that is targeted by the projections from the accessory olfactory bulb (Northcutt and Kicliter, 1980; Neary, 1990), which is now named medial amygdala (Ma) to suggest its homology with its mammalian homonym (Moreno and Gonzalez, 2003). In fact, like the medial amygdala of amniotes, the amphibian Ma gives rise to important projections to the hypothalamus (Neary, 1995; Moreno and Gonzalez, 2003, 2005; Roth *et al.*, 2004), including preoptic and tuberoinfundibular levels (ventromedial hypothalamus). In addition, the amphibian Ma projects to the central amygdala (see below), the BST, the Acb, and ventral lateral septum and nucleus of the diagonal band (Moreno and Gonzalez, 2003). In turn, the hypothalamic targets of the Ma (preoptic, and tuberoinfundibular hypothalamus) project back to it. This set of connections clearly recalls those of the medial amygdala of amniotes.

As we have discussed for reptiles and birds, in amphibians a portion of the BST (mainly its caudal aspect, BSTc; Moreno and Gonzalez, 2003) is interconnected with the Ma. This suggests that amphibians, like amniotes, also possess a medial EA composed of the Ma and the BSTc. Histochemical studies reveal further similarities between the medial EA of amphibians and amniotes. Thus, the medial EA of anurans and urodeles (and even of Gymnophiona; Gonzalez and Smeets, 1997; Hilscher-Conklin *et al.*, 1998) displays a population of cells immunoreactive for arginine vasotocin (Smeets and Gonzalez, 2001) that extends into the preoptic hypothalamus, thus recalling the medial EA of amniotes. This vasotocinergic neuronal population is sensitive to steroid hormones (Boyd, 1994), so that this cell group is sexually dimorphic at least in certain seasons (Boyd *et al.*, 1992). Accordingly, the medial EA of anurans (Morrell *et al.*, 1975) and urodeles (Davis and Moore, 1996), like its counterpart in amniotes, is rich in receptors to sexual steroids, especially estrogens. In addition, vasotocin-immunoreactive fibers innervate some forebrain centers (ventral lateral septum, ventromedial infundibular hypothalamus; Smeets and Gonzalez, 2001) that are reached by projections of the Ma (Moreno and Gonzalez, 2003) and are also sensitive to sexual steroids (Kelley *et al.*, 1975; Morrell *et al.*, 1975; Davis and Moore, 1996). Therefore, like amniotes, amphibians display a sexually dimorphic forebrain circuit in which the medial EA apparently gives rise to vasotocinergic projections. This strongly supports the homology of the medial EA of amphibians and amniotes. In this respect, the absence of a vasotocinergic innervation of a pallial amygdaloid center (specifically in the La), which would be part of this circuit in the amphibian forebrain, again suggests that amphibians lack a cell group equivalent to the AHA of mammals, the VPA of reptiles, and the ventropallial PoA of birds. However, detailed studies of the neurochemistry and connections of the amphibian pallium are needed to determine whether a pallial, vomeronasal-related, and steroid-sensitive pallial amygdaloid center is already present in the brain of amphibians.

15.7.2.5.(ii) The central extended amygdala of amphibians The caudal part of the striatum was renamed central amygdala (Ca) by Marin *et al.* (1998) on the basis of its chemoarchitecture and connections (Marin *et al.*, 1997a, 1997b). The amphibian Ca is interconnected with the parabrachial region, the lateral reticular zone, and the nucleus of the solitary tract (Marin *et al.*, 1997a, 1997b). Moreover, at least some of the projections to the tuberal hypothalamus originally attributed to the striatum are likely to originate in the Ca (Neary, 1995; Marin *et al.*, 1997b). In addition, the Ca receives important afferents from the other two amygdaloid divisions, the Ma (Moreno and Gonzalez, 2003) and La (Moreno and Gonzalez, 2004). Therefore, the afferent and efferent connections of the amphibian Ca resemble those of the mammalian central nucleus of the amygdala.

In amphibians, the projections of the La to the Ca continue rostrally to innervate a continuum of structures that include the rostral BST (BSTr) and the Acb. This recalls the projection from the basolateral to the central EA in amniotes, thus suggesting that the amphibian brain possesses a central EA composed of the Ca plus the BSTr. Available neurochemical data are consistent with this hypothesis. Thus, in *Xenopus* the Acb shows large neurons expressing CRF (Yao *et al.*, 2004), whereas the Ca, Ma, and BSTc display smaller and scattered CRF-immunopositive cells. In addition, the only study of the distribution of NT in the amphibian brain (Bello *et al.*, 1994) reports a few NT-immunoreactive cells in the BSTr.

15.7.3 What's New in the Amniote Amygdala?

The data reviewed above show that amphibians possess an amygdaloid formation with pallial and subpallial components. Within the pallium, the La and maybe the dorsal aspect of the Ma are ventropallial derivatives (Brox *et al.*, 2004; Moreno and Gonzalez, 2004), although the posterior La may be lateropallial (Brox *et al.*, 2004). Both the La and Ma project to the hypothalamus (Moreno and Gonzalez, 2003, 2004). In addition, the La also projects to the dorsal and ventral striatum (Marin *et al.*, 1997a). The subpallial amygdala of amphibians consists of most of the Ma, the Ca, and the BST (Marin *et al.*, 1998; Brox *et al.*, 2003, 2004; Moreno and Gonzalez, 2003, 2004). The latter two structures apparently display long descending projections directed to parts of the hypothalamus (Neary, 1995; Moreno and Gonzalez, 2003), tegmentum, and brainstem, including the parabrachial region (from which they receive in turn an important input) and the dorsal medulla (Marin *et al.*, 1997a, 1997b). In addition, the Ca and BSTr are reached by projections from the presumed amphibian homologue for the basolateral amygdala (Moreno and Gonzalez, 2004).

Therefore, the amygdala of amphibians already shows the pattern of organization present in amniotes. In spite of the relative lack of studies on the amphibian brain, the available data suggest that the subpallial amygdaloid centers (medial and central EA) were already present in the anamniote forebrain and underwent a conservative evolution during the anamniote–amniote transition.

However, the pallial amygdalae of amniotes and anamniotes display relevant differences that deserve being considered. First, it is still unclear whether the existence of a lateropallial amygdala is an acquisition of amniotes or whether anamniotes already possess it (see above). Moreover, the pallium of amphibians shows a low degree of radial migration (as compared with amniote pallium) and this applies for the presumed pallial amygdala. Consequently, in the amphibian pallial amygdala there is no differentiation between cortical (superficial, olfactorecipient) and deep pallial nuclei, but the cells of the pallial amygdala projecting to the EA, striatum, and hypothalamus (which in amniotes are mainly deep) are directly reached by olfactory inputs (Moreno and Gonzalez, 2004, 2005). Although the La of anurans also receives afferents from multimodal dorsal thalamic nuclei (anterior and central nuclei; Moreno and Gonzalez, 2004), this suggests that amygdala of anamniotes is mostly influenced by olfactory and vomeronasal information. In this respect, it is important to note that the cerebral hemispheres of amphibians lack true pallial visual, auditory, and somatosensory centers (thalamic sensory inputs reach only the striatum; see Evolution of the Amphibian Nervous System). In contrast, amniotes display pallial regions (reptiles, ADVR; birds, nidopallial sensory regions; mammals, isocortical sensory areas) that receive sensory thalamic inputs, and project directly and/or indirectly to the deep pallial amygdala (Figures 23 and 24).

15.8 The Amygdala and the Evolution of the Vertebrate Forebrain

15.8.1 The Amygdala: Physiology and Behavior

Considering together all the data reviewed above, it is clear that the amygdala has a long history and that it has undergone a conservative evolution during the phylogeny of tetrapods. This comparative perspective allows us to pose again one of the central questions of the research on the amygdala: whether the amygdala is a functional system (Swanson and Petrovich, 1998; Figure 23a). As we have seen, reptiles, birds, and mammals display a similar set of pallial and subpallial centers in the caudal cerebral hemispheres that can be grouped into two main circuits, structured around the central and medial EA respectively (Figure 23b). The roles of these two circuits in physiology and behavior are discussed below.

15.8.1.1 The roles of the central/basolateral amygdala The central EA is one of the output centers for the basolateral amygdala. Thus, the L, B, and AB of mammals, and their homologues in the remaining amniotes, project massively to the central EA. This projection, however, also extends to other striatal territories, including the whole Acb and the dorsal striatum (the latter projection arising exclusively from the B and its avian and reptilian homologues). This system is clearly multimodal as it receives inputs from cortical, thalamic, and brainstem centers, as well as diverse modulatory (mostly aminergic and cholinergic) afferents. In addition, it receives chemosensory inputs thanks to the presence of important superficial-to-deep projections within the amygdala, which convey the olfactory and vomeronasal stimuli received by the cortical (superficial) amygdala to the basolateral and central amygdala.

The role of this circuit has been extensively studied in mammals using different experimental approaches. The results of these studies indicate that the outputs through the central EA mediate fear/anxiety reactions to incoming stimuli, whereas

the amygdalostriatal pathways are part of the reward system of the brain.

15.8.1.1.(i) Expression and acquisition of fear/aversion In primates and nonprimate mammalian species (Roozendaal *et al.*, 1990; Davis and Shi, 1999; Choi and Brown, 2003; Kalin *et al.*, 2004; Rosen, 2004), lesioning or inactivating the central amygdala and/or anterior and posterolateral BST diminishes the expression of fear and anxiety

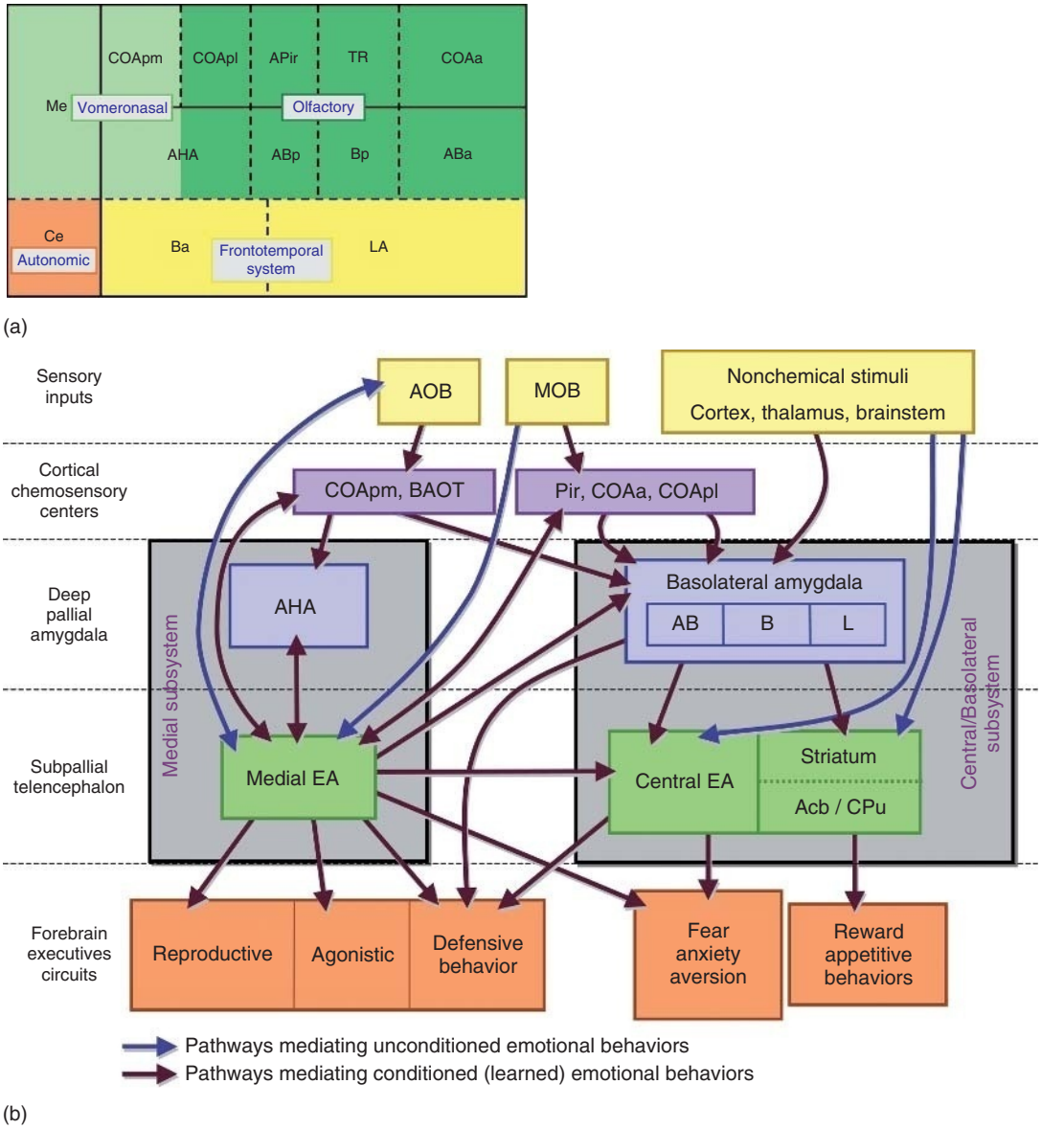

Figure 23 Is the amygdala a functional system? According to the view put forward by Swanson and Petrovich (1998) (a) the amygdala is not a functional system but is composed of structures belonging to the olfactory/vomeronasal, autonomic, and frontotemporal cortical systems. However, the available data on the anatomy and function of the amygdala suggest an alternative scheme on the functional anatomy of the amygdala (b). According to it, the different nuclei of the amygdala are interconnected to conform two functional subsystems, namely the central/basolateral subsystem and the medial subsystem. The former subsystem coordinates innate and learned reactions of fear/anxiety/aversion (through the descending projections of the central EA) or of attraction/reward-directed behaviors (through its projections to the striatum) to virtually any stimulus. The medial subsystem is primarily involved in the coordination of responses to chemosensory stimuli (olfactory and vomeronasal) that constitute species-specific emotional behaviors, such as reproductive/agonistic behaviors to conspecifics (responses to pheromones), and defensive behaviors to conspecifics (as a component of agonistic behaviors) or to predator vomeronasal organ-detected signals. Both subsystems are interconnected, in such a way that olfactory and vomeronasal stimuli can elicit fear/aversion or appetitive behaviors (medial to basolateral/central). On the other hand, nonchemosensory stimuli can modulate the response to pheromones (basolateral/central to medial), although this influence is mediated by direct projections from the basolateral amygdala to the defensive forebrain circuit, rather than through intra-amygdaloid connections.

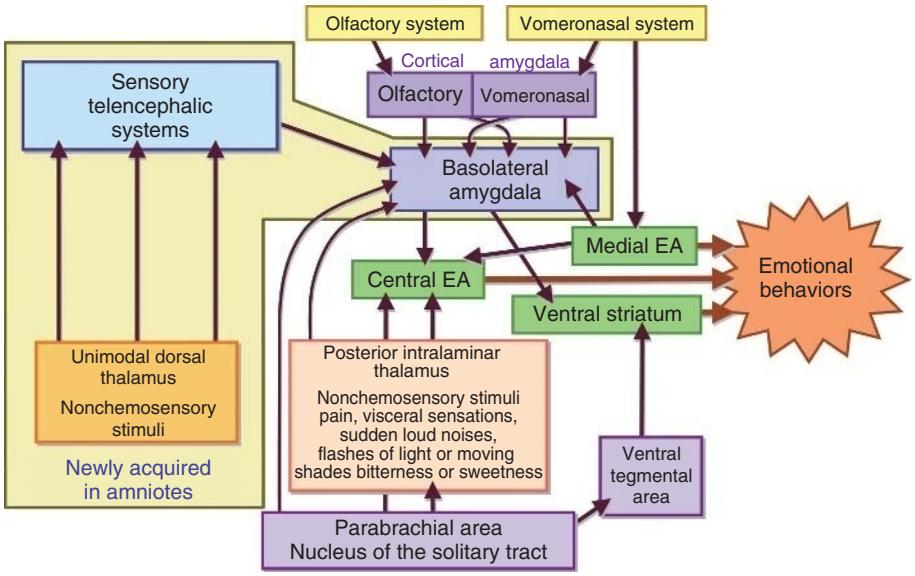

Figure 24 Evolution of the amygdala in tetrapods. The comparative study of the amygdala suggests that all tetrapods have a subpallial amygdala (composed of medial and central EA), which together with the striatum mediates unconditioned emotional behavioral responses to different stimuli (including chemical and nonchemical ones). Moreover, the ancestral amygdala of tetrapods also possesses a cortical amygdala that receives chemosensory information (mainly olfactory), and might contribute to these responses. Two new acquisitions characterize the amygdala of amniotes: (1) the existence of a deep pallial (nuclear) amygdala, namely the basolateral amygdala; and (2) the presence of unimodal nonchemosensory cortical areas in the pallium (dorsal and/or ventral depending on the class). Both have become interconnected, thus allowing processing of stimuli (recognition and identification of their spatial and/or temporal configuration) prior to their emotional evaluation. This has a great adaptive value that has potentiated these traits in all the evolutionary lines of extant amniotes.

against several fear-eliciting stimuli. Comparable functional studies are scarce in nonmammals. However, it has been shown that tonic immobility, a form of prolonged stillness and decreased responsivity induced by threatening stimulation (e.g., physical restraint), which constitutes one of the fear-related ultimate defensive behavioral resources, is reduced by lesions of the former archistriatum of birds (Maser *et al.*, 1973), or the SAT (central EA homologue) in lizards (Davies *et al.*, 2002). Tonic immobility is also part of the behavioral repertoire of some mammals, such as guinea pigs (and maybe humans, where it has been related to catatonia; Moskowitz, 2004). In guinea pigs, tonic immobility seems controlled by the central and basolateral amygdala (Ramos *et al.*, 1999; Leite-Panissi and Menescal-de-Oliveira, 2002). Some of its components, such us the profound analgesia associated with tonic immobility (Leite-Panissi *et al.*, 2001), are dependent on the integrity of the central amygdala and its projection to the periaqueductal gray (Leite-Panissi *et al.*, 2001, 2003).

The role of the descending pathways of the central amygdala in fear expression is further supported by experiments of electrical stimulation. One of the most common behaviors related to stress, discomfort, and anxiety is vocalization. For instance, rats display several kinds of ultrasonic and sonic vocalizations that convey information on their emotional state, some of which are induced by stress and anxiety and are mediated by descending projections arising from the periaqueductal gray to the vocal controlling motor nuclei (Sanchez, 2003). In this respect, electrodes implanted into the amygdala and along the trajectory of the stria terminalis in monkeys elicit different kinds of vocalization (Jurgens, 1982), including purring and chattering calls (which express a self-confident, challenging attitude) and alarm peep and groaning calls indicative of flight motivation and resentment and associated to social stress. Like in rats, the anatomical pathways responsible for these vocalizations in monkeys include periaqueductal regions that are targets for amygdaloid projections (Dujardin and Jurgens, 2005). Moreover, in guinea pigs, the central amygdala is involved in the expression of pain-related vocalization, so that vocalizations induced by noxious stimuli can be decreased by intra-amygdaloid infusions of cholinergic- and opioid-related drugs (Leite-Panissi *et al.*, 2004).

It has also been shown that electrical stimulation of the central amygdala (Rosen and Davis, 1990; Koch and Ebert, 1993) enhances acoustic startle response, one of the well-studied models of

conditioned fear in rats. In fact, it is now well established that conditioned fear to a previously neutral stimulus (conditioned stimulus) is mediated by a process of Pavlovian association between unconditioned (e.g., footshock) and conditioned stimuli (a tone, a light) that apparently takes place in the basolateral amygdala thanks to *N*-methyl-D-aspartate (NMDA)-mediated synaptic plasticity (Davis, 1994; LeDoux, 2000; Lee *et al.*, 2001; Rosen, 2004). In this respect, it has been suggested that the central EA is involved in conditioned, but not unconditioned fear responses (Choi and Brown, 2003; Rosen, 2004). However, the central EA of mammals (more specifically, its capsular and lateral divisions) receives direct nociceptive inputs from the parabrachial area and posterior intralaminar thalamus (Gauriau and Bernard, 2002), which very likely constitute powerful unconditioned fear-eliciting stimuli. These nociceptive-related afferents of the central EA are rich in the peptide CGRP (see above). In this respect, Borszcz (1993, 1995) reported that in order for a painful stimulation (tailshock) to support fear conditioning, it must generate vocalization afterdischarges (vocalizations that extend beyond the termination of the unconditioned stimulus). It is important to note that lesions of the central amygdala abolish the unconditioned vocalization afterdischarges elicited by tailshock (Borszcz and Leaton, 2003). Therefore, the central amygdala (and very likely the whole central EA) is involved in the generation of fear/anxiety or aversion responses to conditioned and, at least, to some unconditioned fear-eliciting stimuli, such as pain.

The presence of a dense CGRPergic innervation of portions of the central EA of reptiles (Martinez-Garcia *et al.*, 2002b) and birds (Lanuza *et al.*, 2000) suggests that this is a general role for the central EA of, at least, amniotes. This is strongly supported by experiments of electrical stimulation of the amygdala in crocodiles (Keating *et al.*, 1970) and iguanas (Distel, 1978), which elicit fear-related behaviors such as fleeing accompanied by vocalization, pupillary dilation, and hyperventilation. In birds, there is evidence that the descending projections arising from the former archistriatal region mediate escape responses (Phillips, 1964). In addition, Phillips and Youngren (1986) demonstrated that kainic acid lesions of the archistriatum of young domestic chicks reduced fear, as expressed by distress calls (peeps). More recently, it has been shown that the archistriatum is indeed involved in the expression of unlearned fear- or anxiety-related behaviors, such as avoidance of the center of an open field (Lowndes and Davies, 1995). It is still unknown whether these reactions are mediated by the archistriatal projections to the BSTl/SpA or by direct descending projections (like the OM). As a conclusion, despite the fact that more functional studies in nonmammals are needed to refine this view, there is ample evidence suggesting that the circuit composed of the basolateral and central amygdaloid divisions is involved in the expression of unlearned fear and anxiety elicited by unconditioned stimuli (at least pain), and in the acquisition and expression of conditioned fear and distress.

15.8.1.1.(ii) Amygdalostriatal pathways: The amygdala and reward In contrast to the data reviewed above, it has been reported that lesions of the basolateral amygdala or of its ventral striatal targets (Acb) in different mammalian species selectively impair learning instrumental responses that result in a delayed reinforcement, which results in impulsive choice (Baxter and Murray, 2002; Cardinal *et al.*, 2004). This indicates that the mammalian amygdalostriatal pathways, together with the prefrontal cortex and the tegmental dopaminergic cell groups, constitute the reward system of the mammalian brain (Baxter and Murray, 2002; Holland and Gallagher, 2004; Schultz, 2004). Therefore, the amygdala is involved not only in the expression of negative emotions (fear, anxiety, aversion) and related learning (conditioned fear and anxiety), but also in positive emotions (reward, attraction, and appetitive behaviors; Kelley, 2004) and related learning (goal-directed behavior through stimulus–reward associations).

In agreement with the current view of homologies between the mammalian and avian brains, lesions of the Acb in birds give rise to a mismanagement of effort economy leading to impulsive behavior when a short delay separates the instrumental response from reward acquisition (Izawa *et al.*, 2001, 2003), or to a complete inability to work for a reward when a long delay is imposed (Kalenscher *et al.*, 2003). To our knowledge there are no data on that issue in any other nonmammalian vertebrate, but the available data in birds suggest that the ventral striatal output of the amygdala of nonmammals, like its mammalian counterpart, is involved in reward expectation and in the generation of behavior using this reward as a goal. This involves not just detecting the reward but also a learning to respond to cues predicting the reward.

15.8.1.2 The roles of the medial extended amygdala The second system of the mammalian amygdala is mainly composed of the secondary vomeronasal centers, since it includes the vomeronasal cortex (COApm) and the medial EA, plus a

deep pallial nucleus, the AHA. Their pattern of connections with the hypothalamus and the septohippocampal system, as well as the presence of receptors to sexual steroids in most of the centers of this circuit, suggests that this system is involved in the control of reproductive and agonistic behaviors elicited by conspecific chemical signals (odorants and/or pheromones). In addition, there is compelling evidence of a role for the medial amygdala of mammals in defensive reactions to some predators. The neural basis of both functions is discussed below.

15.8.1.2.(i) The medial amygdala and reproductive function The medial EA of mammals, together with the COApm and AHA, are surely activated by vomeronasally detected chemical signals from conspecifics of the same or the other gender. Studies carried out in rodents reveal that these pheromones elicit neuroendocrine changes in conspecifics (e.g., Whitten, Vanderbergh, Bruce, and Lee-Boot effects: Halpern, 1987). In addition, vomeronasal organ-detected pheromones elicit behavioral responses that include agonistic/territorial ones (intermale aggression, territorial countermarking), attraction as well as facilitation of courtship and sexual behaviors, including paracopulatory (e.g., vocalizations) and mounting/lordosis (Halpern and Martinez-Marcos, 2003).

The neural mechanisms of the neuroendocrine responses to pheromones (Bronson and Whitten, 1968) or mating (mating-induced ovulation in females; Bakker *et al.*, 2001), probably involve interactions of the medial EA with gonadotropin-releasing hormone-expressing cells of the rostral medial preoptic region (Swanson, 1987). Nevertheless, the whole system of hypothalamic projections of the medial EA seems to be necessary, since lesions of the ventral premammillary nucleus block pheromone-induced ovulation (Beltramino and Taleisnik, 1985). Thus, in many mammalian species exposure of females to male pheromones induces a luteinizing hormone surge mediated by the vomeronasal organ (Beltramino and Taleisnik, 1983) that induces ovulation. A similar effect is found in males in response to female pheromones (Coquelin *et al.*, 1984; Fernandez-Fewell and Meredith, 1998), accompanied by *c-fos* activation in medial preoptic cells (Fewell and Meredith, 2002). In addition, electrical stimulation of the vomeronasal organ (probably involved in pheromone detection (Halem *et al.*, 1999) in hamsters leads to activation of the luteinizing hormone-releasing hormone population of the preoptic area (Meredith and Fewell, 2001). In other species, such as ferrets, ovulation is not spontaneous but it is induced by mating (Carroll *et al.*, 1985). In agreement with this, in most studied mammals mating leads to *c-fos* activation in the luteinizing hormone-releasing hormone-expressing preoptic cells (Fernandez-Fewell and Meredith, 1994; Wersinger and Baum, 1996; Pfaus and Heeb, 1997; Bakker *et al.*, 2001; Meredith and Fewell, 2001), but also of the medial EA. A similar pathway may account for the acceleration or delay of puberty in prepubertal females by conspecific chemosignals. In this respect, it has been shown that vasopressinergic inputs to the gonadotropin-releasing hormone cells, most of which might arise from the medial EA (see above), are important for the modulation of luteinizing hormone surges (Dobson *et al.*, 2003).

As noted above, the medial EA is interconnected with a series of forebrain centers that are involved in reproductive behavior. These include several nuclei of the medial hypothalamus, such as the medial preoptic nucleus, the tuberal nucleus, the ventrolateral aspect of the VMH, and the PMv (Canteras, 2002). Within the cerebral hemispheres, the remaining vomeronasal amygdaloid nuclei (COApm and AHA), together with parts of the septohippocampal system, including the ventral aspect of the lateral septum, are also part of this circuit. The principal outputs of this circuit are the medial preoptic nucleus and VMH, which are involved in the control of sexual behavior of males (Hull *et al.*, 2002) and females (Blaustein and Erskine, 2002) respectively. In agreement with this, most of the nuclei conforming this circuit are sexually dimorphic and express receptors to sexual steroids that very likely mediate the modulatory effects of steroid hormones on copulation, territorial aggression, and other forms of reproductive and agonistic behavior. Therefore, the medial EA, COApm, and AHA are in a good position to facilitate innate agonistic or reproductive behavioral responses (Kollack-Walker and Newman, 1995) to pheromones and other chemical cues from conspecifics.

Functional studies of the counterparts for the medial amygdala and/or AHA of nonmammalian vertebrates are restricted to birds. Thus, lesions of the TnA in male quails (Thompson *et al.*, 1998) result in an impairment of copulatory and paracopulatory behaviors, including courtship vocalizations, thus reinforcing the view that it is homologous to parts of the medial amygdala of mammals. Similar lesions in other avian species, such as ring doves and starlings (Cheng *et al.*, 1999), lead to changes in social behavior such as increased cooing in female doves, interpreted by the

authors as an indifference to concurrent male attacks, and social detachment and lack of social inhibitions in starlings.

The connections from the medial EA to the lateral septum seem to be fundamental to modulate agonistic behavior. Using different bird species, Goodson and collaborators (Goodson, 1998a, 1998b; Goodson and Adkins-Regan, 1999; Goodson *et al.*, 2004) have shown that vasotocinergic (and vasoactive intestinal peptidergic) innervation of the lateral septum, presumably arising from the medial EA (see above), modulates mate competition, aggression, and territorial down song but not courtship. A role for vasotocin in modulating agonistic behavior has also been shown in mammals, amphibians, and fish, thus suggesting that this circuit has a long evolutionary history with a well-conserved role (Goodson and Bass, 2001).

15.8.1.2.(ii) The medial amygdala: Defensive behavior and predator-elicited fear Besides a role in modulating conspecific-related behaviors, the medial amygdala of mammals seems to mediate innate fear to chemical cues derived from common predators, such as cats or foxes (Dielenberg and McGregor, 2001). Confrontation of a rat to cat fur or to a chemical derived from fox feces (2,5-dihydro-2,4,5-trimethylthiazoline, TMT) innately provokes endocrine (increased corticosterone and adrenocorticotropic hormone levels), vegetative (increased arterial pressure) and behavioral components of fear (freezing in some conditions, and escape or hiding if this is allowed; Rosen, 2004).

Although the vomeronasal organ is presumed to be involved in the detection of pheromones (by definition, secreted by conspecifics), there is experimental evidence suggesting that some of the predator-related substances that elicit innate fear are also detected by the vomeronasal organ. Thus, rats do not display fear (increased hiding) if a worn cat collar is present but a mesh wire avoids direct contact with it (Dielenberg and McGregor, 1999), thus indicating that the cat-derived fear-eliciting substance is not volatile, as shown for some vomeronasal organ-detected conspecific pheromones (Moncho-Bogani *et al.*, 2002, 2005; Luo *et al.*, 2003). In agreement with this, cat-derived chemicals induce *c-fos* (Dielenberg and McGregor, 2001) in the medial EA, including the MeP (especially the MePV) and parts of the BST. In addition, the forebrain defensive circuit (Canteras, 2002) seems strongly activated: the ventral lateral septum, the anterior hypothalamus, the dorsomedial aspect of the VMH, and the dorsal premammillary nucleus. Surprisingly, despite the clear signs of fear displayed by the rats studied by Dielenberg and McGregor (2001) when confronted with cat-derived chemicals, neither their central nor their basolateral amygdala was activated by these stimuli. This led the authors to suggest that the medial amygdala is responsible for unconditioned fear, whereas the central/basolateral amygdala is just involved in the expression of conditioned fear (as discussed above; Rosen, 2004). This is partially confirmed by the effects of lesions of the medial or central amygdala on fear elicited by cat odors in rats (Li *et al.*, 2004). The results indicate that the medial but not the central amygdala is involved in generating fear of cat-derived chemicals in rats. A role of the medial amygdala in fear and stress induced by other stimuli (such as acute restraint or footshock) is also possible (Pezzone *et al.*, 1992; Rosen *et al.*, 1998; Dayas *et al.*, 1999; Kubo *et al.*, 2004), although this might be a strain-specific trait (Ma and Morilak, 2004) and the circuitry involved is unknown.

The expression of *c-fos* induced by TMT has recently been studied (Day *et al.*, 2004) and the pattern of cerebral activation differs from that of cat-derived chemicals. Thus, although in both cases the defensive forebrain circuitry is activated, TMT only activates the MeA, but not the MeP. In addition, TMT elicits a strong activation of the central amygdala. These differences can be attributed to the sensory organ used to detect TMT and the cat-related fear-eliciting chemical. Whereas the cat-derived chemical seems a vomeronasal organ-detected stimulus (Dielenberg and McGregor, 2001), TMT is a volatile chemical that displays a strong odor to the human nose, thus it is very likely detected by the main olfactory system. In agreement with this, it strongly activates the granular layer of the main olfactory bulb and those portions of the medial amygdala that show direct inputs from the main olfactory bulb, namely the MeA (see above). Since the MeA projects to the central amygdala, this depicts a circuit for odor-induced unconditioned fear that includes the main olfactory bulb, its direct (and maybe indirect) projections to the MeA and its projection to the CeA (Myers and Rinaman, 2005).

Therefore, the medial amygdala of mammals seems not only involved in coordinating agonistic and reproductive behaviors in response to conspecific pheromones, but also fear/defensive reactions to odors and vomeronasal organ-detected substances secreted by common predators. In other words, the medial amygdala seems the key center to orchestrate innate responses to biologically significant chemicals (pheromones and odorants) derived from conspecifics and predators. Similar functional studies carried out in reptiles suggest that they can also

use chemical stimuli to detect predators and generate anticipatory defensive reactions. Thus, many crotaline snakes display defensive reactions to one of its predators, the kingsnake (*Lampropeltis getula*) which are mediated by the vomeronasal organ (Miller and Gutzke, 1999). Similar functional studies are not available in birds, but since the sign stimuli for detecting predators are not chemical but visual or auditory, the neural circuitry mediating predator-elicited defensive reactions is unlikely to use the medial EA.

15.8.1.2.(iii) The amygdala as a functional system In their insightful review of the structure and function of the amygdala, Swanson and Petrovich (1998) proposed that the term 'amygdala' should be abandoned since it is neither a structural nor a functional unit. From a functional viewpoint, they considered the amygdala as composed of portions of the autonomic (central amygdala), chemosensory (cortical, medial, AHA, ABa, Abp, and Bp) and frontotemporal (the rest of the basolateral amygdala, L and Ba) systems of the brain (Figure 23a).

Our review of the available data in different vertebrates reveals, however, that the amygdala is composed of two functional subsystems that, together, control several aspects of behavior and physiology (Figure 23b). The subsystem that has its output through the central EA also includes portions of the basolateral amygdala that Swanson and Petrovich (1998) consider as part of the olfactory amygdala (ABa, ABp, Bp) and a frontotemporal nucleus (L). Therefore, the central/basolateral subsystem of the amniote amygdala encompasses structures belonging to the three functional divisions of the amygdala proposed by Swanson and Petrovich (1998), thus suggesting that a great part of the amygdala does act as a functional system. Concerning the medial subsystem of the amygdala, it is exclusively composed of nuclei belonging to Swanson and Petrovich's olfactory compartment of the amygdala. However, there is evidence that the medial amygdala is involved in the generation of autonomic and/or endocrine stress responses (Dayas *et al.*, 1999; Kubo *et al.*, 2004), so that it could equally be considered as part of the autonomic amygdala together with the central amygdala.

The central/basolateral and medial subsystems of the amygdala, as proposed here, are also interconnected and connected with similar forebrain centers through which they can manage, jointly, important behaviors. Thus, as we have discussed above, the MeA receives not only vomeronasal but also olfactory inputs, and projects to parts of the central EA. This connection provides a vomeronasal and/or olfactory input to the central EA that could mediate fear/anxiety reactions to this kind of stimuli (Day *et al.*, 2004; Myers and Rinaman, 2005). This does not rule out the possibility that parts of the medial EA mediate some fear/anxiety reactions, without involvement of the central EA, to either chemosensory stimuli (Dielenberg and McGregor, 2001; Li *et al.*, 2004) or nonchemosensory stressors (Dayas *et al.*, 1999; Kubo *et al.*, 2004).

In addition to the role described in mediating reproductive, agonistic, and defensive behaviors in response to chemosensory cues, the medial subsystem of the amygdala is surely involved in the appetitive behaviors due to the reinforcing value of sexual pheromones (Moncho-Bogani *et al.*, 2002, 2005). The rewarding value of these pheromones is likely to be mediated by indirect projections from the medial subsystem of the amygdala to the ventral striatum through the central/basolateral subsystem (Moncho-Bogani *et al.*, 2005) and, therefore, these behaviors are dependent on the interconnection between the two amygdaloid subsystems.

Finally, parts of the central/basolateral subsystem of the amygdala, namely the posterior AB, give rise to a strong projection to the defensive system of the hypothalamus (a portion of the pallial stria terminalis), e.g., the anterior hypothalamus and the dorsomedial (core) VMH. Canteras (2002) interprets this as a pathway mediating defensive reactions to nonchemosensory stimuli, namely visual, somatosensory, and auditory ones.

As a conclusion, the functional division proposed by Swanson and Petrovich (1998) does not seem tenable in the light of the evidence reviewed. In contrast, the amygdala is composed of two functional subsystems, named here as central/basolateral and medial, which, together with other forebrain centers, govern emotional behavioral responses to different kinds of stimuli. These include goal-directed behaviors that relay on delayed reward and involve learning (output to the striatum), fear/anxiety/aversion (central EA), and reproductive/agonistic and defensive behaviors. Interactions of both subsystems are needed to accomplish these functions.

15.8.2 Evolution of the Emotional Brain: The Amygdala and the Evaluation of Incoming Stimuli

Emotional behaviors can be expressed either in response to intrinsically attractive and reinforcing stimuli, such as sexual pheromones, sweet and

salty taste, or in response to intrinsically aversive/fear-eliciting ones, such as pain or disgusting visceral sensations, chemicals from predators, startling loud noises or lights (Figures 23b and 24). The presence of some direct sensory inputs to the EA and to the striatopallidal telencephalon allows quick, automatic responses to these stimuli. Thus, nociceptive stimuli reach directly parts of the central EA through the CGRP-enriched projection from the parabrachial area and intralaminar thalamus (Gauriau and Bernard, 2002), and this pathway seems to mediate unconditioned fear reactions (Borszcz and Leaton, 2003). In addition, fear/defensive reactions to predator-related chemical stimuli (cats) seem mediated by the vomeronasal/olfactory inputs to the medial amygdala (Li *et al.*, 2004). On the other hand, direct access of rewarding stimuli to the ventral striatum mediates most appetitive behaviors related to natural reinforcers, such as food and water intake, salt and sweet appetite, and sexual behavior (Pecina *et al.*, 2003; Kelley, 2004).

As we have seen, the basolateral amygdala of amniote vertebrates receives convergent afferents from all the sensory systems, but it is evident that not every stimulus reaching the amygdala elicits fear or reward. However, when a neutral stimulus coincides with an attractive or aversive stimulus (US), Pavlovian conditioning occurs and the previously neutral stimulus results in a conditioned response similar to the one provoked by the US with which it is associated. In other words, the animals acquire fear or attraction to previously neutral stimuli that become, in that way, emotionally labeled. The synaptic plasticity in the basolateral amygdala might mediate this kind of Pavlovian conditioning, and the conditioned response is mediated by the palliosubpallial projections within the amygdaloid circuit. Our review indicates that this circuit for emotional behavior and emotional learning is present in all the amniotes studied and, therefore, constitutes one of the defining features of the forebrain.

Although most of the models of emotional conditioning use somatosensory or gustatory stimuli as US and auditory or visual ones as conditioned stimuli, vomeronasal stimuli can play an important role as US. Thus, vomeronasal organ-detected chemicals apparently elicit innate fear/defensive reactions (rat defensive behavior to cat fur; Dielenberg and McGregor, 1999). Studies in several rodents reveal that the innate attraction to possible mates (Moncho-Bogani *et al.*, 2002, 2005) as well as different aspects of copulatory (Beauchamp *et al.*, 1982, 1985; Del Punta *et al.*, 2002; Leypold *et al.*, 2002; Stowers *et al.*, 2002) and paracopulatory behaviors (ultrasonic vocalizations; Wysocki *et al.*, 1982) to mates are mediated by nonvolatile pheromones apparently detected by the vomeronasal organ. In addition, similar stimuli seem to elicit agonistic behaviors such as competitive signaling (Hurst and Beynon, 2004) or intermale aggression (Clancy *et al.*, 1984; Del Punta *et al.*, 2002; Leypold *et al.*, 2002; Stowers *et al.*, 2002).

Dealing with this, it is important to stress that there is a significant olfactory–vomeronasal convergence within the basolateral amygdala of amphibians, reptiles, and mammals (see above), which can mediate olfactory–vomeronasal conditioned learning, thus providing olfactory stimuli with an attractive or aversive significance. (Indeed, neurons in the basolateral amygdala respond to a particular odor depending on whether it was previously paired with a pleasant or unpleasant taste (Schoenbaum *et al.*, 1999). A similar neural mechanism would mediate olfactory–vomeronasal associations.) This kind of associations would confer a predictive value to odors that can be detected from a distance due to their volatility. This allows the animal to anticipate its reactions to pheromones or vomeronasal organ-detected predator signals (which are usually nonvolatile; Wysocki *et al.*, 1980; Dielenberg and McGregor, 1999; Moncho-Bogani *et al.*, 2002, 2005; Luo *et al.*, 2003), thus being able to trail the source of attractive pheromones, or to avoid or flee from repulsive or fear-eliciting ones. This has a huge adaptive value and confers advantage to animals showing this ability.

In our view this kind of association might have constituted the primary role of the pallial amygdala in guiding behavior and was already present, at least, in the ancestral tetrapods. Thus, even in amphibians the medial amygdala (which receives the bulk of the projection from the accessory olfactory bulb) and the LA (which receives an important input from the main olfactory bulb) are interconnected (Moreno and Gonzalez, 2003, 2004). In addition, direct thalamic afferents to the amygdala are scarce but present in amphibians, so that they probably occurred very early in the vertebrate evolutionary history. These pathways might primarily convey information on simple but innately significant stimuli (e.g., pain, visceral sensations, sudden loud noises or lights) that would contribute to the emotional tagging of odors. In this way, the vertebrate amygdala became a neural center involved in the emotional labeling of odors by association with either attractive or fear-eliciting chemosensory (mainly vomeronasal) and nonchemosensory stimuli.

15.8.3 The Amygdala and the Evolution of the Pallium in Vertebrates

In all the amniotes studied, the pallial amygdala also receives important afferents from the sensory pallial areas of the telencephalon. There, specialized unimodal regions of the dorsal thalamus convey nonchemosensory stimuli to specific areas of the cortex, where these stimuli are processed by means of complex circuits that include palliopallial excitatory projections and local interneurons mediating feedback and feedforward inhibitory processes. In contrast, amphibians do not possess unimodal thalamocortical sensory pathways (Martinez-Garcia, 2003), but their dorsal thalamic sensory nuclei project to the striatum (Endepols *et al*., 2004; see Evolution of the Amphibian Nervous System). Therefore, sensory cortical areas first appeared in amniotes, where they are represented by the anterior DVR of reptiles and birds (entopallium, nucleus basorostralis of the pallium, field L of the nidopallium), the visual and somatomotor Wulst of the avian hyperpallium, and the sensory isocortex of mammals (Figure 24). The appearance of these cortical sensory areas in amniotes was accompanied by a differentiation within the pallial amygdala of superficial cortical areas receiving direct olfactory inputs, and deep nuclear territories engaged in interconnections with the nonolfactory sensory cortex, e.g., a true basolateral amygdala.

Processing information about the environment prior to conveying it to the amygdala would have been strongly selected during the evolutionary history of amniote vertebrates, since it allowed the animals to react differently to stimuli with a similar configuration but a different emotional value. This may have resulted in an increase in size and complexity of the sensory telencephalon in all the evolutionary lines of amniotes, a phenomenon that seems to have occurred independently in each line. In therapsid reptiles, leading to mammals, sensory processing mainly took place in the mediodorsal pallium, which developed into a complex isocortex with primary sensory and complex associative regions that provide highly processed sensory information to the amygdala. In sauropsids, this sensory processing occurred mainly in the ventral and lateral pallial territories, thus resulting in the development of the DVR, which, at least in birds, includes associative areas that project to the amygdala.

Acknowledgments

This work has been supported by the Spanish Ministry of Education and Science and the FEDER European funds (BFU2004-04272), the Generalitat Valenciana (ACOMP06/258), and La Junta de Comunidades de Castilla-La Mancha (PAC-05-007-2).

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16 The Evolution of Vertebrate Eyes

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Glossary

archaea Also called archaeobacteria, these are genetically and metabolically different from all other known bacteria. They appear to be living fossils, survivors of an ancient group of organisms that bridged the gap in evolution between bacteria and eukaryotes, the multicellu lar organisms.

Cambrian The first period of the Paleozoic era in geology, characterized by desert land areas, warm seas, and rapid early diversification of marine life resulting in the rise of almost all modern animal phyla.

cryptochrome Photosensory receptors mediating light regulation of growth and development in plants; recently found in animals.

eye An organ that can produce an image by comparing the light intensities/ wavelengths coming from different directions.

metazoan Multicellular organism.

phenotype The observable physical or biochemical characteristics of an organism, as deter mined by both genetic makeup and environmental influences.

phylogeny The evolutionary development and history of a species or higher taxo nomic grouping of organs or organisms.

rhodopsin The pigment sensitive to light in verte brate retinal rods of the eyes, consisting of the seven transmembrane domain protein, opsin, and retinal.

16.1 Introduction

Light from the sun carries energy essential for all life on earth and has been a profound selective force, driving the evolution of cellular and molecular processes that harvest the sun's energy. Light is also the premier source of information for many species, and this selective pressure led to the evolution of light-sensing organs, including eyes, that harvest information carried by light. Basically, from the beginning of biological evolution on our planet over 5 billion years ago, sunlight has both fueled and informed life. Light, and the light/dark cycle from our rotating planet are arguably second only to sex as the most important selective forces ever to act on biological organisms. Energy and information are also essential inside cells in the form of DNA and mitochondria and, similar to light, they have an evolutionary history essential to life. One of the most remarkable consequences of light on earth has been the evolution of mechanisms that convert photons not only into energy but also into signals useful to organisms. The evolution of eyes and other structures that collect and use light to represent information from incoming photons has left a remarkable evolutionary trail. And in understanding the genetic, biochemical, and structural remnants of eye evolution, one must follow Ernst Mayr's dictum: "evolution is an affair of phenotypes." Nowhere is this more evident than in the varieties of eyes and the diversity of mechanisms to convert photons into energy useful to the owners of those eyes.

How did eyes evolve? Darwin, the great English naturalist who first brought the systematic

explanatory power of evolution to bear on the bewildering biological complexity of our planet, felt that eyes offered a special challenge to evolutionary thinking because they are such "... organs of extreme perfection and complication ..." (1859). He was quite explicit on this point, saying "... that the eye ... could have been formed by natural selection seems, I freely confess, absurd in the highest possible degree." Although this is most often cited in relation to Darwin's thinking on eyes, he also wrote:

> Reason tells me, that if numerous gradations from a simple and imperfect eye to one complex and perfect can be shown to exist, each grade being useful to its possessor, as is certainly the case; if further, the eye ever varies and the variations be inherited, as is likewise certainly the case; and if such varia tions should be useful to any animal under changing conditions of life, then the difficulty of believing that a perfect and complex eye could be formed by natural selection, though insuperable by our imagination, should not be considered as subversive of the theory.

Indeed, there are features of eye evolution that challenge the imagination, but we are coming closer to a fuller understanding of how eyes evolved.

More than a century later, new discoveries and new insights that reach from molecular to macroscopic levels of analysis reinforce Darwin's prescient writing. Although we still have much to learn from the evolution of eyes, both about the existing eyes as well as the processes of evolution that produced them, several new findings have guided our understanding about the origins of eyes (see The Role of Vision in the Origin and Evolution of Primates).

Excitement about eye evolution comes from discoveries across the spectrum of biological investigation. Molecular biologists who seek fundamental similarities among organisms have found some clusters of genes implicated in eye development that are conserved in eyes across large phylogenetic divides. We also now know that vertebrate genomes contain nearly twice as many genes encoding light-transducing opsin proteins as were once thought to be present. Moreover, physiologists have identified two fundamentally different kinds of eye phenotypes in single organisms. In fact, within the eye of at least one vertebrate, there are now known to be two fundamentally different kinds of phototransduction, each apparently serving separate but overlapping functions. Evolutionary biologists interested in understanding why organisms and their parts are so different have found new types of eyes, both in the fossil record and in living animals. What do these different approaches to the evolution of eyes tell us? Together they offer complementary views of eye evolution and possibly the beginnings of a clearer story about how and how often eyes arose during evolution.

16.2 Eye Variation: Structural and Functional Adaptations

16.2.1 Adaptations to General Constraints

In his monumental book, Walls (1942) provided remarkable insights into all aspects of the vertebrate eye. Moreover, this classic has numerous illustrations, many drawn by Walls himself, with details about the range and variety of vertebrate eye phenotypes. Indeed, the variety of eyes is astonishing, reflecting the range of adaptations produced by selective pressures for vision in different habitats. There are many features common to all eyes, however, which are a consequence of fundamental physical constraints on their construction. Since eyes collect and focus light, their structure ultimately depends on the physical properties of light, which set limits on the optical features of eyes. For example, eyes have evolved to be sensitive within a narrow range of wavelengths, relative to the broad spectrum of energy produced by sunlight (see Figure 1). This is most likely due to the fact that early evolution occurred in water, which strongly filters light (Fernald, 1988). Selection for biochemical mechanisms sensitive to this limited range of wavelengths predisposed the sensitivity that emerged during subsequent evolution. Even though many species long since moved onto land where they

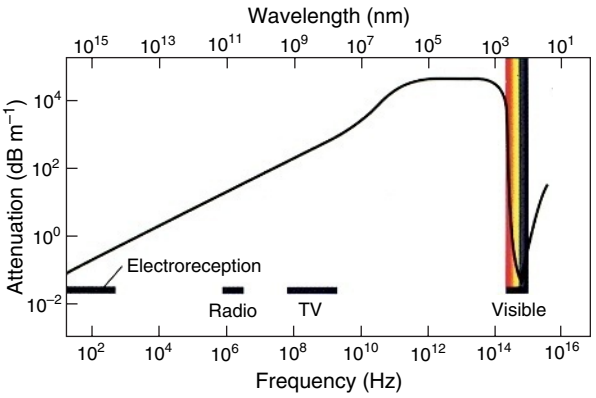

Figure 1 The attenuation (decibels/meter) of electromagnetic (EM) radiation in water as a function of wavelength (nm) and frequency (Hz). This illustrates that attenuation of EM radiation by water is generally quite high except for two ranges: under 10^3 Hz and from 10^{14} to 10^{15} Hz. This accounts for the usefulness of low-frequency signaling and electroreception to weakly electric fish and to the range of frequencies we now term visible light. The band of EM radiation we now consider visible light is transmitted through water with an attenuation six orders of magnitude lower than that of adjacent wavelengths. Redrawn from Fernald, R. D. 1988. Aquatic adaptations in fish eyes. In: Sensory Biology of Aquatic Animals (*eds.* J. Atema, R. R. Fay, A. N. Popper, and W. N. Tavolga), pp. 185 208. Springer.

are exposed to the broader spectrum of electromagnetic radiation from the sun, most animal eyes remain limited to seeing within the narrow band. However, insects and some species of fish and birds later evolved additional receptor types for ultraviolet (UV) light (e.g., Viltala *et al.*, 1995). Thus, the narrow range of wavelength sensitivity is a residual reflection of our aquatic origins and illustrates how early evolutionary solutions persist in the evolved organs.

Of the approximately 33 animal phyla, about one-third have no specialized organ for detecting light, one-third have light-sensitive organs, and the remaining third are animals with what we would consider eyes (Land and Nilsson, 2002). Image-forming eyes appeared in 6 of the 33 extant metazoan phyla (Cnidaria, Mollusca, Annelida, Onychophora, Arthropoda, and Chordata), and these 6 contribute about 96% of the known species alive today (Land and Fernald, 1992), suggesting that eyes are, indeed, useful. Existing eyes have many shapes and sizes, reflecting the diverse solutions to the problem of obtaining an image. Eyes can range in size from a fraction of a millimeter to tens of centimeters in diameter. The range of eye types, sizes, and locations suggests that they can evolve relatively easily (see below).

16.2.2 Optical Systems of Eyes

Eye optical systems fall into three classes based on their image-forming mechanisms: images formed via shadows, images formed via refraction (e.g., lens and/or cornea), and images formed via reflection. These different optical types were first systematically analyzed by Land (1981), who has contributed significantly to our understanding of eyes and particularly their optical function. The physical laws governing the behavior of light are well known and these fundamentally limit how an eye can be formed, whether it produces an image, records the direction of incident light, or simply the presence of light. For this reason, similar structures have arisen in distinctly unrelated animals such as fishes and cephalopods. The chambered or camera eyes in these two lineages are similar in a large number of details, even though their owners are phylogenetically distant (Packard, 1972). Both evolved spherical lenses to achieve sufficient refractive power for focusing light underwater, but the inverted retinal layers of fishes (and all vertebrates) are distinctly different from the noninverted, somewhat simpler retinas of cephalopods. Macroscopically, these eye types and the animals bearing them are not homologous, even though there are striking similarities and even homologies at the molecular and developmental levels, which are at the heart of understanding eye evolution.

The major optical types of eyes (Figure 2) consist of systems that detect shadows, refraction, and reflection. This range of eye types reveals that a limited number of optical solutions actually have persisted in organisms.

The greatest variety of eyes exists among invertebrates. These animals have both camera eyes (e.g., cephalopods) and compound eyes (e.g., *Drosophila*). Moreover, invertebrates also have the greatest variety of eyes as regards number and location on given species. Whereas vertebrates settled on paired, chambered eyes with lenses on the head, invertebrate species may have multiple, nonpaired eyes and eyes in remarkable locations. For example, certain butterflies have light detecting organs located such that darkness signals successful copulation (Arikawa *et al.*, 1996a, 1996b). In addition, Nilsson and colleagues (Nordström *et al.*, 2003) recently described a visual system in the planula of a box jellyfish *Tripedalia cystophora*, with eyecups directly connected to motor cilium. In this case, there is no nervous system to process visual information because the eyes are a complete sensorimotor system unto themselves.

While primitive eyes provide information about intensity and possibly the direction of a light source, more advanced eyes also inform their owners about wavelength and contrast and can provide high-resolution images of an illuminated scene via the concentration of cone photoreceptors in one area such as the fovea in many vertebrates.

There is great variation in the capacities of eyes depending on development and ultimately their structure. For example, resolution of an image, as measured in subtended degrees, differs by approximately 13-fold among vertebrates and even more between vertebrates and invertebrates. Eagles have the greatest acuity that is around 10 000-fold greater than that found in planaria (Land and Nilsson, 2002). Similarly, a comparison of relative sensitivities among vertebrates reveals a range of 4×10^5 between highly sensitive deep-sea animal vision and human foveal vision (Land and Nilsson, 2002).

Another remarkable adaptation is differential wavelength sensitivity of photoreceptor types resulting in the ability to distinguish colors. The selective pressures for evolution of such wavelength discrimination appear to have been quite pervasive. Very likely the added value of better contrast discrimination, which increases the likelihood of identifying food, mates, and predators,

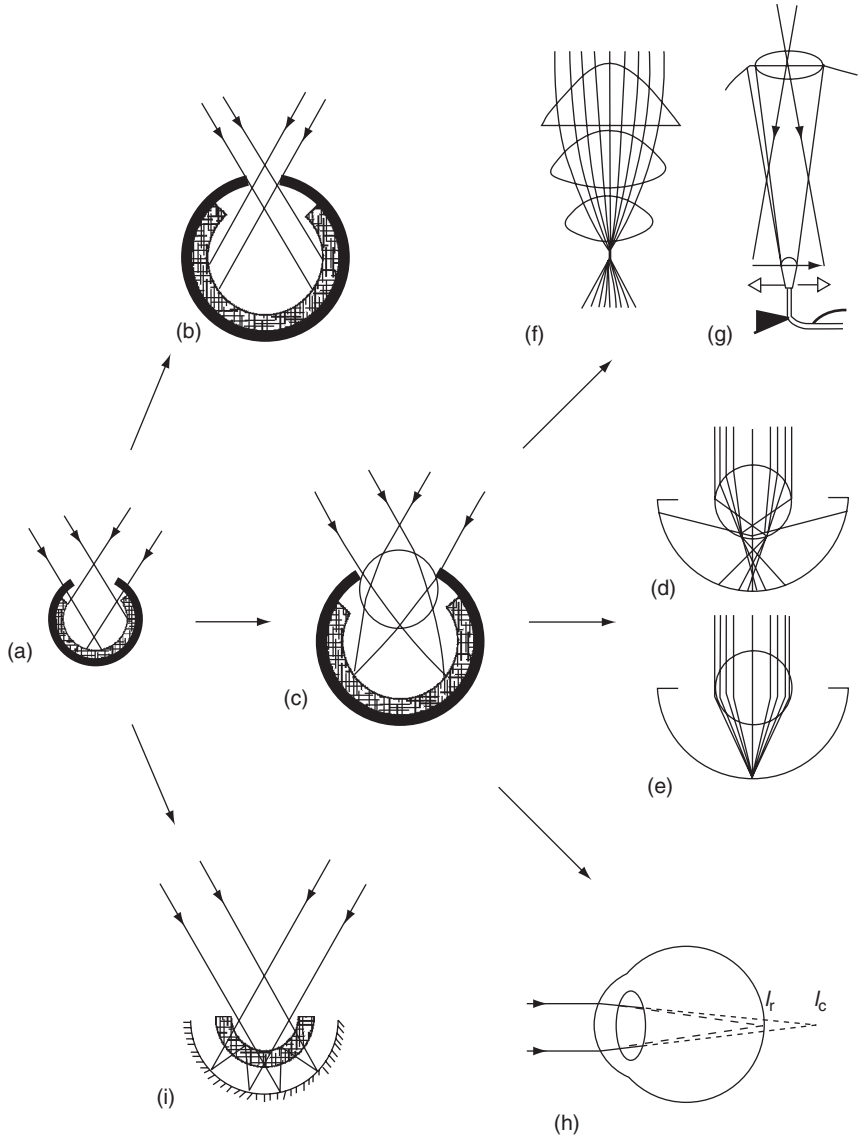

Figure 2 Likely evolutionary sequence of single-chambered eyes. Arrows indicate functional developments, not specific evolutionary pathways. a, Pit eye, common throughout the lower phyla. b, Pinhole of *Haliotis* or *Nautilus*. c, Eye with a lens. d, Eye with homogeneous lens, showing failure to focus. e, Eye with lens having a gradient of refractive index. f, Multiple-lens eye of male *Pontella*. g, Two-lens eye of *Copilia*. Solid arrow shows image position and open arrow the movement of the second lens. h, Terrestrial eye of *Homo sapiens* with cornea and lens; I_c, image formed by cornea alone; I_r, final image on the retina. i, Mirror eye of the scallop *Pecten*. Redrawn from Fernald, R. D. 2000. Evolution of eyes. *Curr. Opin. Neurobiol.* 10, 444 450.

would have been enhanced with chromatic information (e.g., Nagle and Osorio, 1993; Osorio and Vorobyev, 1996). Indeed, recent work comparing eight primate taxa suggests that trichromatic vision evolved where leaf consumption was critical (Lucas *et al.*, 2003). In support of this idea, many species of diurnal reptiles and birds have colored retinal filters, composed of oil droplets, which appear to have evolved to increase the number of colors that can be discriminated, suggesting selective pressure for improved color vision (Vorobyev, 2003).

16.2.3 Lenses: Multiple Protein Types and Gene Sharing

Eyes collect light through an aperture and focus it with a lens onto photoreceptor cells specialized to convert photons into neural signals. Some eyes exist without pupils and even without lenses (*Nautilus*), but eyes that evolved to give their owners a clear

view of the environment on a short timescale do have lenses. Lenses are constructed of tightly packed proteins, so could the composition of lenses yield insight into how eyes evolved?

In vertebrates, lenses are formed from modified epithelial cells and contain high concentrations of soluble proteins, known as crystallins because of their organized packing into arrays. In contrast, in most invertebrates, the lens proteins are secreted by specialized cells of the eye. Recently, lenses of mitochondrial origin have been found in the two pairs of eyes of the parasite *Neoheterocotyle rhinobatidis* (Rohde *et al.*, 1999). Despite their distinct cellular origins, for a lens to function optically, its constituent proteins must be distributed to produce a radial gradient of refractive index that is low at the edge of the lens and high in the center (see Kroeger *et al.*, 1999; Land, 2000). An exact gradient of refractive index is essential for vision in animals living in water but is also found in terrestrial vertebrates and invertebrates. Perhaps most remarkably, cephalopods assemble their spherical lens from two distinct embryological sources, yet manage to produce the required gradient of refractive index (Jagger and Sands, 1999).

Until quite recently, the 10 or so crystalline proteins found in lenses were thought to be unique to lens tissue and were also thought to have evolved for this function. Of the large number of crystallins, alpha and beta-gamma crystallins are indeed specialized lens proteins in vertebrates, related to heat shock protein and schistosome egg antigen, respectively. However, the remaining vertebrate lens proteins are not conserved, but rather comprise a diverse group, many of which are used as enzymes elsewhere in the body. Surprisingly, most of these taxon-specific lens proteins are actually products of the same genes as the enzymes; this double use has been termed gene sharing by Wistow (1993a, 1993b). For example, a crystalline protein in the duck lens was shown to be similar to a metabolic enzyme, argininosuccinate lyase, and the lens protein and metabolic enzyme are encoded by the same gene, not from duplicated genes. Such sharing might possibly have been a prelude to gene duplication. This molecular opportunism is so effective that it has also occurred both in cephalopods (Tomarev and Zinovieva, 1988) and in *Drosophila* (Janssens and Gehring, 1999). One possibility is that since lenses need the production of a relatively large amount of protein, genes that have been upregulated in other tissues might be selected as appropriate.

Perhaps the most remarkable example of a lens from an unusual source is found in the brittlestar (*Ophiocoma wendtii*). These animals form crystal lenses as a part of their skeletal armor from calcite crystals. The crystals, oriented to bring light onto the photoreceptive surfaces in the body, focus the light much as corrective lenses might and effectively concentrate the light by approximately 50 times (Aizenberg *et al.*, 2001).

The common cellular strategy of assembling lenses from diverse proteins seems to be a convergent evolutionary solution that has occurred in many vertebrates independently. The exquisite gradient of the refractive index that evolved in vertebrates and invertebrates alike resulted because it is the only way known for making an optically useful lens. What remains unknown is how such diverse protein species are assembled through folding and organization that preserves key properties of transparency and suitable refractive index gradient along the axis of the lens. The challenge for understanding lens development is to identify the mechanisms responsible for organizing diverse proteins into a functioning lens. This knowledge could provide useful insights into eye evolution from the perspective of lens assembly.

16.2.4 Capturing Light: The Opsin/Retinal Solution

Evolution has left its mark in the DNA sequences of the main light-capturing molecule, opsin, in the biochemistry of transduction, and in the association between the active proteins and other molecules essential for phototransduction. Vertebrate visual pigments (opsins) appeared before eyes (Land and Fernald, 1992) and evolved along at least seven lines, diverging from an ancestral type, before teleost fish diverged from other vertebrates (e.g., Hisatomi *et al.*, 1994) and indeed before deuterostomes split from the protostomes (Terakita, 2005), suggesting that a common ancestor had multiple opsin genes. This surmise has been confirmed with recent evidence (see below). That visual pigments evolved along parallel lines following an ancient divergence is widely accepted, though there are some differences in exact interpretation (Okano *et al.*, 1992).

Opsins are seven transmembrane proteins (30–50 kDa) that associate with a nonprotein moiety, the chromophore retinal. Among the approximately 1000 opsin forms that have been described to date, the phylogenetic differences among the seven major groups correspond to specific functional classifications (Figure 3). These classes differ in several ways, including their transduction via different G-proteins. For example, vertebrate and invertebrate photosensitive opsins are heterotrimeric guanine nucleotide-binding protein (G-protein)-coupled receptors that use 11-*cis*-retinal or a close variant as their chromophore. Vertebrate rod and cone

opsins signal through photoreceptor-specific G-proteins called transducins, whereas invertebrate opsins signal through the Gq family of G-proteins. Photo responses are terminated by a combination of phosphorylation of the excited opsin, the binding of arrestin proteins, which is then followed by regeneration of the active chromophore form needed for photosensitivity.

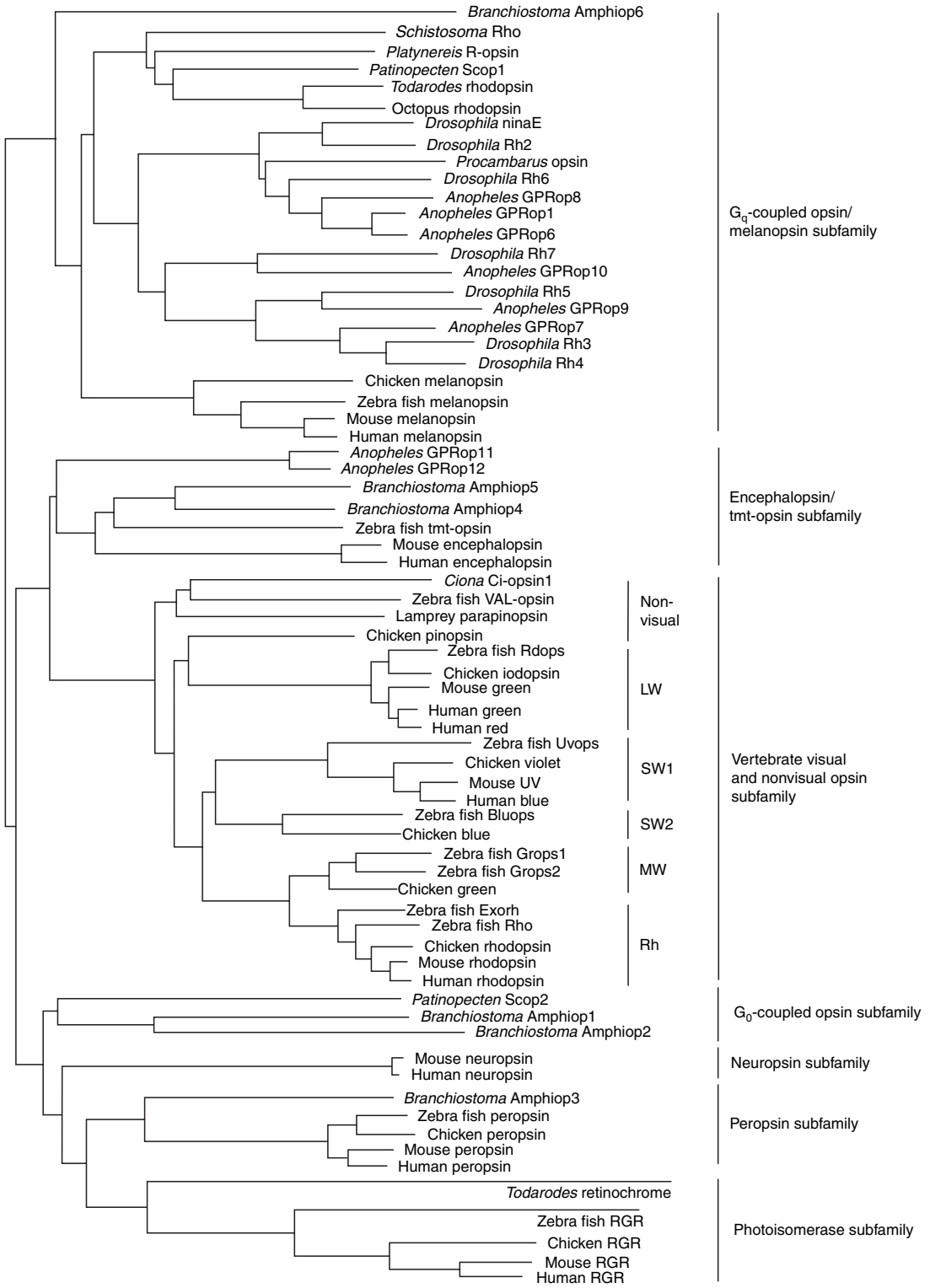

Figure 3 Phylogenetic tree of known opsins. These naturally segregate into seven families. Reprinted from Terakita, A. 2005. The opsins. *Genome Biol.* 6, 213.

A great deal is known about the detailed evolutionary relationships among rhodopsin molecules; some of this based on understanding the interaction between retinal and opsin (Marsh and Griffiths, 2005). Indeed, rhodopsin function is very well understood (e.g., Menon *et al.*, 2001) and the adaptive radiation of pigment types due to natural selection for particular wavelength responses has been described for some special cases (e.g., East African cichlids, Sugawara *et al.*, 2002; squirrelfish, Yokohama and Takenaka, 2004). However, there has been considerable variance in spectral sensitivities that likely resulted from specific selective advantages for one solution over another. Detailed comparisons between terrestrial vertebrates and insects, for example, reveal that there are not unique solutions to encoding both spatial and spectral information. Mammals and bees use long wavelength receptors for luminance and color vision, whereas flies and birds have evolved separate sets of photoreceptors for the two purposes (Osorio and Vorobyev, 2005).

Primate photopigments also offer examples of recent evolutionary change in these important molecules. For example, Old World monkeys, apes, and humans have trichromatic vision, while New World monkeys are polymorphic, having dichromatic or trichromatic color vision (Jacobs, 1996). In this context, *Homo sapiens* may be unique in the polymorphism found in our color vision system (e.g., Neitz *et al.*, 1996). This variance in the number and kinds of photopigments in the human retina might reflect the reduced selective pressure on color vision. The subtlety of selective pressures on chromatic detection can be found in many species. It is particularly evident in the variation within a single species of bluefin killifish, where the relative abundance of cone types depends on whether the animals live in springs or swamps (Fuller *et al.*, 2003). The novel differential spectral sensitivity in these populations is produced through differential expression of cone classes in the retina, rather than via modification of the spectral tuning of opsin molecules, showing that there are different ways to achieve different kinds of chromatic sensitivity.

Another mechanism for temporal modulation of wavelength sensitivity in cone photoreceptors has been described in Pacific salmon (*Oncorhynchus gorbuscha*). As salmon move from being planctivores living in surface waters where UV light is abundant to fish-eating predators in deeper waters where blue-green light prevails, they remodel their UV-sensitive cones with insertion of an opsin that is tuned to blue wavelengths (Cheng and Flamarique, 2004). A similar mechanism has been previously reported in winter flounder (*Pseudopleuronectes americanus*) in which a single opsin type in juveniles, located in hexagonally arranged single cones, is replaced by three different opsin types in photoreceptors arranged in a square array in the metamorphosed adult (Evans and Fernald, 1993; Evans *et al.*, 1993).

These examples show that animals have evolved eyes with resolution, sensitivity, and wavelength detection to match their needs, even as those needs change during their life history. The best understood aspects of visual transduction is that which is used for the main visual input in both vertebrates and invertebrates. The role of the other opsin families is beginning to be understood, although a great deal remains mysterious.

16.3 Evolutionary Issues

16.3.1 Origins of Eyes

Logically, eyes might be monophyletic, having evolved from a single progenitor, or polyphyletic, having arisen more than once during evolution. Salvini-Plawen and Mayr (1977) compared overall structure, photoreceptor types, developmental origins of eye tissue, position of receptor axons, and other anatomical markers among eyes using current fauna. Based on this analysis, they came to the conclusion that eyes evolved not once but at least 40 different times, and possibly many more (reviewed in Land and Fernald, 1992). This multiple-origins hypothesis, based on morphological evidence, has been challenged more recently by results from molecular experiments. Specifically, Gehring and Ikeo (1999) proposed that because a single, well-conserved master gene, *Pax6*, can initiate eye construction in diverse species, eyes must have arisen from a single ancestor. Did eyes appear many times in the course of evolution making them polyphyletic, as claimed by Salvini-Plawen and Mayr based on phenotype, or have all eyes descended directly from a common, primitive form, making them monophyletic, as claimed by Gehring and Ikeo (1999) based on genes controlling development? Since this original debate erupted, there have been several salient discoveries that suggest eyes arose more than once and we carry the evidence within our own eyes!

By the Cambrian period (570–500 Mya), eyes were present in the form of very simple eyecups, useful for detecting light but not for processing directional information. Although the causes are unknown, explosive speciation, or the big bang of animal evolution, happened during the Cambrian (Conway-Morris, 1998). Existing eye types improved radically, coincident with the appearance of carnivory and predation. The evolution of ocular structures

has proceeded in two stages (Figure 2; Land and Fernald, 1992). First was the production of simple eyespots, which are found in nearly all the major animal groups and contain a small number of receptors in an open cup of screening pigment (Land and Fernald, 1992). This kind of detector cannot play a role in recognizing patterns but rather in distinguishing light from dark. The second stage in eye evolution is the addition of an optical system that can produce an image. Image-forming eyes occur in 96% of known species distributed among six phyla. Among the known eye types are at least 11 distinct optical methods of producing images, the most recently described is a telephoto lens, identified in the chameleon in 1995. Indeed, six of the optical mechanisms have only been discovered in the past 25 years.

Since camera-type eyes are demonstrably superior in several respects (Nilsson, 1989), why do all animals not have them? Certainly, camera-type eyes require big heads and bodies to hold them and this likely restricted the number of animals that have followed this evolutionary path. Also, it is probable that, having evolved one eye type, conversion to another type requires intermediate stages that are much worse or useless compared with the existing design. This would make a switch essentially lethal to animals that depend on sight. Although this argument makes sense intuitively, some existing cases of novel optical combinations suggest this is probably not the whole story.

Textbooks tend to group animal eyes into two groups, the camera-type or simple eyes and the compound eyes, which may be didactically useful since such a dichotomy reflects a real and fundamental difference in optical mechanisms, but it conceals a remarkable diversity of optical systems subsumed under each heading.

For example, Nilsson and Modlin (1994) described a mysid shrimp (*Dioptromysis paucispinous*) that has a combined simple and compound eye: partly compound with multiple facets exactly like the eye of an insect, and partly simple with a single lens focusing an image on a sheet of receptors like that of a human. These shrimp are about 5 mm long with nearly spherical eyes at the ends of stalks. In addition to the facets (approximately 800–900), there is a single giant facet facing the shrimp's tail, which the shrimp frequently rotates forward, probably to get a better look at something since that facet has roughly five times the acuity (but much lower sensitivity) than the rest of the eye. It is as if the shrimp is carrying a pair of binoculars for the occasional detailed look at something ahead of it. The discovery that simple and compound eye types can be found in a single animal raises the question of how a developmental program could produce this outcome.

16.3.2 Developmental Evidence of Eye Evolution

Classical experimentation on ocular development focused on vertebrate eyes, a specialized extension of the brain. Experimental models were primarily limited to mice and chicks due to their extensive prior exploitation as model organisms. The beautiful images available today make the often subtle but distinctive morphological changes during eye development seem much more obvious than they were when first observed. With scanning electron microscopy and sophisticated methods of timing the state of tissue development, it is possible to watch unfolding of the production of an eye (e.g., see University of North Carolina website, 'Relevant Website').

Eyes develop from the prospective forebrain, beginning in the eyefields, which are made up of cells of the anterior neural plate. As the prosencephalon grows, this region moves forward until the optic groove forms, and the neuroectoderm of the groove locally contacts the surface ectoderm, inducing the lens placode. As the placode invaginates to form the lens vesicle, the optic vesicle forms the bilayered optic cup, which ultimately becomes the eye. The interaction between the optic vesicle and the lens placode was identified as the organizer of the lens by Spemann (1924). The presumptive lens arises from the lens placode, a thickening of the ectoderm in contact with the optic vesicle. Coincident with this change is the onset of expression of proteins that will form the lens. Other structures of the eye are formed by large- and small-scale tissue movements, caused and accompanied by the expression of tissue-specific genes at that site. The cornea arises from the surface ectoderm over the lens and from migrating mesenchyme derived from the neural crest. Many of the original observations about the role of specific tissue bits in these processes resulted from exquisite embryonic manipulations related to transplantation experiments. For example, Nieuwkoop (1963) identified, among other things, the source tissue essential for the induction of eye production.

With well-described macroscopic change in hand, the next challenge is to synthesize the phenomenological, macroscopic morphological observations with molecular explanations of eye development and understand what this tells us about evolution.

The morphological process of eye development has been viewed as a set of steps toward a final tissue arrangement. Underlying this apparently straightforward sequence of large-scale events, however, are distributions of gene expression with substantial overlap in both time and space. Gene expression is

closely regulated, and specific gene products are used repeatedly, which makes the causal relationships difficult to conceptualize. Nonetheless, progress in characterizing the genes responsible for particular steps in eye development has been reasonably rapid, as shown in several recent reviews (Harland, 2000; Chow and Lang, 2001; Graw, 2003). Functions for at least 15 transcription factors and several signaling molecules have been described in human and mice eyes, based on developmental disorders and/or molecular manipulations (e.g., Graw, 2003). As with other molecular actors, both the transcription factors and signaling molecules are expressed during ocular development and also in a wide range of other tissues. This suggests that the particular combination of expression patterns is important for the proper functioning of these genes in eye development.

As is now well known, the paired box gene 6 (*PAX6*), a member of the family of genes that encode transcription factors with a homeodomain and a paired domain, appears to be important in eye formation across many species. The remarkable demonstration that *PAX6* can induce eyes where they should not be (ectopic) in *Drosophila* (Halder *et al.*, 1995), and similar subsequent demonstration in vertebrates (Chow *et al.*, 1999), led to the suggestion that there might be master control genes responsible for development and differentiation of ocular tissue in many species. Subsequent work has suggested that the term 'master control gene' is a misnomer, however, since a suite of genes is required, collectively, to initiate eye development, and transcription factors are a necessary part of the initiation process. Moreover, as noted above, the genes in question actually have dynamic spatial and temporal expression during many stages of eye development, in addition to expression for essential purposes in other tissues. Nonetheless, it is remarkable that some of the same genes appear in the context of eye development, despite great evolutionary distance among the owners of the eyes. How this might have occurred is discussed below.

For *Drosophila* eyes, it is now known that a collection of seven genes, encoding transcription factors and two signaling molecules collaborate to make eyes (reviewed in Kumar, 2001). These nuclear factors (*eyeless* (*ey*), *twin of eyeless* (*toy*) – both of which are *PAX6* homologues – *sine oculus* (*so*), *eyes absent* (*eya*), *dachshund* (*dac*), *eye gone* (*eyg*), and *optix*), and signaling systems, including the Notch and receptor tyrosine kinase pathways, act via a complex regulatory network that is reasonably well understood (see Kumar, 2001, Figure 1). The master gene hypothesis is not supported, because deletion of any of these genes causes loss or radical reduction in the *Drosophila* compound eye and, surprisingly, any gene except *sine oculus*, in collaboration with certain signaling molecules, can cause ectopic expression of an eye in a limited set of imaginal disks. This means that the whole troupe is needed to produce a reasonable eye. Why this might be so is suggested by recent work showing that the *eya* gene products are phosphatases, the first case in which a transcription factor can itself dephosphorylate other proteins to fine-tune gene expression (Li *et al.*, 2003). This elegant work demonstrated the details of interactions among *Six1*, *Dach*, and *Eya* in the formation of the kidney, muscle, and inner ear, as well as eyes, suggesting that this suite of genetically interacting proteins has been recruited repeatedly during evolution for organogenesis of different structures.

It is difficult to abandon the heuristic of hierarchical regulatory processes in development originally proposed by Lewis to characterize homeotic properties of bithorax and antennapedia genes, but molecular analysis of eye development shows that this concept may not be useful in this case. Instead, eye development appears to need new ways of thinking about how complex tissues are made and how such organs arose in evolution. The widespread and redundant activities of specific genes during ocular development (e.g., Chauhan *et al.*, 2002; Baumer *et al.*, 2003) suggest that hierarchies, if they exist, are unknown and the more likely scenario is the orchestrated activity of a suite of molecular actors.

As described above, the diversity of eyes confirms their dynamic evolutionary past. Explosive speciation, or the big bang of animal evolution, occurred during the Cambrian (Conway-Morris, 1998), when existing eye types appear to have improved radically, coincident with the onset of carnivory and predation. Many selective forces were likely at work (Fernald, 2000), including perhaps the first instances where light enabled behavioral signals (Parker, 1998), so no predominant selective force can be claimed. The rapidity of eye evolution has always been a question, but, using a simulation, Nilsson and Pelger (1994) suggested that about 2000 sequential changes could produce a typical image-forming eye from a light-sensitive patch. With reasonable estimates, this suggests that an eye could evolve in less than half a million years, making the virtual explosion of eyes during the Cambrian seem reasonable (Land and Nilsson, 2002). After the Cambrian, three phyla emerged: arthropods, mollusks, and chordates. Although these groups all use the opsin molecule to capture

light, details of the structure and function of their eyes differ considerably.

One of the most interesting developmental differences among extant eyes is the embryonic origin of the different structures in vertebrate and cephalopod eyes (summarized in Nilsson, 1996). Cephalopod eyes form from an epidermal placode through successive infoldings, whereas vertebrate eyes emerge from the neural plate and induce the overlying epidermis to form the lens as described above. It is also noteworthy that the cephalopod eyes lack a cornea, which is present in all vertebrates whether aquatic or not.

In addition to the differences in embryonic origin, photoreceptor cells divide into either ciliary or microvillar structures to provide the membrane surface for the opsin molecule (Salvini-Plawen and Mayr, 1977). Microvilli predominate in invertebrates, whereas vertebrate photoreceptors are ciliary. Physiological responses are also quite different, with the microvillous receptors of arthropods and mollusks depolarizing to light, and the ciliary receptors of vertebrates hyperpolarizing to light. In phototransduction, vertebrate photoreceptors exploit cyclic guanosine 5′-monophosphate (GMP) as a second messenger system, while invertebrates use inositol trisphosphate (Fernald, 2000). And, even though opsin is the key molecule for detecting light, mechanisms for regeneration (e.g., reisomerization) of the chromophore/opsin system are dramatically different among phyla (Gonzalez-Fernandez, 2003).

16.3.3 Functional Evidence about Eye Evolution

Until recently, the photodetection systems we understood well were localized primarily to eyes and pineal glands and a few other sites in the body such as the skin. For each of these, a canonical opsin and related transduction cascade were known. Specifically, ciliary structures associated with specific G-proteins are known from vertebrate eyes and microvilli associated with inositol phosphate signaling cascades are known from invertebrate eyes (see above). Then, in several laboratories, each of these phototransduction cascades was found in unexpected organisms. Arendt *et al.* (2004) found that the polychete ragworm (*Platynereis dumerilii*), in addition to the rhadomeric photoreceptors in its eyes, had ciliary photoreceptors in the brain. They also showed that the typical types of opsins associated with each photoreceptor type were both expressed in the ragworm and localized only with that type (e.g., vertebrate c-opsin in the brain and invertebrate r-opsin in the eye). This means that the two main types of eyes exist in a worm.

The idea that two kinds of photoreceptors might exist in a single organism was first suggested by the pioneering work of Gorman, who with colleagues showed physiological and morphological data suggesting that both types of photoreceptors exist in a scallop, *Pecten irradians* (Gorman and McReynolds, 1969, 1971). These investigators found depolarizing and hyperpolarizing responses to light stimuli from cells located in different layers of the scallop retina, with depolarizing potentials arising from the proximal layer and hyperpolarizing potentials form the distal layer. The investigators interpreted their data solely with respect to the various kinds of selective advantages each response type might have but did not consider the evolutionary implications, though their data support the existence of the two canonical receptor types in one organism.

Meanwhile, in vertebrates, a parallel set of results has been appearing. A small population of intrinsically photosensitive retinal ganglion cells have been discovered that play key roles in the regulation of nonvisual photic responses. These rely on melanopsin (see Figure 3), an opsin first identified in vertebrate melanophores, brain, and eyes by Provencio *et al.* (1998). The melanopsin in the retina was soon shown to be in the form of photosensitive ganglion cells (Berson *et al.*, 2002), required for normal light-induced circadian phase shifting (Panda *et al.*, 2002), and yet could not function without normal rods and cones (Ruby *et al.*, 2002), meaning that its signals are combined with those from rods and cones somewhere in the visual system. Photosensitive ganglion cells comprise a non-image-forming system that can detect the presence or absence of light but not much more. Subsequent functional analyses showed that retinal melanopsin functions via a phototransduction cascade that resembles invertebrate opsins and, in another similarity to invertebrates, has intrinsic photoisomerase activity (Panda *et al.*, 2005; Qiu *et al.*, 2005). Adding to the remarkable set of discoveries, melanopsin-expressing ganglion cells in the primate retina have been shown to signal color and radiance levels to the lateral geniculate nucleus (Dacey *et al.*, 2005). So, not only do vertebrates carry a version of the invertebrate visual transduction system with them, but it is used in a variety of ways, including to provide information to the image-forming visual system.

Taken together, these findings show that at least two kinds of photoreception existed in the urbilateria, before the split into three Bilateria branches at the Cambrian (Figure 4), and, importantly, each of these branches still carry versions of

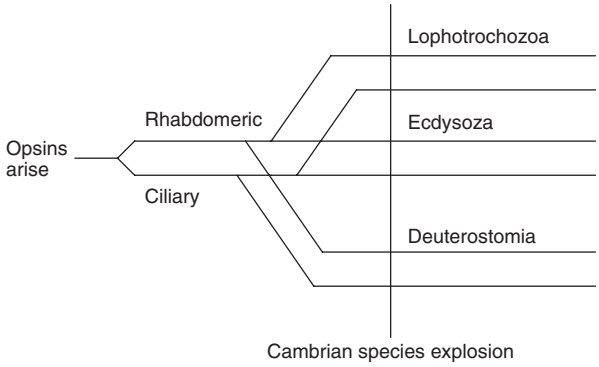

Figure 4 Schematic phylogeny of the Bilateria showing that the distinct rhabdomeric and ciliary organization of opsins preceded the splitting of the urbilateria. Based on Nilsson, D. E. 2005. Photoreceptor evolution: Ancient siblings serve different tasks. *Curr. Biol.* 15, R94 R96.

these two systems. In addition, cryptochromes, also discovered very recently (Cashmore *et al.*, 1999), are another photoreceptive system that is not based on opsin, has no molecular amplification, and is found in both plants and animals. To date, cryptochromes have been shown to play a role in circadian rhythms (Green, 2004) and control of the iris muscle in birds (Tu *et al.*, 2004) as well as many functions in plants. Considering that seven families of opsin have been described in humans (see Figure 3), we can expect more surprises in the detection of light. The additional opsins discovered recently have not yet been functionally characterized, but the evidence suggests that there are no more opsins to be discovered (Kumbalasiri and Provencio, 2005). Even so, figuring out how all the existing opsins work together is a daunting challenge.

16.3.4 Parallel Evolutionary Universe?

One of the persistent issues in the evolution of eyes, as noted above, is whether eyes evolved once or many times. Though it seems quite clear that there were at least two kinds of phototransduction (e.g., ciliary and rhabdomeric) before the urbilateria split into three families (see Figure 2), energy and information are harvested in archaea and eukaryotic microbes using a system that clearly arose independently, via convergent evolution. Microbial, or type 1 rhodopsins, named to distinguish them from the visual pigments or type 2 rhodopsins, function to harvest light for energy, to guide phototaxis, and probably many yet undiscovered functions (Spudich *et al.*, 2000). While the number of known type 2 (visual) rhodopsins has increased dramatically over the past several years (see above), the number of known type 1 rhodopsins has rapidly increased with the harvesting and genetic sequencing of ocean samples from a handful to over 800 (Spudich and Jung, 2005). These type 1 rhodopsins are widely dispersed on the planet, found in organisms living in both freshwater and seawater, salt flats, and glacial seas, among others.

There are several fundamental differences between types 1 and 2 rhodopsins. First, there is no evident phylogenetic relationship between the genetic sequences of type 1 and type 2 rhodopsins. As more type 1 opsins are discovered, a connection may become apparent, but given the current state of knowledge, this seems unlikely. Second, the type 1 rhodopsins reveal convergent solutions to the mechanisms for converting photon energy. Both rhodopsin types consist of seven transmembrane domain proteins and, in each, retinal is attached in a Schiff base linkage via a lysine residue in the seventh helix (Spudich *et al.*, 2000). However, type 1 rhodopsin (25–30 kDa) has a different organization of its intramembrane domains from type 2 rhodopsin (35 kDa), which reflects the fundamental difference in their signaling cascades. Whereas type 1 rhodopsins function within the membrane to pump ions or signal to other integral membrane proteins, type 2 rhodopsins signal via G-proteins, receptor kinases via the cytoplasmic loops (see above and Spudich *et al.*, 2000). Retinal is used in association with both apoproteins, but these are photoisomerized quite differently. In the familiar, type 2 rhodopsins, 11-*cis* retinal is transformed to all *trans* upon absorbing light, whereas in type 1 rhodopsins, all *trans* retinal is transformed to 13-*cis* when absorbing light.

Taken together, the remarkable convergence of type 1 and 2 rhodopsins suggests that in the course of evolution, an opsin apoprotein associated with retinal has been discovered and exploited twice. Clearly, when the seven transmembrane protein is appropriately solvated with retinal, it is useful for transforming the energy of photons into more useful forms. This also suggests that progenitors of the type 1 opsins may have existed in earliest evolution before the divergence of archaea, eubacteria, and eukaryotes. This means that the light-driven ion transport mechanism for deriving energy used in association with retinal 1 preceded the evolution of photosynthesis as a means for using the sun's energy (Spudich and Jung, 2005). We can now wonder whether a proto eye-like structure using rhodopsin 1 remains to be found that would allow a comparison of an additional independent solution to extracting information from light.

16.4 How Did Eyes Evolve?

Eyes exist in a variety of shapes, sizes, optical designs, and locations on the body, but they all

provide similar information about wavelength and intensity of light to their owners. Different tissues have been recruited to build lenses and retinas across the phyla. In contrast, all eyes share the same mechanism of absorbing photons, i.e., the opsin–chromophore combination has been conserved across phylogeny. Despite new findings yielded by powerful molecular techniques, all evidence still suggests that eyes have a polyphyletic origin, particularly since the discovery that two photodetection systems had evolved prior to the split of the urbilateria into three families. Clearly, eyes as we know them contain homologous molecules responsible for many structural, functional, and even developmental features. Given a growing list of homologous gene sequences among molecules in the eye across vast phylogenetic distances, the challenge is now to discover what makes the eyes of *Drosophila*, squid, and mouse so different. Understanding what makes eyes different may be a bigger challenge than finding what they have in common.

It seems increasingly evident that as eyes evolved, different functional mechanisms have been generated by recruiting existing gene programs. From genome sequencing, we know that there are far fewer genes in organisms than previously thought, so the use and reuse of genes and their products in combinatorial assemblies as reported for known genomes make sense. In the development of eyes, this seems to be the rule not the exception. Specifically, in the evolution of eyes, it seems likely that light sensitivity evolved early in the Cambrian in the form of a proto-opsin molecule in association with the chromophore, retinal. This molecular combination, sensitive to light, became associated with the genes *pax6* (Sheng *et al.*, 1997), and possibly *eya* (based on its phosphatase activity (Li *et al.*, 2003)). One can imagine that this combination was recruited and worked well in early evolved eyespots and other light-sensing organs. It would not be surprising, for example, to find these genetic players in the recently described eye without a nervous system (Nordström *et al.*, 2003). As different eye types evolved over time, there was probably repeated recruitment of particular gene groups, not unlike improvisational groups of actors, interacting to produce candidates for selection. The evolutionary fiddling through which various combinations or routines were tried could have led to numerous parallel evolutionary paths for eyes as we now envisage.

From this, two different mechanisms for transmitting the photic information to surrounding cells were selected for, one in ciliary and one in rhadomeric photoreceptors. These two systems are likely present in all organisms, as described above for worms and mice. The big surprise is that both of these transduction systems persisted, with each selected as the primary visual system for a major branch of animals. So the answer to the question of whether eyes evolved from a single prototypical eye (monophyletic), or whether they evolved repeatedly (polyphyletic), appears to be that quite evidently eyes arose at least twice and probably many times. And, as described above, given the vast number of organisms using rhodopsin 1, we should not be surprised if additional eyes appear in the biological world in the future.

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17 Vertebrate Olfactory Subsystems and their Evolution

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Glossary

olfactory system	The olfactory system in any animal is the primary sensory system that responds to chemical stimuli emanating from a distant source.
pheromone	A chemical cue that, when released by an individual, elicits specific behavioral or physiological responses from conspecifics.
terminal nerve	The most anterior cranial nerve in vertebrates. The terminal nerve releases compounds into the nasal epithelia, modulating activity of sensory receptor cells.
vomeronasal system	A discrete olfactory subsystem present in tetrapods that differs morphologically from the olfactory system. Its function is unclear.

17.1 Introduction

17.1.1 What is Olfaction?

The term olfaction is commonly applied to chemosensory systems that detect chemicals emanating from a distant source. Other chemosensory systems generally require physical contact with the source for detection, and this sensory modality is called gustation. The vertebrate olfactory and gustatory systems are anatomically distinct; the latter is discussed in a separate article (Evolution of Taste). Fibers of the trigeminal nerve also detect chemical stimuli, as dochemosensors in the respiratory, circulatory, and digestive systems that detect gasses, ions, and nutrients. In general, these chemosensory systems consist of isolated sensory cells that project to the spinal cord or hindbrain. These systems will not be discussed here.

In this article, we will consider three interrelated olfactory subsystems: the main olfactory system, or olfactory system proper; the vomeronasal system; and the terminal nerve. The main olfactory system comprises receptor neurons located in a specialized sensory epithelium in the nasal cavity, as well as the central projections of these neurons. The receptor cells of the olfactory epithelium develop from the nasal placode, and the ingrowing fibers of the developing sensory neurons are involved in development of the olfactory bulb at the rostral pole of the prospective telencephalon (Gong and Shipley, 1995; Graziadei and Monti-Graziadei, 1992; Long *et al.*, 2003).

The vomeronasal system, or accessory olfactory system, also develops from the nasal placode, and is present as a discrete sensory system only in tetrapods. The vomeronasal epithelium is sequestered in the vomeronasal organ, also known as Jacobson's organ (Figure 1). The sensory epithelium contains some what different cell types than does the main olfactory epithelium, and the vomeronasal receptor neurons terminate in microvilli, whereas olfactory receptor neurons can terminate in cilia or microvilli or both. In some vertebrates,

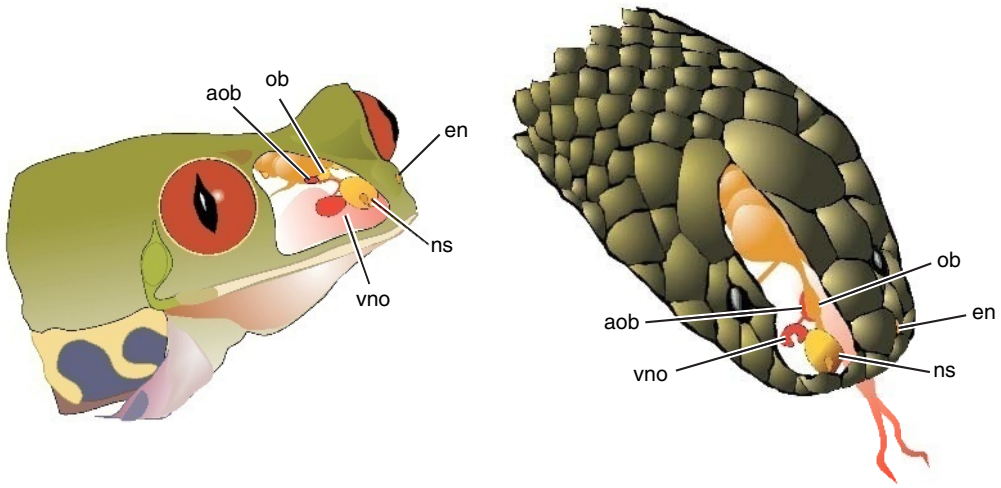

Figure 1 Illustration of the locations of the olfactory epithelium, inside the nasal sac (ns), and of the vomeronasal organ (vno) in a frog and a snake. The nasal sac opens to an external nostril (en) on the dorsal surface of the snout, whereas the vomeronasal organ opens to the nasal sac in frogs and oral cavity in snakes. The axons of the olfactory receptor neurons project to the olfactory bulb (ob), and those of the vomeronasal receptor neurons project to the accessory olfactory bulb (aob).

the axons of the vomeronasal receptor neurons form a separate cranial nerve, the vomeronasal nerve, but in others these axons run alongside the olfactory nerve and the two cannot be distinguished using conventional light microscopy. Vomeronasal receptor cell axons project to the accessory olfactory bulb, a structure that is histologically distinct from the main olfactory bulb (Figure 1). The secondary projections of the olfactory and accessory olfactory bulbs differ. The molecular, physiological, and anatomical differences between the vomeronasal and olfactory system suggest that the two subsystems serve different behavioral functions, but the nature of this difference is unclear. Although the vomeronasal system is often presumed to be specialized for detecting pheromones, it responds to a variety of odorants with differing behavioral significance in all groups of tetrapods, and this presumption is unwarranted (see Baxi *et al.*, 2006; Halpern and Martínez-Marcos, 2003; Restrepo *et al.*, 2004; Shepherd, 2006). A different hypothesis posits that the vomeronasal system is specialized for detecting nonvolatile molecules. Although better supported than the pheromone hypothesis, this hypothesis is still problematic (Baxi *et al.*, 2006).

Throughout this article, we will refer to the nasal chemosensory system present in all vertebrates as the 'olfactory system' rather than the 'main olfactory system'. Similarly, although it is common to use the term 'main olfactory bulb' to refer to the primary target of the olfactory receptor cells in animals that possess a separate vomeronasal system, we will refer to this structure simply as the 'olfactory bulb' to facilitate comparisons across vertebrates.

The terminal nerve, or nervus terminalis, is the most anterior of the cranial nerves. It extends between the nasal cavity and basal forebrain, and, because of its anatomy, has been thought to serve sensory function (e.g., Demski and Northcutt, 1983; Rossi *et al.*, 1972). Nevertheless, recordings from the terminal nerve have failed to detect sensory activity (Bullock and Northcutt, 1984; White and Meredith, 1995), and the terminal nerve is now thought to function in modulating activity in the olfactory epithelium (reviewed in Oka, 1992; Wirsig-Wiechmann *et al.*, 2002a). The terminal nerve usually contains a ganglion, the location of which varies across groups of vertebrates. The neurites extending outward from these bipolar neurons sometimes comprise a separate nerve, but the fibers of the terminal nerve can also run within the olfactory or vomeronasal nerve, as illustrated in Figure 2. The cells and fibers of the terminal nerve contain neuromodulatory compounds, including gonadotropin releasing hormone (GnRH) and acetylcholine (reviewed in Wirsig-Wiechmann *et al.*, 2002a). The nerve can also be immunohistochemically labeled with antisera directed against neuropeptide Y (NPY) and the molluscan cardioexcitatory neuropeptide FMRFamide, but the two antisera may cross-react with a single peptide (Chiba, 2000). We include the terminal nerve in this article not only because of its role as a centrifugal portion of the olfactory system, but also because the primary

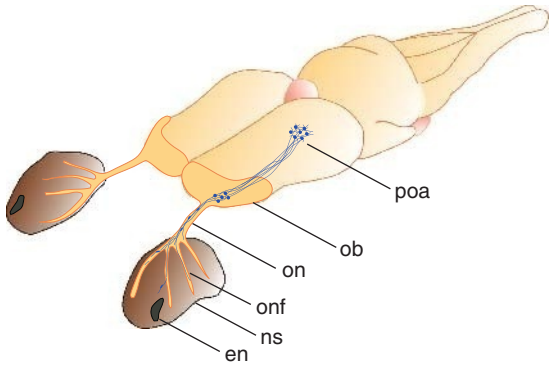

Figure 2 Location of the terminal nerve around the nasal cavities and in the brain of a salamander. Anterior is down and to the left. The fibers of the terminal nerve extend between the preoptic area and nasal cavities, wrapping around the outside of the olfactory epithelium (lower left). en, external nostril; ns, nasal sac; ob, olfactory bulb; onf, olfactory nerve fascicles; on, olfactory nerve; poa, preoptic area.

neurons of the terminal nerve develop from the nasal placode (Schwanzel-Fukuda and Pfaff, 1989), as do the olfactory and vomeronasal receptor neurons.

17.1.2 Components of the Vertebrate Olfactory System

Elements of the olfactory system have been described from an evolutionary or comparative perspective in several reviews (Ache and Young, 2005; Eisthen, 1992, 1997, 2002; Hildebrand and Shepherd, 1997; Nieuwenhuys, 1967). Here, we will describe the general features of the olfactory system in nonmammalian vertebrates, illustrated in Figure 3, to provide a context for understanding the changes that have occurred over the course of vertebrate evolution, as well as variations that occur within specific lineages. In the sections that follow, we will survey the structure and function of the olfactory system in each class of vertebrates, noting features that are new, unusual, or taxon-specific. We will not describe the results of neurobiological studies designed to investigate general features of olfactory system function in vertebrates unless the results have interesting implications for understanding olfaction in the group under investigation. At the end of the article, we will discuss innovations and variations, and their possible functional implications.

In all vertebrates, the olfactory epithelium is sequestered inside the nasal cavity. Because adaptation occurs fairly quickly, the odorant-containing medium must be kept moving over the surface of the sensory epithelium. A variety of mechanisms are employed to achieve this end, including nasal cavities that allow water or air to flow through when the animal is locomoting or breathing, specialized pumping mechanisms, and nonsensory cells with motile cilia that create constant movement. Within the nasal cavity, the pseudostratified sensory epithelium contains three basic cell types (Figure 3b): olfactory receptor cells; sustentacular cells, a class of supporting cells; and basal cells, the progenitor cells that give rise to new receptor and sustentacular cells throughout life. The olfactory receptor cells are bipolar neurons with a dendrite that terminates in cilia or microvilli or both. The membrane-bound odorant receptors are localized to these processes, which are therefore presumed to be the site of transduction (Menco, 1997). The odorant receptors are part of the large superfamily of G-protein-coupled receptors (GPCRs) that have seven membrane-spanning domains (reviewed in Gaillard *et al.*, 2004; Mombaerts, 2004). The large family of odorant receptor genes has been suggested to contain two fundamentally different classes that differ in the size of the third extracellular loop (Freitag *et al.*, 1995, 1998), although others have suggested that a larger number of groupings better describes the evolutionary history of the gene family (Niimura and Nei, 2005). As depicted schematically in Figure 3c, the odorant receptor is coupled to an olfactory-specific G-protein ($G\alpha_{olf}$), with alpha subunits that are expressed in few other tissues; when stimulated, the G-protein activates type III adenylyl cyclase (Nakamura, 2000; Ronnett and Moon, 2002). The details of olfactory transduction are well understood for only a small number of vertebrate species, and involve myriad mechanisms (Firestein, 2001; Schild and Restrepo, 1998). One interesting feature of olfactory transduction is that it often involves several steps, in which ions entering through one channel gate another (Eisthen, 2002).

Even before odorants contact receptors, they interact with other molecules in the nasal cavity. The dendrites of the receptor neurons protrude into a specialized mucus produced by a combination of glands and secretory cells, including goblet and sustentacular cells as well as Bowman's glands. This mucus contains a variety of compounds, including mucopolysaccharides, peptides, and amines (Getchell and Getchell, 1992; Getchell *et al.*, 1993; Zancanaro *et al.*, 1997). In some vertebrates, it also contains specialized odorant binding proteins, soluble lipocalins produced in the lateral nasal glands. The role of odorant binding proteins in olfactory processing is unresolved (Pelosi, 2001; Tegoni *et al.*, 2000).

At the opposite pole of the olfactory receptor neuron, the unmyelinated axon projects to the

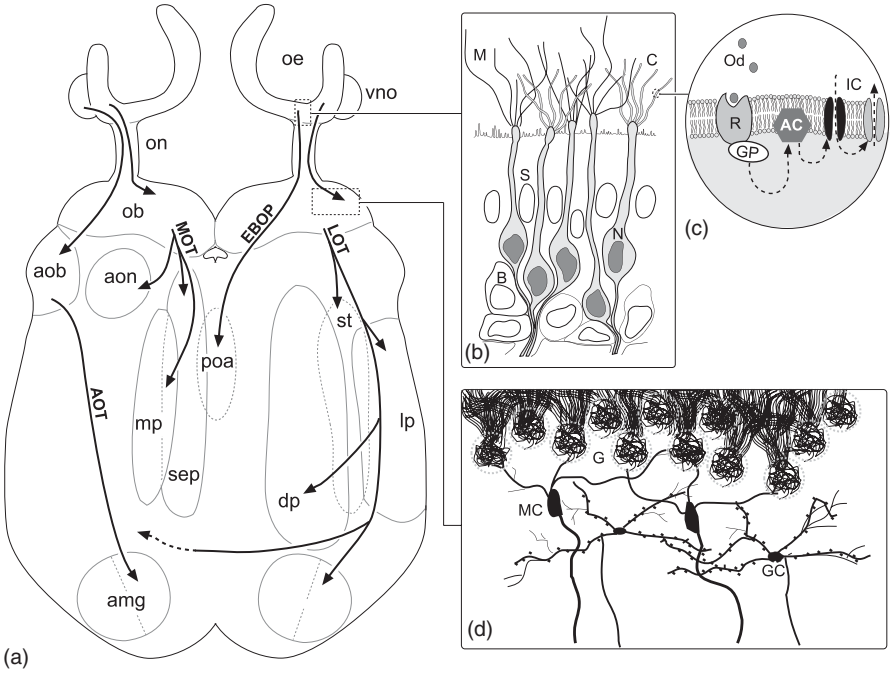

Figure 3 Diagram illustrating the basic elements of the olfactory system in nonmammalian vertebrates. a, Schematic dorsal view of the forebrain and nasal sensory epithelia of a generalized nonmammalian vertebrate. The olfactory epithelium (oe) and vomeronasal organ (vno) lie rostral to the brain, and the axons of the receptor neurons project through the olfactory nerves (on) to the olfactory bulb (ob) and accessory olfactory bulb (aob), respectively. In general, four tracts project centrally. The accessory olfactory tract (AOT) projects from the accessory olfactory bulb to the amygdala (amg). The medial olfactory tract (MOT) projects to the anterior olfactory nucleus (aon), septum (sep), and medial pallium (mp). The extrabulbar olfactory pathway (EBOP) contains axons of primary olfactory receptor neurons that bypass the olfactory bulbs, projecting directly to the preoptic area (poa). The lateral olfactory tract (LOT) projects bilaterally to the striatum (st), lateral pallium (lp), dorsal pallium (dp), and the amygdala. b, The olfactory sensory epithelium consists of three types of cells: receptor neurons (N), sustentacular cells (S), and basal cells (B). The receptor neurons in vertebrates terminate in either cilia (C) or microvilli (M). c, Transduction occurs on ciliary or microvillar membranes. The receptor protein (R) has seven membrane-spanning regions and is coupled to a G-protein (GP). Odorant (Od) binding activates adenylyl cyclase (AC), gating an ion channel (IC). Often, ions entering through this channel gate another ion channel. d, Organization of the olfactory bulb. The axons of olfactory receptor neurons enter glomeruli (G), where they interact with dendrites from mitral cells (MC), the large output neurons of the olfactory bulb. Granule cells (GC) are also present in a deeper layer of the olfactory bulb.

olfactory bulb in the rostral telencephalon. In the olfactory bulb, each unbranching axon forms synapses with many other cells in tangles of fibers known as glomeruli, which are characteristic of olfactory systems in a variety of animals (Eisthen, 2002). The olfactory bulb has a laminar organization. In many vertebrates these layers are not clearly differentiated, but in almost all the layers form concentric rings around the ventricular region in the center of the olfactory bulb. The outermost layer consists of the axons of the olfactory receptor cells, which course over the surface of the bulb before terminating in a single glomerulus in the subjacent layer (Figure 3d). This glomerular layer may contain periglomerular interneurons. Below this, an external plexiform layer is sometimes present, overlying a layer containing the cell bodies of the mitral cells, the output cells of the olfactory bulb. The mitre-shaped cell body that gives these cells their name is only obvious in tetrapods, leading to some confusion concerning the number of classes of output cells in nontetrapods. Mitral cells are large, and generally possess several dendrites that project into different glomeruli. Within a glomerulus, each mitral cell dendrite arborizes extensively, making large numbers of synapses with the axons of the olfactory receptor neurons. The granule cell layer lies between the mitral cell layer and the layer of ependymal cells surrounding the ventricle. The mitral and granule cell layers may be separated by an internal plexiform layer. In some vertebrates, the granule cell layer contains two classes of cells: the granule and stellate cells. Both types of cells generally have axons. Stellate cells possess multiple dendrites that arborize in the glomeruli. In contrast, the granule cells have an oval-shaped soma, and the dendrites interact with neurites of other cells below the glomerular layer or outside glomeruli. In some animals, granule cell dendrites bear spiny processes, whereas those of stellate cells do not. In the

following discussion, we will use these definitions in describing cell types present in diverse animals, regardless of the labels used by the authors of the papers cited.

The fibers projecting centrally from the olfactory bulb consist of the axons of mitral cells. In some groups of vertebrates, axons of other classes of cells, such as the granule and stellate cells, may contribute to these tracts. In most vertebrates, two tracts extend from the olfactory bulb (Figure 3a). The medial tract generally projects to ipsilateral ventral forebrain areas such as the septum and, in tetrapods, to the anterior olfactory nucleus and medial pallium/hippocampus. The lateral olfactory tract projects bilaterally to lateral and dorsolateral pallial areas and to the amygdala, and ipsilaterally to the striatum. Thus, unlike other sensory systems, olfactory projections to the cortex are not routed through the thalamus, contributing to Edinger's (1904) famous hypothesis that the telencephalon was originally an olfactory structure that was invaded by other sensory systems over the course of vertebrate evolution. In addition to the fiber tracts that arise from output cells of the olfactory bulbs, many vertebrates possess a small extrabulbar olfactory pathway that consists of axons of primary olfactory receptor neurons that bypass the olfactory bulb and project directly to the preoptic area (Hofmann and Meyer, 1989; Szabo *et al.*, 1991). Because of their similar projections, many older papers appear to confound the terminal nerve and extrabulbar olfactory pathway (Eisthen and Northcutt, 1996). In the following discussion, we will refer the projection that arises from olfactory receptor neurons as the extrabulbar olfactory pathway, regardless of the name the authors ascribed to it.

The olfactory system does not seem to contain simple maps or even one-to-one functional relationships. Olfactory receptor neurons tend to be broadly tuned, and a given cell will respond to many different odorants, sometimes in different ways; similarly, a given odorant can evoke different responses from different receptor neurons (e.g., Dionne, 1992; Dionne and Dubin, 1994). In mammals, olfactory receptor neurons expressing the same receptor genes project to the same glomeruli, which are located in relatively stable positions within the olfactory bulb (Mombaerts *et al.*, 1996; Schaefer *et al.*, 2001). The neurons that project to a given glomerulus tend to respond to the same sets of odorant stimuli (e.g., Bozza and Kauer, 1998), but a given odorant can activate many glomeruli across the olfactory bulb (e.g., Wachowiak *et al.*, 2002). The effect of changing odorant concentration on spread of activation among glomeruli is unclear, probably in part due to differences in recording methods, odorants used, and species examined in different studies (e.g., Wachowiak and Cohen, 2003; Wachowiak *et al.*, 2002). Nevertheless, the olfactory bulb does not appear to contain a simple map in which an odorant can be identified by the location of the glomeruli that are stimulated; instead, temporal features of the response also play an important role in odorant recognition (e.g., Friedrich and Laurent, 2001; Laurent, 2002).

The vomeronasal epithelium contains the same basic cell types found in the main olfactory epithelium, but lacks Bowman's glands (Parsons, 1967). Vomeronasal receptor neurons express GPCR genes, but the two families of GPCRs found in the vomeronasal organ do not share strong sequence similarity with those expressed in the olfactory epithelium (Dulac and Axel, 1995; Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). Transduction in vomeronasal receptor neurons seems to involve different G-proteins, second messengers, and ion channels than in olfactory receptor neurons, although only a handful of species have been examined to date (reviewed in Halpern and Martínez-Marcos, 2003). The axons of vomeronasal receptor neurons project to the accessory olfactory bulb, caudal to the olfactory bulb. In general, the accessory olfactory bulb contains only mitral, granule, and periglomerular cells, and the layers are less distinct than are those in the olfactory bulb (reviewed in Lohman and Lammers, 1967; Nieuwenhuys, 1967). The accessory olfactory tract projects from the accessory olfactory bulb primarily to portions of the amygdala that differ from those receiving olfactory input via the lateral olfactory tract.

Although there are theoretical reasons for envisioning that the receptors in a chemosensory system should differ in their breadth of tuning (Hildebrand and Shepherd, 1997), it is not clear that vertebrates possess any narrowly tuned receptor neurons. To illustrate this point, we will briefly discuss two examples: ciliated versus microvillar olfactory receptor neurons in teleost fishes, and olfactory versus vomeronasal receptor neurons in mammals. Researchers studying teleost fishes have used electro-olfactogram (EOG) recordings to examine odorant responses in patches of epithelium that are dominated by one receptor cell type. The results indicate that ciliated cells respond to bile acids, which can serve as pheromones in fishes, and that microvillar cells respond to amino acids (salmonids; Thommesen, 1983); or that ciliated cells respond to

amino acids, and that bile acids, steroids, and prostaglandin pheromones are preferentially detected by microvillar cells (goldfish; Zippel *et al.*, 1997). A similar study with channel catfish indicated that both ciliated and microvillar cells respond well to both amino acids and bile acids (Erickson and Caprio, 1984), but a later study in the same species using pharmacological agents to disrupt signaling in different cell types demonstrated that ciliated olfactory receptor neurons respond to bile acids and amino acids, and that microvillar receptor neurons respond to nucleotides and amino acids (Hansen *et al.*, 2003). Whole-cell recordings from olfactory receptor neurons in rainbow trout indicate that microvillar neurons respond selectively to amino acids, and that ciliated neurons respond more broadly to amino acids, a steroid, and conspecific urine (Sato and Suzuki, 2001). Agmatine labeling suggests that a greater proportion of microvillar than ciliated olfactory receptor neurons respond to amino acids in zebra fish (Lipschitz and Michel, 2002). Thus, the relationship between cell morphology and odorant responses is unclear, and may be species-specific. To consider a different comparison for a moment, researchers often assume that vomeronasal receptor neurons in tetrapods are much more narrowly tuned than are olfactory receptor neurons. Because of this bias, few studies have investigated the breadth of tuning of vomeronasal receptor neurons (Baxi *et al.*, 2006). Nevertheless, a few studies that have examined the issue conclude that vomeronasal receptor neurons respond to a broad array of chemicals, both naturally occurring and artificial (e.g., Sam *et al.*, 2001; Tucker, 1971).

The olfactory system, broadly defined, plays an important role in the behavior of most vertebrates. Olfactory cues may be involved in orientation and homing, habitat selection, territoriality, species recognition, kin recognition, individual recognition, parent–offspring interactions, mate choice, courtship and mating behavior, predator avoidance, foraging, and food choice. A thorough review of the behavioral functions of the olfactory system in each class of vertebrates is far beyond the scope of this article. Instead, for each group, we will provide a brief overview in which we illustrate the behavioral significance of olfactory input.

17.2 Evolutionary Changes in the Vertebrate Olfactory System

17.2.1 Chordates and Basal Craniates

Animals in many phyla possess a sensory system for detecting chemicals at a distance, and these sensory systems are generally labeled 'olfactory'. Nevertheless, there is no evidence that the vertebrate olfactory system is homologous with those present in other phyla; instead, it appears that similar features have evolved independently several times for use in sensing odorants (Eisthen, 2002). The nearest relatives to vertebrates are the urochordates (ascidians or tunicates), cephalochordates (lancelets), and hagfishes, which are considered craniates but not vertebrates. Ascidian larvae possess unpaired anterior sense organs, including a photosensitive system and a balance sensor, but no candidate homologue of the vertebrate olfactory system (Lacalli, 2001; Nieuwenhuys, 2002). Thus, the vertebrate olfactory system may not have arisen from a system shared with the common ancestor with urochordates.

Lancelets may or may not possess an olfactory system that is homologous with that of vertebrates. Lancelets respond to various classes of sensory stimuli, including chemical cues (Parker, 1908). They lack a discrete olfactory organ, but a class of potentially chemosensory cells that occurs in the rostral epithelium has been described (Lacalli and Hou, 1999). A GPCR gene that bears some sequence similarity to vertebrate olfactory receptor genes is expressed in bipolar rostral sensory cells in *Branchiostoma belcheri* (Satoh, 2005). Given that these cells bear axons, they appear to be one of the classes of type I sensory cells described by Lacalli and Hou (1999), who argued on morphological grounds that these cells are likely mechanosensory. In addition, the sequence was not subjected to rigorous phylogenetic analysis nor compared with those of many other GPCRs. Given that the use of GPCRs in chemosensory receptor cells appears to be a trait that has evolved several times independently (Eisthen, 2002), it is difficult to accept this observation as strong support for the presence of a vertebrate-like olfactory system in lancelets.

The lancelet *Branchiostoma floridae* possesses paired anterior nerves that, based on consideration of their external topography, have been suggested to be homologues of the vertebrate olfactory nerves (Lacalli, 2002). Although the author interprets the central target of these nerves as a possible homologue of the telencephalon, this region can also be interpreted as the equivalent of the vertebrate mesencephalon or rostral rhombencephalon; if so, these fibers are probably not homologues of the olfactory nerves (Northcutt, 2005). If Northcutt and Gans's 'new head' hypothesis is correct, then many rostral structures are evolutionarily new in craniates; among these structures are the ectodermal

placodes, including the nasal placode, and portions of the forebrain, including the olfactory system (Gans and Northcutt, 1983; Northcutt, 2005; Northcutt and Gans, 1983).

The condition in hagfishes should be instructive, as features shared by hagfish and vertebrates may have been present in earliest vertebrates. Hagfish are scavengers as well as opportunistic predators (Shelton, 1978). They respond vigorously to odorants from dead fish, and can use chemical cues to find carrion (Greene, 1925; Tamburri and Barry, 1999). In addition to their olfactory system, hagfish possess Schreiner organs, unusual chemosensory organs that are distributed across the body surface (Braun, 1995; see The Evolution of Taste Systems). The relative contributions of the olfactory and Schreiner organ system to behavior have not been determined, but odorants such as L-amino acids, GABA, and hydroxyproline evoke strong physiological responses from the olfactory epithelium (Døving and Holmberg, 1974).

Hagfish possess a single midline olfactory organ, with the sensory epithelium folded across a radial array of lamellae (e.g., Døving and Holmberg, 1974). The olfactory epithelium of the Atlantic hagfish *Myxine glutinosa* contains both ciliated and microvillar receptor neurons. Instead of the 9+2 microtubule arrangement that is typical of many cilia, including those on vertebrate olfactory receptor neurons, the cilia have a 9+0 arrangement (Theisen, 1973).

In *Myxine glutinosa* and *Eptatretus burgeri*, the olfactory bulb contains mitral, stellate, and granule cells, but the layers are indistinct, with somata of each cell type occurring in several different layers (Iwahori *et al.*, 1998; Jansen, 1930; Holmgren, 1919, in Nieuwenhuys, 1967). All three cell types have axons, and the dendrites of the mitral cells extend into several glomeruli. Periglomerular cells do not seem to be present, but all authors describe intraglomerular mitral cells with a single dendrite that arborizes in one glomerulus, and an axon that presumably exits the olfactory bulb (Holmgren, 1919; Iwahori *et al.*, 1998; Jansen, 1930).

Projections of the olfactory bulb have been investigated in the Pacific hagfish, *Eptatretus stouti* (Wicht and Northcutt, 1993). A short fiber tract projects from the medial olfactory bulb to the ipsilateral septum and through a dorsal commissure to the contralateral olfactory bulb. Fibers extend from the lateral olfactory bulb project bilaterally and widely in the forebrain, with targets that include the striatum, all layers of the pallium, and the central prosencephalic nucleus. An additional group of fibers extends from the ventrolateral portion of the olfactory bulb to terminate diffusely along a path extending into the diencephalon, including the hypothalamus and dorsal thalamus (Wicht and Northcutt, 1993). Given its location and targets, this tract may constitute the extrabulbar olfactory pathway. Immunocytochemical data suggest that hagfish lack a terminal nerve (Braun *et al.*, 1995; Crim *et al.*, 1979b; Jirikowski *et al.*, 1984; Wicht and Northcutt, 1992).

17.2.2 Lampreys

Lampreys have a biphasic lifecycle. The ammocoete larvae hatch in streams and rivers, then live buried in sediment for years, filter feeding, before metamorphosing and swimming downstream to live in larger bodies of water. Juvenile lampreys are generally parasitic, attaching to other fish to consume their blood and flesh. When sexually mature, adult lampreys stop feeding and migrate upstream in streams and rivers to spawn, after which they usually die.

In both *Ichthyomyzon fossor* and *Petromyzon marinus*, the complexity and relative size of the nasal cavity increases greatly during metamorphosis from ammocoete to the juvenile form, suggesting that olfaction may be important for free-swimming lampreys than for larvae (Leach, 1951; VanDenbossche *et al.*, 1995, 1997). Metamorphosed *P. marinus* increase activity in response to odorants from trout and fish-derived amines, but not amino acids (Kleerekoper and Mogensen, 1960).

Adult male *P. marinus* migrate and build nests in the spawning area before females arrive. The males emit a unique bile acid that is attractive to females, and to which females are highly sensitive (Li *et al.*, 2002; Siefkes and Li, 2004). Migratory lampreys do not home to particular streams; rather, they appear to simply seek suitable habitat for spawning (Bergstedt and Seelye, 1995). The means by which they do this is wonderful in its simplicity, for adult lampreys are attracted to odorants produced by healthy ammocoetes. Specifically, adults of several species have been shown to be extremely sensitive to bile acids produced by ammocoetes (Fine *et al.*, 2004; Li and Sorensen, 1997; Li *et al.*, 1995), as well as to two unique steroids that are released by ammocoetes (Sorensen *et al.*, 2005b). Adults are highly sensitive to these compounds, and both types of cues are released in sufficient quantities to attract them (Polkinghorne *et al.*, 2001; Sorensen *et al.*, 2005b). Interestingly, the bile acids are not released by nonfeeding ammocoetes, suggesting that these cues serve as a good indicator of habitat suitability (Polkinghorne *et al.*, 2001).

Lampreys possess a single midline nostril and nasal cavity that develops from a single nasal placode. Although earlier authors suggested that monorhiny is the ancestral condition for vertebrates, the presence of paired olfactory organs during development in *P. marinus* suggests that monorhiny may be a derived condition (Kleerekoper and Van Erkel, 1960). If so, it arose independently in hagfishes and lampreys. Within the nasal cavity, the olfactory epithelia of *Lampetra fluviatilis* and *P. marinus* contain only ciliated receptor cells, indicating that lampreys lack microvillar receptor neurons (Bronshtein and Ivanov, 1965; Thornhill, 1967; VanDenbossche *et al.*, 1995).

In contrast with the numerous and large families of odorant receptor genes described in other groups of vertebrates, lampreys (*L. fluviatilis*) appear to possess only a few small families of odorant receptors (Berghard and Dryer, 1998; Freitag *et al.*, 1999). Of course, it is difficult to rule out the possibility that lampreys possess many additional receptor genes that have not yet been identified. Although the sequences possess features that are characteristic of vertebrate odorant receptor genes, including a large third extracellular loop (Berghard and Dryer, 1998; Freitag *et al.*, 1999), phylogenetic analysis indicates that the lamprey odorant receptor gene family diverged before the origin of the two main classes of odorant receptor genes present in other vertebrates (Freitag *et al.*, 1999). The odorant receptor genes from lampreys also share strong similarity with histamine receptors, indicating that the odorant receptor genes in lampreys and other vertebrates may have been independently co-opted out of the larger GPCR superfamily (Berghard and Dryer, 1998; Niimura and Nei, 2005). The genes are expressed in the olfactory epithelium of both ammocoetes and adults, suggesting that the quantitative differences in the size of the olfactory system in the two forms do not necessarily indicate qualitative differences in the odorants detected (Berghard and Dryer, 1998).

In *Petromyzon* larvae, the olfactory bulb glomeruli are histochemically heterogeneous and form six distinct territories, suggesting a rough functional specialization (Frontini *et al.*, 2003). Whether this organization is particular to ammocoetes or remains throughout the lifecycle is not known. A single layer of glomeruli encircles the bulb (Heier, 1948; Iwahori *et al.*, 1987). As in hagfish, the layers are indistinct, and the somata of some mitral cells can be found in the glomerular layer. The olfactory bulb of *L. fluviatilis* contains mitral, stellate, and granule cells, all of which have axons that exit the olfactory bulb (Heier, 1948; Nieuwenhuys, 1967). Two classes of mitral cells appear to be present: those with cell bodies in the mitral cell layer, which extend a single dendrite to arborize in one glomerulus; and those with cell bodies in the glomerular layer, which have dendrites that arborize in more than one glomerulus (Heier, 1948; Iwahori *et al.*, 1987; Nieuwenhuys, 1967). The latter class of cells may function more like periglomerular cells than like mitral cells. A more recent description of the olfactory bulb of *L. japonica* suggests that either granule or stellate cells are absent in this species (Iwahori *et al.*, 1987). The cells that Iwahori *et al.* call 'granule' approximate our description of stellate cells, above. These cells lack axons, but possess several dendrites that arborize in glomeruli, often spanning the width of the bulb (Iwahori *et al.*, 1987). It is difficult to reconcile these different descriptions of bulbar organization, particularly for two species of *Lampetra*, but perhaps the discrepancies are due to developmental or methodological differences.

The olfactory bulb gives rise to four major centripetal pathways in silver lampreys, *Ichthyomyzon unicuspis*, illustrated in Figure 4 (Northcutt and Puzdrowski, 1988). A group of fibers that may comprise a homologue of the medial olfactory tract originates from the ventrolateral olfactory bulb and projects to the ipsilateral septum, preoptic area, and possibly to the rostral portion of the striatum (Figure 4a). A lateral olfactory tract projects ipsilaterally throughout the lateral pallium and either to or through the dorsal and medial pallia, as well as to the posterior tuberculum and hypothalamus; some fibers project contralaterally to a dorsal portion of the lateral pallium, septum, striatum, and the posterior tuberculum and hypothalamus (Figure 4b). A third group of fibers extends through the ventromedial olfactory bulb to the striatum and preoptic regions, as well as to the hypothalamus and throughout the posterior tuberculum (Figure 4c; Northcutt and Puzdrowski, 1988). This tract has been interpreted as an extrabulbar olfactory pathway (Eisthen and Northcutt, 1996). Finally, silver lampreys possess an olfactory pathway through a dorsal commissure to the adjacent dorsomedial neuropil, a fibrous region that spans the length of the olfactory bulb, and to glomeruli in the contralateral olfactory bulb (Figure 4d; Northcutt and Puzdrowski, 1988). Fibers projecting to the contralateral olfactory bulb have also been described in *L. fluviatilis* (Heier, 1948) and in hagfish (Wicht and Northcutt, 1992), suggesting that a dorsal commissure carrying secondary olfactory fibers to the contralateral olfactory bulb may have been present in the earliest vertebrates.

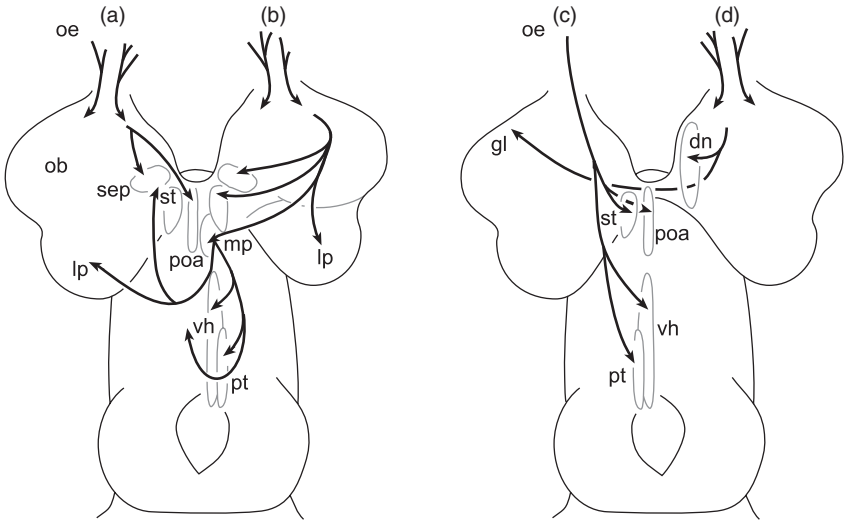

Figure 4 Schematic dorsal view of the forebrain in the silver lamprey (*Ichthyomyzon unicuspis*), illustrating the major olfactory projections. a, Projections of the medial olfactory tract. b, Projections of the lateral olfactory tract. c, Projections of the extrabulbar olfactory pathway. d, Projections through the dorsal commissure to the contralateral olfactory bulb. dn, dorsomedial neuropil; gl, glomerular layer; lp, lateral pallium; mp, medial pallium; ob, olfactory bulb; oe, olfactory epithelium; poa, preoptic area; pt, posterior tubercle; sep, septum; st, striatum; vh, ventral hypothalamus. Based on Northcutt, R. G. and Puzdrowski, R. L. 1988. Projections of the olfactory bulb and nervus terminalis in the silver lamprey. *Brain Behav. Evol.* 32, 96 107.

Heier (1948) first suggested that lampreys lack a terminal nerve, and since that time its presence in lampreys has been questionable. In other vertebrates, the terminal nerve can be labeled with antisera directed against GnRH, NPY, or FMRFamide (Wirsig-Wiechmann *et al.*, 2002a). These antisera fail to label any structures reminiscent of the terminal nerve in larval or adult *I. unicuspis*, *Lampetra japonica*, *L. planeri*, *L. richardsoni*, *Lethenteron japonica*, or *P. marinus* (Chiba, 1999; Crim *et al.*, 1979b, 1979a; Eisthen and Northcutt, 1996; King *et al.*, 1988; Meyer *et al.*, 1987; Ohtomi *et al.*, 1989; Tobet *et al.*, 1995; Wright *et al.*, 1994). Fibers that project from the nasal sac through olfactory bulb to the ventral forebrain have been labeled with injections of horseradish peroxidase or cobalt lysine into the nasal sac of larval or adult lampreys (*L. planeri*, Meyer *et al.*, 1987; *I. unicuspis*, Northcutt and Puzdrowski, 1988; von Bartheld *et al.*, 1987; von Bartheld and Meyer, 1988). Although this projection was originally interpreted as a terminal nerve, it may instead be the extrabulbar olfactory pathway, which was unknown at the time these studies were conducted (Eisthen and Northcutt, 1996).

17.2.3 Cartilaginous Fishes: Sharks, Skates and Rays, and Chimaeras

The class chondrichthyes consists of cartilaginous fishes (sharks, ratfish or chimaeras, and skates and rays) that are widely distributed in the world's oceans. Some species enter freshwater, and a few, like the rays *Paratrygon motoro* and *Himantura signifer*, live exclusively in freshwater (Compagno and Roberts, 1982; Müller and Henle, 1841).

Olfaction plays a major role in the life of cartilaginous fishes. Their olfactory ability is legendary, and olfaction is important for prey detection (Kleerekoper, 1978; Sheldon, 1911). Nevertheless, the popular notion that sharks can detect even a small amount of blood diluted in the ocean over many miles is an exaggeration. Electrophysiological experiments demonstrate that sharks are able to detect components of human and bovine blood, as well as other stimuli like amino acids and crab or squid extract, but responses to blood are no stronger than the responses to other stimuli (Hodgson and Mathewson, 1978; Kajiura *et al.*, 2004b; Silver, 1979; Zeiske *et al.*, 1986).

The role of olfaction in prey localization in sharks has been the subject of much study. Some species approach prey from downstream by swimming into the current (rheotaxis). Tests in the open sea indicate that lemon sharks (*Negaprion brevirostris*) use rheotaxis to find odorants from prey, but will continue to swim against the current past the stimulus source, suggesting that additional sensory cues are involved in short-range localization (Hodgson and Mathewson, 1971). Other species use a strategy called klinotaxis in which they turn in the direction of the nostril that is more strongly stimulated. Early

naris-occlusion experiments with smooth dogfish (*Mustelus canis*) demonstrated that the animals turn persistently toward the open nostril when activated by an olfactory stimulus (Sheldon, 1911). Subsequent experiments with other sharks and rays indicate that klinotaxis is common in cartilaginous fishes (reviewed in Kleerekoper, 1978).

Prey localization through klinotaxis has been suggested to constitute an important pressure driving evolution of increasing head width in hammerhead sharks (Sphyrnidae). Recent work by Kajiura *et al.* (2004a) demonstrates that the ability to localize odorants increases with increasing head width in sphyrnid sharks, although their olfactory sensitivity does not differ significantly from that of other families of sharks (Kajiura *et al.*, 2004b). In addition to its role in foraging, olfaction has also been implicated in reproductive behavior in sharks (e.g., Bleckmann and Hofmann, 1999; De Martini, 1978; Forlano *et al.*, 2000). Nevertheless, this area of research remains largely unexplored.

The olfactory organ of cartilaginous fishes consists of a chamber inside a cartilaginous capsule. Incurrent and excurrent nostrils on the ventral snout allow water to flow over the olfactory mucosa. The anterior margin of the nasal flap is sufficiently diverse that it was used in species identification until researchers learned that within-species variability is also high (Tester, 1963). In chimaeras, the two olfactory chambers are adjacent, with a cartilaginous septum that separates them down the midline. The incurrent nostrils are located medially above the mouth, and the excurrent nostrils open laterally near the edge of the mouth (Zeiske *et al.*, 1992). In pelagic sharks, water is driven over the olfactory epithelium by swimming movements, as illustrated in Figure 5. In sedentary animals, flow is driven by respiratory movements. Some species, such as spiny dogfish (*Squalus acanthias*) and the small-spotted catshark (*Scyliorhinus canicula*), use specialized valve mechanisms to regulate flow over the olfactory epithelium (Theisen *et al.*, 1986).

Inside the nasal cavity, the olfactory organ consists of a rosette composed of two rows of olfactory lamellae situated on each side of a transverse raphe. In some species, such as Oman sharks (*Iago omanensis*), secondary lamellae greatly increase the surface area of the sensory epithelium (Fishelson and Baranes, 1997). In addition to the usual receptor neurons, supporting cells, and basal cells present in all vertebrates, the olfactory epithelium of some cartilaginous fishes also contains goblet cells (Zeiske *et al.*, 1986). In sharks, the ancestral condition appears to be the presence of only microvillar olfactory receptor neurons (Reese and Brightman, 1970; Theisen *et al.*, 1986; Zeiske *et al.*, 1986, 1987). The spiny dogfish *Squalus acanthias* was originally reported to possess both ciliated and microvillar receptor cells (Bakhtin, 1976, 1977), but a later study concluded that only microvillar receptor neurons are present (Theisen *et al.*, 1986). Similarly, the olfactory epithelium of the ratfish *Chimaera monstrosa* and those of skates and rays contain only microvillar receptor cells (Holl, 1973; Meng and Yin, 1981). Nevertheless, one study with Oman sharks demonstrates the presence of unusual olfactory receptor neurons that resemble the crypt-type olfactory receptor neurons present in ray-finned fishes (Fishelson and Baranes, 1997; see discussion in Hansen and Finger, 2000).

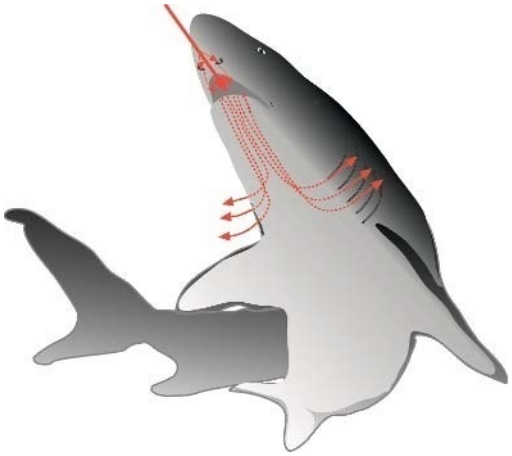

Figure 5 Schematic ventral view of a shark, showing the characteristic nostrils with prominent nasal flap, and the direction of water flow (arrows).

In cartilaginous fishes, the olfactory bulb is adjacent to the olfactory sac, and is connected by short olfactory nerves; thus, the tracts are much longer than the nerves. The length of these tracts varies across species, and in all cases they are formed by mitral cell axons with a characteristic black myelin sheath (Dryer and Graziadei, 1996). The olfactory bulb is somewhat laminated, but no plexiform layers separate the cell layers (Dryer and Graziadei, 1993; Franceschini and Ciani, 1993; Nieuwenhuys, 1967). Periglomerular cells appear to be lacking. Sterzi (1909, in Nieuwenhuys, 1967) described the olfactory bulb of spiny dogfish (*Squalus acanthias*) as containing a clear layer of mitral cells with primary dendrites that arborize in multiple glomeruli as well as secondary dendrites that interact with fibers of other cells outside the glomeruli. Granule cells have both axons and smooth dendrites, and stellate cells also appear to be present; Sterzi referred to the latter as 'triangular cells'. In contrast, a more recent study of sharks

(*Sphyrna tiburo* and *Rhizoprionodon terranovae*) and rays (*Dasyatis sabina*) found two types of mitral cells present, one with a loose dendritic arborization and the other with a more dense one (Dryer and Graziadei, 1993, 1994, 1996). The dendrites of both types arborize in a single glomerulus. Given the irregular laminar borders described by Dryer and Graziadei, it seems possible that their mitral cells with loose dendritic arbors are actually stellate cells.

The central projections of the olfactory bulb have not been examined in detail in cartilaginous fishes. The lateral olfactory tract in sharks and rays projects ipsilaterally to the area retrobulbaris, striatum, lateral pallium, and area superficialis basalis (Ebbesson and Heimer, 1970; Smeets, 1983). The existence of a medial olfactory tract is uncertain, and an earlier description of a widely projecting pathway (Smeets, 1983) has been reinterpreted as an artifact of the degeneration technique used (Northcutt, 1995). An extrabulbar olfactory pathway has not been described in this group (Hofmann and Meyer, 1995).

The terminal nerve was originally discovered in a small shark (*Mustelus asterias*) by Fritsch (1878), who referred to it as a 'supernumerary nerve'. Locy (1905) later described this structure in a variety of sharks and rays, and named it the *nervus terminalis* because the nerve enters the brain through the lamina terminalis. The exact point of entry in the brain varies somewhat among species; for example, in *Squalus acanthias* the terminal nerve enters the telencephalon dorsally (Locy, 1905). In most cartilaginous fishes, the terminal nerve is completely separate from the olfactory nerve, and includes a visible ganglion located medial and external to the nasal cavity. In some species, additional ganglia occur along the nerve (Locy, 1905).

Electron microscopic examination of the terminal nerve in sharks and rays demonstrates that the cells are heterogeneous. Most fibers are unmyelinated, but a few fibers in the proximal part of the nerve are myelinated (Demski and Schwanzel-Fukuda, 1987; White and Meredith, 1987). The ganglion consists largely of unipolar cells, with a few bipolar and multipolar cells present (White and Meredith, 1995; Wu *et al.*, 1992). Cells and fibers of the terminal nerve display GnRH-like immunoreactivity in many species of sharks and rays (Chiba, 2000; Chiba *et al.*, 1991; Demski and Schwanzel-Fukuda, 1987; Stell, 1984; White and Meredith, 1995). Data from two species of sharks (*Sphyrna tiburo* and *Scyliorhinus torazame*) suggest that in cartilaginous fishes, separate populations of terminal nerve neurons contain GnRH and FMRFamide-like compounds (Chiba, 2000; White and Meredith, 1995). Additional FMRFamide immunoreactive fibers have been described within the olfactory nerve in sharks and rays (Wu *et al.*, 1992), but it is not yet clear whether these fibers constitute part of the terminal nerve pathway.

Electrical stimulation of the terminal nerve ganglion in Atlantic stingrays (*Dasyatis sabina*) leads to an increase in GnRH levels in the brain (Moeller and Meredith, 1998), but electrical stimulation of the terminal nerve does not significantly alter activity in the olfactory bulb (Meredith and White, 1987). Recordings from the terminal nerve in sharks and rays have failed to detect sensory activity, although efferent activity from the brain has been recorded (Bullock and Northcutt, 1984; Demski and Schwanzel-Fukuda, 1987; White and Meredith, 1995). Further, activity of terminal nerve ganglion cells can be modulated by application of acetylcholine or norepinephrine, suggesting that centrifugal fibers may regulate activity of these cells (White and Meredith, 1993, 1995). Overall, the available data indicate that the terminal nerve in cartilaginous fishes is not sensory, although it has not yet been demonstrated to serve a modulatory function.

17.2.4 Ray-Finned Fishes

Actinopterygii, the ray-finned fishes, is the largest class of vertebrates, comprising nearly 25 000 species. One division of ray-finned fishes, the teleost fishes, contains over 23 000 species (Nelson, 1994). In addition to teleosts, the class Actinopterygii contains paddlefish and sturgeons, gars, bowfins, and bichirs or reed fishes. Ray-finned fishes occupy an enormous diversity of aquatic ecological niches and possess many specialized adaptations. We will describe here the main features of the olfactory system that are shared by the species that have been examined to date.

Olfactory cues are critical for foraging in some species of ray-finned fishes (Atema, 1980; Bateson, 1890), and electrophysiological recordings from goldfish and carp (*Carassius*) reveal a high sensitivity to food-related odorants, with thresholds for amino acids generally in the range of 10^{-6}–10^{-9} M (Goh and Tamura, 1978; Zippel *et al.*, 1993). This sensitivity is greater than that typically measured in other aquatic vertebrates (Hamdani *et al.*, 2001). In addition to locating food by following trails of extremely dilute odorants, many salmonid species return to their natal streams to spawn after spending years developing in oceans, homing in part based on olfactory cues. In anadromous species, the developing fish undergo behavioral, anatomical, and physiological changes necessary to survive the marine environment as they migrate downstream.

During this period, olfactory sensitivity increases, allowing the animals to imprint on odorants that will guide their homing behavior when they are reproductively mature (reviewed in Nevitt and Dittman, 2004).

The complex nature of pheromonal signaling during courtship is understood better in goldfish (*Carassius auratus*) than in any other vertebrate. Vitellogenic females release 17β-estradiol, which attracts males (Kobayashi *et al.*, 2002). As the female approaches ovulation, it begins to release sex steroids. During the 12 h period leading up to ovulation, the ratio of the three released steroids changes, allowing males to assess the female's reproductive state with great temporal precision (Scott and Sorensen, 1994). The steroid that dominates the mixture early in this process inhibits male responses to one of the steroids that dominates later, perhaps as a mechanism to prevent inappropriate responses to a signal that is present in small quantities throughout the ovulatory period (Stacey and Sorensen, 2002). At ovulation, the female begins to release prostaglandins, attracting males, which are extremely sensitive to this mixture (Sorensen *et al.*, 1988). Males also release a pheromone that attracts females, which has been suggested to function in sex recognition during competition for mates (Sorensen *et al.*, 2005a).

The mechanisms underlying olfactory transduction of prostaglandins and sex steroids are substantially similar in goldfish, and differ from those used to transduce amino acid odorants (Sorensen and Sato, 2005). In zebra fish (*Danio rerio*), pheromonal cues stimulate a different region of the olfactory bulb than do other odorants (Friedrich and Korsching, 1998), and processing of these two types of information may be segregated in goldfish as well (Hanson *et al.*, 1998). In addition, in goldfish and its congener the Crucian carp (*C. carassius*), the lateral olfactory tract carries information related to foraging behavior, whereas the medial tract is involved in pheromonal mediation of courtship and spawning behavior (Dulka, 1993; Hamdani *et al.*, 2001; Kyle *et al.*, 1987; Sorensen *et al.*, 1991).

Many teleost pheromones also function as hormones, or are metabolites of hormones, and may be passively released through diffusion from the blood in gills during respiration (e.g., Vermeirssen and Scott, 1996). In contrast to the common conception of pheromones as cues specifically produced as communication signals, such observations have led to the hypothesis that pheromonal communication in fishes originally evolved as a mechanism by which animals could assess the reproductive status of conspecifics through detection of cues that the releaser cannot control (e.g., Sorensen and Scott, 1994). In addition to sex pheromones, teleost fishes appear to produce alarm pheromones, which also raise interesting evolutionary issues. The phenomenon of alarm cues was first described by von Frisch (1938, 1941), who noted that a minnow, *Phoxinus phoxinus*, releases a chemical ('Schreckstoff') when injured; the release of the chemical results in a behavioral alarm response by conspecifics. This phenomenon has since been documented in many teleost species, particularly in Ostariophysi (reviewed in Døving *et al.,* 2005). Nevertheless, because its adaptive value is difficult to understand, some have questioned the very existence of the phenomenon (e.g., Magurran *et al.,* 1996). The problem is that production of alarm substances is energetically costly (Wisenden, 2000), but of questionable benefit to the animal that produces them while being consumed by a predator, even if kin are nearby (Williams, 1992). A resolution to this apparent paradox may be that release of alarm substances may attract secondary predators that will chase or consume the initial predator, allowing the injured individual to escape (Mathis *et al.*, 1995). This hypothesis has received some empirical support (Chivers *et al.*, 1996a; but see Cashner, 2004).

An interesting adaptation in some teleosts is the use of the olfactory system for detecting changes in external salinity; for example, Atlantic salmon (*Salmo salar*) have polyvalent cation-sensing receptors in the olfactory epithelium (Nearing *et al.*, 2002). Other species can compensate for changes in salinity. In sea bream (*Sparus auratus*), a euryhaline marine species, olfactory receptor neurons increase their firing rate to compensate for reduction in extracellular calcium levels (Hubbard *et al.*, 2000). Olfactory responses to odorants are similar in freshwater and seawater in rainbow trout (*Oncorhynchus mykiss*), suggesting that the compensatory mechanism resides within the olfactory epithelium (Shoji *et al.*, 1996).

Ray-finned fishes usually have two pairs of nostrils on the dorsal surface of the snout, with the anterior and posterior nares separated by a strip of skin. Water flows into the anterior (incurrent) nares, passes over the olfactory epithelium in the nasal sac, and then exits through the posterior (excurrent) nares. Water flow can be generated by swimming movements. In teleosts with an accessory nasal sac, opercular movements during respiration alternately compress and expand the accessory nasal sacs, pumping water through the nasal sac (Døving *et al.*, 1977; Nevitt, 1991).

As in other fishes, the olfactory epithelium in ray-finned fishes is organized into a rosette of lamellae

radiating outward from a central raphe. Both the shape and complexity of lamellar organization differs among species (Yamamoto, 1982; Zeiske *et al.*, 1992). The number of lamellae is also variable, increasing during development to a species-typical level, such as the 168 lamellae present in adult undulated moray eels (*Gymnothorax undulatus*) or the 230 lamellae in adult barred snappers (*Hoplopagrus guenteri*) (Fishelson, 1995; Pfeiffer, 1964). Some groups, such as salmonids, have secondary lamellae that greatly increase the size of the epithelial surface (Yamamoto and Ueda, 1977).

As illustrated in Figures 6a and 6c, the olfactory epithelium of teleost fishes contains both ciliated and microvillar receptor cells (extensively reviewed in Eisthen, 1992). Within these two broad categories, many subtypes can be distinguished; these subtypes differ morphologically and project to distinct regions of the olfactory bulb (Morita and Finger, 1998).

Ray-finned fish are the only group of vertebrates in which the olfactory epithelium clearly contains receptor neurons that do not fit into the preceding categories. Hansen *et al.* have described 'crypt' receptor neurons with a dendritic surface that bears apical microvilli as well as a large concave section that is filled with cilia (Figure 6b; Hansen and Zeiske, 1998). Another unusual feature of crypt cells is that they express two types of G-proteins (Hansen *et al.*, 2004). A recent study of the electrophysiological properties of crypt cells in the Pacific jack mackerel (*Trachurus symmetricus*) found that, among the relatively small number of neurons examined, some responded to amino acid odorants, but that none responded to polyamines or bile acids (Schmachtenberg, 2006). The axons of crypt cells terminate in the olfactory bulb, indicating that they are true olfactory receptor neurons (Hansen *et al.*, 2003). Crypt cells are present in the olfactory epithelium of many, but not all, species of teleosts that have been examined to date, and are present in a polypteriform fish, *Polypterus senegalus*, but not in shortnose gars, *Lepisosteus plastostomus* (Hansen and Finger, 2000). Like teleosts, sturgeons, and paddlefish possess ciliated, microvillar, and crypt olfactory receptor neuron (Bakhtin, 1976; Hansen and Finger, 2000; Pyatkina, 1976; Zeiske *et al.*, 2003). Overall, these data suggest that ciliated, microvillar, and crypt-type olfactory receptor neurons are broadly present in ray-finned fishes, but that the distribution of each cell type is somewhat variable.

In addition to these receptor cell types, both 'rod' and 'rodlet' cells have been proposed to function as olfactory receptor neurons in teleosts. Instead, the former are probably unhealthy or degenerating cells (Muller and Marc, 1984; Zeiske and Hansen, 2005), and the latter may be migrating secretory cells, a type of white blood cell, or even parasites (Bielek, 2005).

Both olfactory-type and vomeronasal-type odorant receptor genes are expressed in the olfactory epithelium of teleosts, although the size of the gene families appears to be smaller than in mammals. In channel catfish (*Ictalurus punctatus*) fewer than 100 members of the olfactory receptor gene family have been found (Ngai *et al.*, 1993a, 1993b). In zebra fish, the entire odorant receptor gene repertoire appears to consist of 143 genes, with even smaller numbers found in pufferfish (44 genes in *Takifugu rubripes* and 42 in *Tetraodon nigroviridis*) (Alioto and Ngai, 2005). Members of the V2R gene family have been sequenced from both pufferfish (*Takifugu*

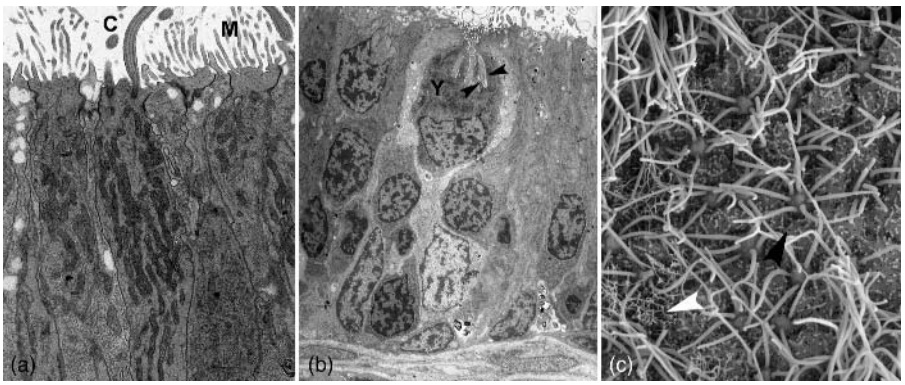

Figure 6 Electron micrographs of the three classes of olfactory receptor neurons in ray-finned fishes. a, Transmission electron micrograph of dendrites of ciliated (C) and microvillar (M) olfactory receptor neurons in goldfish, *Carassius auratus*. b, Transmission electron micrograph of a crypt cell (Y) in zebra fish, *Danio rerio*. Crypt cells are characterized by cilia sunken within the cell (arrowheads), as well as microvilli on the surface. c, Scanning electron micrograph of the dendritic surface of ciliated (black arrowhead) and microvillar (white arrowhead) olfactory receptor neurons in a fathead minnow (*Pimephales promela*). Micrographs provided courtesy of Dr. Anne Hansen.

rubripes) and goldfish (Cao *et al.*, 1998; Naito *et al.*, 1998). A single member of the V1R gene family has been sequenced in zebra fish and found to be expressed in the olfactory epithelium, and V1R-like genes with high sequence variability are also present in the genomes of several other teleost species (*Oryzias latipes*, *Danio malabaricus*, *Takifugu rubripes*, and *Tetraodon nigroviridis*) (Pfister and Rodriguez, 2005). In goldfish, the olfactory sensory neurons that bear cilia express olfactory receptor genes and $G\alpha_{olf}$, and those with microvilli express V2R genes and $G\alpha_o$, although some are immunoreactive for $G\alpha_{i\text{-}3}$ or $G\alpha_q$ instead (Hansen *et al.*, 2004). V2Rs are also expressed in crypt cells (Hansen *et al.*, 2004). In zebra fish, ciliated olfactory receptor neurons express the olfactory-type receptor genes also express olfactory marker protein and the cyclic nucleotide-gated channel A2 subunit that is typical of mammalian olfactory receptor neurons; microvillar neurons express V2R-type receptors and the TRPC2 channel that are typical of mammalian vomeronasal receptor neurons (Sato *et al.*, 2005). Taken together, these data suggest that a vomeronasal-type system is present in teleosts, but that the receptor neurons are mixed together with olfactory receptor neurons in the epithelium (discussed in Alioto and Ngai, 2005; Eisthen, 2004).

The organization of the olfactory bulb is moderately laminar. As in other fishes, distinct plexiform layers are lacking, and the cell bodies are somewhat intermingled across layers (Nieuwenhuys, 1967). Periglomerular cells may be lacking. Each mitral cell has from one to five primary dendrites, and each dendrite ends in one or more glomeruli (Nieuwenhuys, 1967). Another class of output cell is called the 'ruffed' cell, due the presence of a ruff of processes at the base of the axon (Kosaka and Hama, 1979a, 1979b). This type of cell may be broadly present in teleosts, but has not been described in other classes of vertebrates (Alonso *et al.*, 1987; Fuller and Byrd, 2005; Kosaka and Hama, 1980). In his study of the olfactory bulb of teleosts, Catois (1902) referred to an additional class of large output cells with an elongated, horizontally oriented cell body as 'fusiform' cells. Morphologically similar cells are apparent in illustrations of the olfactory bulb of sturgeons (Johnston, 1898), and may be present in other ray-finned fishes as well. Granule cells and stellate cells appear to be present in both teleosts and sturgeons (Johnston, 1898; Nieuwenhuys, 1967).

The forebrain of ray-finned fish is everted and contains many discrete cell groups, the homologies of which are difficult to establish. Because connectivity is often used by comparative neuroanatomists as a key criterion for homology, any attempt to compare the similarity of olfactory projection patterns between teleosts and other vertebrates can become circular; therefore, independent evidence, such as histochemical data, is necessary to corroborate hypotheses of homology. The central projections of the olfactory bulb have been examined in some detail in goldfish, *Carassius auratus* (Levine and Dethier, 1985; Northcutt, 2006; von Bartheld *et al.*, 1984). As depicted in Figure 7a, the medial olfactory tract projects bilaterally to the ventral forebrain areas Vs, Vl, and Vv, which may be equivalent to part of the septum (Northcutt and Braford, 1980); to Vd, which may be the equivalent of part of the striatum (Northcutt and Braford, 1980); to Dm, which may be the equivalent of the amygdala (Northcutt, 2006); and to the preoptic area, with some fibers terminating as far caudally as the hypothalamus. The lateral olfactory tract of goldfish projects mainly to dorsolateral pallial areas, as well as projecting bilaterally to the hypothalamic region and nucleus tuberis (Figure 7b). One of the targets of the lateral olfactory tract is Dl, which may be the equivalent of the medial pallium/hippocampus (Northcutt, 2006). Topographically similar projections have been described in a salmonid, *Oncorhyncus mykiss* (Folgueira *et al.*, 2004). The presence of an extra-bulbar olfactory pathway is well-established in teleosts (Anadón *et al.*, 1995; Bazer *et al.*, 1987; Hofmann and Meyer, 1995; Szabo *et al.*, 1991) as well as in *Amia*, sturgeons, and polypteriform species (Hofmann and Meyer, 1995; Huesa *et al.*, 2000). The details of the targets differ among species, but the fibers generally project to ventral forebrain areas (Figure 7c).

Among nonteleost ray-finned fishes, the olfactory projections have been most carefully examined in the bichir, *Polypterus palmas* (Braford and Northcutt, 1974; von Bartheld and Meyer, 1986). In *Polypterus*, a lateral olfactory tract projects largely to the pallial area P3 and may contain some contralaterally projecting fibers, and a medial tract projects ipsilaterally to the pallial areas P1 and bilaterally to ventral telencephalic regions, with some fibers extending as far caudal as the hypothalamus. Similar projections have been described in the sturgeon *Acipenser baeri* (Huesa *et al.*, 2000). Based on its position and its massive input from the olfactory bulb, P1 is generally considered to be the homologue of the lateral pallium (Northcutt and Davis, 1983; von Bartheld and Meyer, 1986), and topological considerations have led to the suggestion that P3 is the homologue of the medial pallium / hippocampus (Braford, 1995; Northcutt and Davis, 1983; von Bartheld and Meyer, 1986). If these interpretations

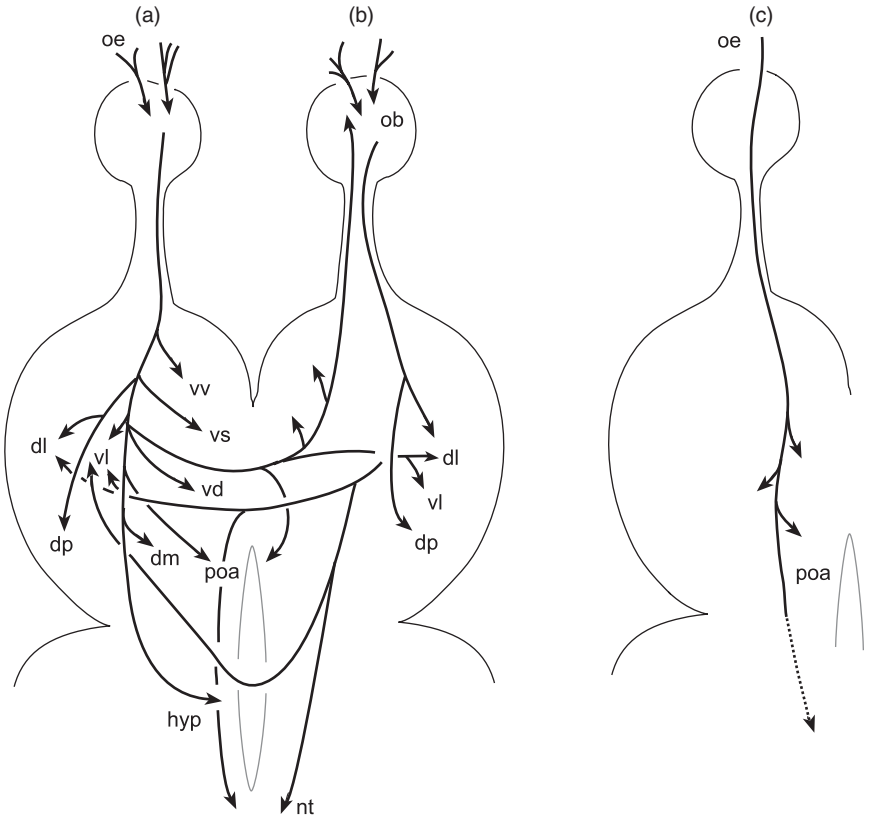

Figure 7 Schematic dorsal view of the forebrain in the goldfish, *Carassius auratus*. a, Projections of the medial olfactory tract. b, Projections of the lateral olfactory tract. c, Projections of the extrabulbar olfactory pathway. dl, lateral division of dorsal telencephalic area; dm, medial division of dorsal telencephalic area; dp, posterior division of dorsal telencephalic area; hyp, hypothalamus; nt, nucleus tuberis; ob, olfactory bulb; oe, olfactory epithelium; poa, preoptic area; vd, dorsal nucleus of the ventral telencephalic area; vl, lateral nucleus of the ventral telencephalic area; vs, supracommissural nucleus of the ventral telencephalic area; vv, ventral nucleus of the ventral telencephalic area. Based on Dulka (1993), Levine and Dethier (1985), Northcutt (2006), von Bartheld *et al.* (1984), and Polese (2004).

are correct, then the lateral olfactory tract of the bichir *Polypterus palmas* projects to the homologue of the medial pallium/hippocampus and the medial tract projects to the homologue of the lateral pallium, which is the reverse of the general vertebrate pattern. Perhaps the apparent medial and lateral tracts have been reversed in *Polypterus*, in conjunction with eversion of the telencephalon. If this were the case, then we would expect that the tracts in other ray-finned fish, such as goldfish, would be similarly reversed. Unfortunately, the data from goldfish are ambiguous: the lateral olfactory tract projects to a lateral pallial area, as is typical of the lateral tract, but also to a region that may be the equivalent of the septum, as is typical of the medial olfactory tract in tetrapods. Further, the medial olfactory tract of goldfish projects to ventral areas that are suspected homologues of portions of the septum and medial pallium, which is typical of the medial tract of other vertebrates, but also to a potential homologue of the amygdala, as it is typical of the lateral olfactory tract of other vertebrates. Thus, the relationships between the medial and lateral olfactory tracts in ray-finned fishes and other vertebrates are unresolved, and may bear no one-to-one correspondence with those in other vertebrates.

Unlike in cartilaginous fishes, the terminal nerve in ray-finned fishes projects alongside the primary olfactory fibers in the olfactory nerve, and is not visible externally. The ganglion cells of the terminal nerve develop from the nasal placode (Parhar, 2002; Whitlock and Westerfield, 2000). In most teleosts the terminal nerve ganglion consists of a cluster of cells located between the olfactory bulb and the ventral telencephalon, with fibers that extend centrally to the ventral forebrain and peripherally to both the olfactory epithelium and retina (Brookover and Jackson, 1911; Kim *et al.*, 1995; Oka *et al.*, 1986; Parhar *et al.*, 1996; Rossi *et al.*, 1972; Yamamoto *et al.*, 1995). Because of this association with both the olfactory system and retina, the terminal nerve ganglion in teleosts is also

frequently called the nucleus olfacto-retinalis (Münz *et al.*, 1981). The exact location and the morphology of the ganglion cells varies somewhat among species (reviewed in Münz and Claas, 1987). In some teleosts, such as the dwarf gourami (*Colisa lalia*), the fibers of the terminal nerve project to many disparate regions of the brain (Oka, 1992; Oka and Matsushima, 1993).

The teleost terminal nerve contains at least one form of GnRH (Yamamoto *et al.*, 1995), and many of the cells and fibers of the terminal nerve are GnRH-immunoreactive (e.g., Oka and Ichikawa, 1990; Schreibman *et al.*, 1979). Some of these cells also display immunoreactivity to NPY or to FMRFamide (Ekström *et al.*, 1988; Mathieu *et al.*, 2002; Östholm *et al.*, 1990; Pinelli *et al.*, 2000; Rama Krishna and Subhedar, 1992; Walker and Stell, 1986). The terminal nerve displays similar immunoreactivity in polypteriform fishes (*Polypterus palmas*, *P. senegalus*, and *Calamoichthys calabaricus*), sturgeons (*Acipenser ruthenus*), and gars (*Lepisosteus oculatus*) (Chiba, 1997, 2005; Pinelli *et al.*, 2000; Wright and Demski, 1996). Curiously, GnRH immunoreactivity has also been described in primary olfactory receptor neurons in the carp *Cirrhinus mrigala*; expression is seasonal, and occurs only in adult females (Biju *et al.*, 2003, 2005). Perhaps these cells are evolutionarily derived from terminal nerve cells that did not migrate properly during development, and differentiated into olfactory receptor neurons under the influence of local cues. Nevertheless, given that the neurons involved are not ganglion cells, they do not appear to be part of the terminal nerve system.

The function of the terminal nerve has been the subject of much study in teleosts, particularly in the dwarf gourami, *Colisa lalia*. Electrophysiological experiments demonstrate that the majority of the ganglion cells fire spontaneous action potentials brought about by the interaction of a tetrodotoxin-resistant, persistent sodium current that depolarizes the cell and a persistent potassium current that repolarizes the cell (Abe and Oka, 1999; Oka, 1992, 1996). The firing frequency of the cells is modulated by the same form of GnRH that is present in the nerve, causing an initial decrease in firing rate, followed by a later increase (Abe and Oka, 2000, 2002). This modulation of firing rate by GnRH may function to synchronize firing (Abe and Oka, 2000). Studies such as these were the first to suggest that the terminal nerve may function as a neuromodulatory system, rather than as a sensory system, as originally thought. Additional evidence for this hypothesis comes from studies showing that exposure to odorants does not alter the firing rate of terminal nerve ganglion cells (Fujita *et al.*, 1991), and that lesions of the terminal nerve impair initiation of nest-building behavior, but do not abolish reproduction, in dwarf gouramis (Yamamoto *et al.*, 1997). In addition, compounds present in the terminal nerve alter the activity of retinal ganglion cells, suggesting that the retinopetal branch of the terminal nerve in teleost fishes is neuromodulatory (Huang *et al.*, 2005; Maaswinkel and Li, 2003; Walker and Stell, 1986). Interestingly, a tract-tracing study with dwarf gouramis and tilapia (*Oreochromis niloticus*) indicates that the terminal nerve receives input from olfactory areas in the forebrain, as well as from the nucleus tegmento-olfactorius, a midbrain region that receives input from the reticular formation as well as areas involved in visual and somatosensory processing (Yamamoto and Ito, 2000). Taken together, the results of these studies suggest that the teleost terminal nerve functions to modulate activity in the olfactory epithelium and retina, in part in response to visual and olfactory input.

17.2.5 Lobe-Finned Fishes: Lungfishes and Coelacanths

Three genera of lungfishes and one of coelacanths are alive today. Although they are dispersed around the globe, each species lives in a relatively restricted area. Lungfishes live in freshwater in Africa (*Protopterus*), South America (*Lepidosiren*), and Australia (*Neoceratodus*), and the two known extant species coelacanths (*Latimeria*) live in the deep ocean near Madagascar and Indonesia. In addition to their wide geographical separation, the living lobe-finned fishes share only distant common ancestors, and each group possesses unique features that are poorly understood. *Protopterus*, for example, has a reduced olfactory system that is thought to be the result of adaptation to drought and starvation (Derivot, 1984).

In lungfish, the olfactory organ is located ventrally. The incurrent nostril opens on the dorsal surface of the snout, but, unlike other fishes the excurrent nostril opens inside the mouth (Huxley, 1876). This internal naris functionally connects the nasal cavity and respiratory system, causing water to flow across the surface of the olfactory epithelium during breathing (Huxley, 1876). The degree to which the gills and lungs are involved in respiration varies among groups.

As in other fishes, the nasal cavity of lungfishes contains a series of lamellae, on the surface of which lies the olfactory epithelium (Derivot, 1984; Pfeiffer, 1969; Theisen, 1972). The morphology of

olfactory receptor neurons varies among taxa. The African lungfish (*Protopterus annectans*) has both ciliated and microvillar olfactory receptor neurons, but the Australian lungfish (*Neoceratodus forsteri*) lacks ciliated olfactory receptor neurons (Derivot *et al.*, 1979; Theisen, 1972). The microvillar olfactory receptor neurons in these animals are unusual: the cells lack centrioles, and the microvilli contain microtubules, which have not been described in the microvilli on olfactory receptor cells in other vertebrates (Theisen, 1972). Both species lack the crypt-type olfactory receptor neurons characteristic of ray-finned fishes (Hansen and Finger, 2000).

The olfactory bulbs are located adjacent to the telencephalon in *Protoperus* and *Lepidosiren*, but those in *Neoceratodus* are connected to the telencephalon by short, hollow peduncles (Holmgren and van der Horst, 1925). The structure of the olfactory bulb in *Protoperus* has been described by Rudebeck (1945). Clear laminae are present, including plexiform layers, although the internal plexiform layer is not as robust as in *Neoceratodus* (Holmgren and van der Horst, 1925). Periglomerular cells are scattered among the glomeruli, and their morphology is unlike those in other vertebrates: each cell has a single dendrite that arborizes in a glomerulus, and the axons of most periglomerular cells form a distinct tract that passes over the dorsal surface of the olfactory bulb and extends to the anterior pallium (Rudebeck, 1945). The mitral cells have primary dendrites that arborize in the glomerular layer as well as secondary dendrites that extend through the external plexiform layer. The granule cells have spiny dendrites and unmyelinated axons. Stellate cells may not be present.

Although *Protopterus* has been suggested to possess a vomeronasal nerve and accessory olfactory bulb (Schnitzlein and Crosby, 1967), more recent studies have demonstrated that this is not the case (Derivot, 1984; Reiner and Northcutt, 1987). Thus, a discrete vomeronasal system is lacking in lobe-finned fishes.

The central projections of the olfactory system in lungfishes have not been examined using modern methods, but were described by Holmgren and van der Horst (1925) and Rudebeck (1945) based on normal material (reviewed in Nieuwenhuys, 1967). A central olfactory tract consisting of axons from both mitral and granule cells passes through the telencephalic hemisphere in the pallium and subpallium, forming internal and external fiber layers. The internal fiber layer is confined to the pallium whereas the external fiber layer extends to the striatum and the lateral parts of the olfactory tubercle, continuing posteriorly to the stria medullaris. A medial olfactory tract projects to the septum. A contralateral projection is present, but the terminations of decussating fibers has not been determined. The presence of primary olfactory fibers that project bilaterally to the di- and mesencephalon has been detected in *Protopterus* using horseradish peroxidase injections into the nasal cavity (von Bartheld and Meyer, 1988), and a similar projection has been described in *Neoceratodus* (Schober *et al.*, 1994). Thus, lungfishes appear to possess an extrabulbar olfactory pathway.

Initial studies of the anatomy of the terminal nerve in lungfish (*Protopterus annectans*; Pinkus, 1894 and *Neoceratodus forsteri*; Sewertzoff, 1902) described a discrete ganglion close to the olfactory sac with a nerve that runs independent of the olfactory nerve, entering the brain at the level of the preoptic area. Because of this topology, it was named 'nervus praeopticus' (Sewertzoff, 1902). The terminal nerve has now been examined in all three genera of lungfish, and in each it has two roots: an anterior root that runs within the olfactory nerve, consisting of a fascicle of fibers and bipolar cells, and a posterior root that corresponds to Sewertzoff's 'nervus praeopticus' (Holmgren and van der Horst, 1925; Rudebeck, 1945). In *Neoceratodus*, the two roots are joined at the level of the ganglion (Fiorentino *et al.*, 2002; Holmgren and van der Horst, 1925; Rudebeck, 1945). Experimental embryological work with larval *Neoceratodus* demonstrates that the terminal nerve ganglion and both roots originate within the olfactory placode (Fiorentino *et al.*, 2002). In *Neoceratodus* and *Protopterus*, only the posterior root displays GnRH immunoreactivity (Schober *et al.*, 1994), but in *Neoceratodus*, both roots display FMRFamide-like immunoreactivity (Fiorentino *et al.*, 2002). Perhaps the anterior root is homologous with the bundle of FMRFamide-immunoreactive fibers that run within the olfactory nerve of some sharks, described by Wu *et al.* (1992).

Two species of coelacanths are known, and because of the rarity of material the olfactory system has been examined only superficially in *Latimeria chalumnae*. This species has an incurrent nostril on the dorsal side of the snout, and a valvular excurrent nostril that opens just in front of the eye. The olfactory epithelium is radially organized around a central axis where the three dorsal and two ventral lamellar lobes converge (reviewed in Zeiske *et al.*, 1992). Although the fine structure of the olfactory epithelium has not been examined, odorant receptor genes have been sequenced from coelacanths (Freitag *et al.*, 1998). Both classes of receptor genes described by Freitag *et al.* (1995, 1998),

purportedly for odorant detection in air and in water, are present.

A bundle of receptor cell axons emerges from each lobe, and the bundles merge to become a short olfactory nerve that projects into the nearby olfactory bulb (Northcutt and Bemis, 1993). The olfactory bulb is located adjacent to the nasal cavity, and is connected to the telencephalon by a long olfactory peduncle (Nieuwenhuys, 1965). The organization of the olfactory bulb resembles that of other fishes, but clear plexiform layers are lacking (Nieuwenhuys, 1965). The olfactory tracts run through the peduncle. Some tracts terminate within the olfactory bulb (corpus rostrale), whereas the others form a central olfactory tract that becomes thinner as it proceeds caudally through the pallium. The termination sites of these fibers are unclear (Nieuwenhuys, 1965). Neither an extrabulbar olfactory pathway nor a terminal nerve has been identified in *Latimeria* (Northcutt and Bemis, 1993).

17.2.6 Tetrapods: Amphibians, Reptiles, and Mammals

The tetrapods include all extant amphibians, reptiles (including birds), and mammals. Early tetrapods were aquatic, and the last common ancestor of tetrapods is now generally accepted to have been fully aquatic (Lebedev and Coates, 1995; Panchen, 1991). Thus, many features in amphibians and amniotes (reptiles and mammals) that represent adaptations to terrestrial life arose independently.

In early tetrapods, the external nostril shifted position to lie low on the snout, and became enlarged (Clack, 2002). In addition, olfaction became coupled to respiration, and remains so in many extant tetrapods (Clack, 2002). The olfactory system of tetrapods is dramatically reorganized compared with that of other vertebrates, as the vomeronasal system now forms a discrete sensory system. The olfactory system of terrestrial tetrapods must contain features that facilitate functioning to detect odorants in air, instead of in water, yet few concrete examples have been identified. For example, the vomeronasal system was once thought to have arisen as an adaptation to terrestrial life (Bertmar, 1981), but this idea has since been discarded (Eisthen, 1992, 1997).

17.2.6.1 Amphibians Amphibians comprise three distinct groups: anurans, or frogs and toads; urodeles, or salamanders, including newts; and apodans or caecilians, legless neotropical animals that are relatively poorly understood. Amphibians lack a diaphragm, and as they draw air into and out of the lungs by expanding and contracting the buccopharyngeal cavity, air passes across the sensory epithelium in the nasal cavity (Jørgensen, 2000). Salamanders have been observed to do the same underwater, allowing for olfactory sampling of the aqueous environment (Jørgensen, 2000).

The vomeronasal system is generally present throughout life in amphibians. Among salamanders, the system is present in both aquatic and terrestrial species, including those that never metamorphose. The vomeronasal system has been described in cryptobranchids, sirenids, ambystomatids, salamandrids, amphiumids, and plethodontids, but is absent in members of the proteid family (Anton, 1908, 1911; Eisthen, 2000; Eisthen *et al.*, 1994; Saito *et al.*, 2003; Schmidt and Roth, 1990; Seydel, 1895; Stuelpnagel and Reiss, 2005). Given the phylogenetic relationships among salamander families (Frost *et al.*, 2006), either a vomeronasal-like system arose independently at least four times in salamanders, or it was present in the last common ancestor of extant salamanders and lost in proteids. Clearly, the latter is the most parsimonious hypothesis. The vomeronasal system is also present throughout life in both metamorphosing and direct-developing frogs, including those that are fully aquatic as adults (Cooper, 1943; Hansen *et al.*, 1998; Jermakowicz *et al.*, 2004; Nezlin and Schild, 2000; Reiss and Burd, 1997a, 1997b; Scalia, 1976; Zwilling, 1940), as well as in caecilians (Billo and Wake, 1987; Schmidt and Wake, 1990).

Although anurans are often considered to rely almost entirely on visual and acoustic cues, olfaction plays an important role in the lives of some species (Waldman and Bishop, 2004). For example, tadpoles reduce their activity or seek refuge when exposed to odorants from sympatric predators, injured conspecifics, or predators that have consumed conspecifics (e.g., Laurila *et al.*, 1997; Marquis *et al.*, 2004). Sustained exposure to such cues can also lead to more dramatic changes in morphology and life history (Chivers *et al.*, 2001). Juvenile toads (*Bufo cognatus* and *B. microscaphus*) avoid chemical cues from predatory garter snakes, *Thamnophis* (Flowers and Graves, 1997). Wood frog tadpoles (*Rana sylvatica*) prefer to school with kin (Waldman, 1982, 1984), and kin recognition is mediated by olfactory cues (Waldman, 1985). Some tree frogs that lay eggs in small pools of water in plants provide additional trophic eggs to ensure adequate food supplies for tadpoles. In one such species, *Chirixalus eiffingeri*, tadpoles become highly active when exposed to water conditioned by

adult females (Kam and Yang, 2002), suggesting that tadpoles associate odorants from females with food.

Olfactory cues are also used by adult frogs, particularly in relation to reproductive behavior. Olfaction is involved in orientation to breeding ponds (Ishii *et al.*, 1995), and some frogs prefer their own odorants to those from conspecifics (Waldman and Bishop, 2004). Male magnificent tree frogs, *Litoria splendida,* produce a pheromone that attracts females (Wabnitz *et al.*, 1999). Males of some other *Litoria* species have a rostrally directed spike on the tip of the snout, and the surface epithelium is rich in secretory glands (Menzies, 1993). Although the function of this spike is unknown, its sexually dimorphic distribution and glandular surface suggest that it is involved in chemical communication related to reproduction (Menzies, 1993). Adult *Xenopus* can also use odorant cues in air to find food (Shinn and Dole, 1978).

The role of olfaction in salamander behavior has been examined much more extensively than that in frogs. Like tadpoles, salamanders respond to odorants from injured conspecifics (Chivers *et al.*, 1996b, 1997). Larval tiger salamanders (*Ambystoma tigrinum*) are facultative cannibals, and larvae are more likely to become cannibals in mixed-sibship groups than when surrounded by siblings (Pfennig and Collins, 1993). Cannibals prefer to consume conspecifics that are not kin, but this discrimination disappears when the nostrils are plugged, implicating olfaction in the kin recognition process (Pfennig *et al.*, 1994).

Chemical cues are involved in social behavior in salamanders, and odorants from conspecifics are involved in aggregation (Secondi *et al.*, 2005), individual recognition (Jaeger, 1981; Ovaska, 1988), and marking territories (Chivers *et al.*, 1996b; Jaeger, 1986; Ovaska and Davis, 1992; Simons *et al.*, 1994). Salamanders also use chemical cues to discriminate the sex and reproductive condition of conspecifics (Marco *et al.*, 1998; Park *et al.*, 2004; Verrell, 1985), and the use of pheromones in courtship behavior appears to be widespread. For example, in two species of fire-belly newts (*Cynops*), males produce a species-specific peptide that attracts females (Kikuyama *et al.*, 1995; Yamamoto *et al.*, 2000). In plethodontid salamanders, (*Desmognathus ochrophaeus* and *Plethodon jordani*), male pheromones can increase female receptivity, and in red-spotted newts (*Notophthalmus viridescens*) unreceptive females can become receptive when exposed to chemical cues from a male (Houck and Reagan, 1990; Rogoff, 1927; Rollmann *et al.*, 1999). Interestingly, in red-spotted newts, males are less attracted to odorants from females engaged in courtship behavior than females that are not, even when male odorants are present in both situations (Park and Propper, 2001; Park *et al.*, 2005). Nevertheless, not all pheromone effects in salamanders are mediated by nasal chemosensory systems: in many plethodontid salamanders, males use their teeth to inject pheromones through the skin of females, thereby increasing their receptivity (Houck and Arnold, 2003).

The behavior of caecilians has not been examined in detail. Nevertheless, Himstedt and Simon (1995) showed that plugging the nostrils in *Ichthyophis kohtaoensis* disrupts foraging, suggesting that olfaction plays an important role in this behavior. Chemical cues have been shown to play a role in both aggregation and individual recognition in *Typhlonectes natans* (Warbeck and Parzefall, 2001).

The relative contributions of the olfactory and vomeronasal systems to amphibian behavior are unclear. The vomeronasal organ plays a role in predation in the red-backed salamander, *Plethodon cinereus* (Placyk and Graves, 2002). In both salamanders and caecilians, the relative size of the vomeronasal organ is larger in aquatic or semiaquatic species than in terrestrial species (Dawley, 1998; Schmidt and Wake, 1990), suggesting that aquatic amphibians may rely more on vomeronasal input than do terrestrial species. The vomeronasal organ tends to be larger in male than female plethodontid salamanders, and organ size increases during the breeding season, suggesting a role in reproductive behavior (Dawley, 1998; Dawley *et al.*, 2000). Indeed, in female salamanders (*Cynops pyrrhogaster* and *Plethodon jordani*), male-produced attraction pheromones elicit physiological responses from the vomeronasal organ, but not from the olfactory epithelium (Kikuyama and Toyoda, 1999; Wirsig-Wiechmann *et al.*, 2002b). Nevertheless, EOG recordings demonstrate that both the olfactory and vomeronasal epithelia respond to pheromones in female red-bellied newts (*C. pyrrhogaster*) and male red-spotted newts (*N. viridescens*), and that both epithelia respond more strongly to odorants from the opposite sex than the same sex in male and female axolotls, *Ambystoma mexicanum* (Park *et al.*, 2004; Park and Propper, 2002; Toyoda *et al.*, 1999).

The morphology of the nasal cavity varies considerably among amphibian groups, and often changes during metamorphosis. Many of the anatomical differences associated with life in water and on land have recently been reviewed by Reiss and Eisthen (2006). Perhaps the most dramatic changes occur during metamorphosis in frogs. For example, larval African clawed frogs, *Xenopus laevis*, possess

only one olfactory chamber, which is used for olfaction in water. This chamber contains both ciliated and microvillar receptor neurons (Hansen *et al.*, 1998). During metamorphosis, this chamber is transformed into the principal cavity, which functions for detecting odorants in air, and a new middle chamber develops (reviewed in Hansen *et al.*, 1998; Reiss and Eisthen, 2006). In other frogs, this middle cavity is nonsensory, but in *Xenopus* the middle cavity is lined with sensory epithelium and functions in olfaction underwater (Altner, 1962; Paterson, 1951). Adult *Xenopus* are almost entirely aquatic, and sample odorants in both at the surface and in water. A muscular valve shunts the medium into one chamber or the other (Altner, 1962). In all frogs studied to date, including *Xenopus*, the adult principal cavity contains only ciliated receptor neurons, but the middle cavity of *Xenopus* contains both ciliated and microvillar receptor neurons (Bloom, 1954; Hansen *et al.*, 1998; Mair *et al.*, 1982; Menco, 1980; Oikawa *et al.*, 1998; Reese, 1965; Taniguchi *et al.*, 1996). Because the principal cavity that detects waterborne odorants in larvae is transformed into the principal cavity that detect airborne odorants in adult, a clear correlation emerges: in *Xenopus*, ciliated receptor neurons are used for olfaction in air, and both ciliated and microvillar receptor neurons are used for olfaction in water (Hansen *et al.*, 1998).

In addition to these morphological differences, the sensory epithelia in the principal and middle cavities of *Xenopus* differ at the molecular level. Freitag *et al.* (1995, 1998) divide the odorant receptor genes in frogs into two classes: class I genes, with a large loop in the third extracellular domain and sequences similar to those found in teleosts, and class II genes, similar to those found in mammals. In *Xenopus*, class II genes are expressed in principal cavity and are proposed to function for detecting odorants in air, and class II are expressed in middle cavity and are proposed to function for detecting odorants in water (Freitag *et al.*, 1995; Mezler *et al.*, 2001). The principal cavity also contains a novel form of $G\alpha_s$ that is more closely related to the mammalian $G\alpha_{olf}$ than to other forms of $G\alpha_s$ in frogs, and that induces cAMP formation. In contrast, the epithelium in the middle cavity contains $G\alpha_{o1}$, which stimulates formation of IP_3 (Mezler *et al.*, 2001). Two different homologues of mammalian olfactory marker protein have been found in *Xenopus*, and they are differentially expressed in the two olfactory cavities (Rössler *et al.*, 1998).

Odorant binding proteins, suggested to be a mammalian adaptation, have been found in *Rana pipiens*, *Xenopus laevis*, and *Xenopus tropicalis* (Lee *et al.*, 1987; Millery *et al.*, 2005). As in mammals, odorant binding proteins in frogs appear to be lipocalins. Interestingly, in both species of *Xenopus*, the protein is expressed in principal cavity but not middle cavity (Millery *et al.*, 2005). This observation lends support to the hypothesis that the function of odorant binding proteins is related to physical constraints imposed by the problem of detecting odorants in air; for example, odorant binding proteins may function to transport hydrophobic molecules through aqueous lymph or mucus to the odorant receptors (Bignetti *et al.*, 1987; Vogt, 1987).

The nasal sac of salamanders is essentially a simple oval-shaped tube that is almost completely lined with sensory epithelium, which is one of the reasons why some electrophysiologists favor salamanders as model animals for olfactory research (e.g., Kauer, 2002). The olfactory epithelium of most adult salamanders, even those that are fully terrestrial, appears to contains both ciliated and microvillar receptor cells (Breipohl *et al.*, 1982; Eisthen, 2000; Eisthen *et al.*, 1994; Farbman and Gesteland, 1974; Jones *et al.*, 1994). In contrast, four types of olfactory receptor neurons have been described in *Dicamptodon tenebrosus*: those with cilia, with long microvilli, with unusual short microvilli, and those with both cilia and extremely short (<0.5 μm) microvilli (Stuelpnagel and Reiss, 2005). This finding suggests that other salamanders may possess additional types of neurons that have not been distinguished. Thirty-five putative odorant receptor genes have been sequenced from tiger salamanders (*A. tigrinum*), and all appear to be class II genes (Marchand *et al.*, 2004). Because terrestrial tiger salamanders were used, this result appears to support the Freitag *et al.* (1998) hypothesis that odorant receptors in the class II gene family function to detect odorants in air. Nevertheless, mudpuppies (*Necturus maculosus*), which are members of the fully aquatic proteid family, possess both class I and class II odorant receptor genes (Zhou *et al.*, 1996), casting doubt on the distinction. Olfactory transduction in mudpuppies also involves a novel cyclic nucleotide-dependent chloride current (Delay *et al.*, 1997). The existence of this current in mudpuppies may represent an environmental adaptation to life in freshwater, in which the ionic concentrations of the olfactory mucus may be difficult to regulate. If so, the external calcium required to gate the chloride channels widely involved in olfactory transduction may not reliably be present, leading to the use of a different gating mechanism in these animals (Delay *et al.*, 1997).

Caecilians have paired nares that open into the nasal cavity, which contains the olfactory

epithelium. Adult caecilians also have short tentacles between the naris and eye that are both chemosensory and somatosensory (Badenhorst, 1978). Caecilians are blind, and the tentacles are derived from modified eye structures (Billo and Wake, 1987). The lumen of the tentacle carries secretions from the Harderian gland and communicates with the vomeronasal organ (Badenhorst, 1978; Schmidt and Wake, 1990), an arrangement that allows the animal to detect chemicals with the vomeronasal organ even while burrowing or swimming, when the external nares are closed (Prabha *et al.*, 2000). The nasal cavity of *Typhlonectes compressicaudum* contains two morphologically distinct types of olfactory epithelium. The dorsocaudal portion of the nasal cavity contains an epithelium with only ciliated olfactory receptors neurons, whereas the anterior ventral epithelium contains both ciliated and microvillar receptor neurons (Saint Girons and Zylberberg, 1992). Given their relative locations, these observations suggest that, as in *Xenopus*, caecilians use morphologically different receptor neurons for detecting odorants in water and air (Reiss and Eisthen, 2006).

As in other tetrapods, the vomeronasal receptor neurons in frogs, salamanders, and caecilians terminate in microvilli (Eisthen, 2000; Eisthen *et al.*, 1994; Franceschini *et al.*, 1991; Kolnberger, 1971; Kolnberger and Altner, 1971; Oikawa *et al.*, 1998; Saint Girons and Zylberberg, 1992). In addition to the receptor neurons, sustentacular cells, and basal cells that are generally present in the vertebrate olfactory and vomeronasal epithelia, the vomeronasal epithelium of frogs and salamanders contains large ciliated supporting cells that may function to move fluid across the surface of the epithelium (Eisthen, 1992). The vomeronasal epithelium in *Xenopus* has been shown to express members of the V2R family of vomeronasal receptor genes as well as $G\alpha_o$, the G-protein that is co-expressed with V2Rs in mammals (Hagino-Yamagishi *et al.*, 2004). Although a portion of the principal cavity containing olfactory epithelium was reported to express V2R genes (Hagino-Yamagishi *et al.*, 2004), the authors appear to have misidentified the posterior portion of the vomeronasal organ, which is longer in *Xenopus* than in other frogs (Reiss and Eisthen, 2006).

The olfactory bulbs of amphibians display a moderate degree of lamination, and include both internal and external plexiform layers in all groups (Herrick, 1948; Hoffman, 1963; Nieuwenhuys, 1967). Unlike in any other group of vertebrates, the olfactory bulb of salamanders does not consist of concentric layers; rather, the olfactory nerve enters from the rostrolateral pole, and the layers progress in wide bands from rostral to caudal (e.g., Herrick, 1948). In frogs, the layers are not completely concentric (Scalia *et al.*, 1991b).

In addition, in frogs the two olfactory bulbs are fused across the midline (Hoffman, 1963), a condition also observed in some teleost fishes and bird species (Nieuwenhuys, 1967). Morphologically, the two bulbs in frogs appear to be organized almost as a single unit. For example, the axons of some olfactory receptor neurons cross the midline to project to the contralateral bulb (Hoffman, 1963). The laminae within the bulb are continuous across the midline (Hoffman, 1963; Scalia *et al.*, 1991b), and the processes of both mitral and granule cells cross the midline to interact with elements in the contralateral olfactory bulb (Herrick, 1921; Ramón y Cajal, 1922; Scalia *et al.*, 1991b). Field potential recordings demonstrate that the responses to unilateral stimulation of the olfactory nerve are similar in both olfactory bulbs, suggesting that signal transmission is fully bilateral (Andriason and Leveteau, 1989). However, recordings from mitral cells indicate that unilateral nerve stimulation leads to an inhibition of evoked responses on the contralateral side (Leveteau *et al.*, 1993). The authors of this study suggest that the contralateral inhibition is a mechanism for enhancing contrast between the two sides, facilitating odorant localization. If so, perhaps the joint olfactory bulbs of frogs allow them to better localize odorants than salamanders, which have separate olfactory bulbs.

In both salamanders and frogs, the axons of olfactory receptor neurons have been shown to branch upon entering the outer fiber layer of the bulb and innervate glomeruli that are widely spaced (Herrick, 1924, 1931; Nezlin and Schild, 2005). Periglomerular cells are present, although they do not surround and isolate individual glomeruli to the same extent observed in mammals (Herrick, 1924, 1931; Nezlin *et al.*, 2003; Nezlin and Schild, 2000; Ramón y Cajal, 1890). The primary dendrites of the mitral cells arborize in multiple glomeruli (Herrick, 1948; Hoffman, 1963). Mitral cells also bear secondary dendrites that do not terminate in glomeruli and appear to make synapses with processes of granule cells; these neurites are more prominent in frogs than in salamanders (Scalia *et al.*, 1991b). Herrick (1924) describes 'atypical mitral cells' in the external plexiform layer in tiger salamanders that could be homologous with the tufted cells found in mammals, and Scalia *et al.* (1991b) described candidate tufted cells in a similar location in northern leopard frogs (*Rana pipiens*). Axonless granule cells are present. The dendrites of these cells are spiny in

frogs and tiger salamanders, but smooth in mudpuppies (Herrick, 1924, 1931; Hoffman, 1963; Scalia *et al.*, 1991b). Amphibians appear to lack stellate cells. The structure of the accessory olfactory bulb is similar to that of the olfactory bulb, but plexiform layers are absent and the boundaries of the layers are less distinct (Herrick, 1924, 1931; Nieuwenhuys, 1967). In salamanders, some mitral cells have dendrites that project to glomeruli in both the olfactory and accessory olfactory bulbs (Herrick, 1924).

Central olfactory projections have been examined in several anurans, including *Xenopus* and ranid and hylid frogs, as well as in tiger salamanders, *Ambystoma tigrinum* (Kemali and Guglielmotti, 1987; Kokoros and Northcutt, 1977; Northcutt and Royce, 1975; Roden *et al.*, 2005; Scalia, 1972; Scalia *et al.*, 1991a). As shown in Figure 8a, the medial olfactory tract projects to the postolfactory eminence and medial pallium as well as to the lateral and medial septal nuclei (Northcutt and Royce, 1975; Roden *et al.*, 2005; Scalia *et al.*, 1991a). The lateral olfactory tract projects ipsilaterally to the lateral pallium, dorsal striatum, lateral amygdala, and a region interpreted as either the dorsal pallium or the dorsal portion of the lateral pallium (Figure 8b; Moreno *et al.*, 2005; Northcutt and Royce, 1975; Scalia *et al.*, 1991a). Fibers of the medial and lateral olfactory tracts project in combination to the contralateral amygdala and lateral pallium. The accessory olfactory tract projects bilaterally to the medial amygdala (Figure 8d), the rostral portion of which also receives olfactory input (Kemali and Guglielmotti, 1987; Moreno and Gonzalez, 2003; Moreno *et al.*, 2005; Scalia *et al.*, 1991a). The extrabulbar olfactory pathway of *Xenopus* has been examined in detail by Hofmann and Meyer, who have found that fibers originating in the olfactory epithelium bypass the olfactory bulb and terminate in the ipsilateral preoptic area and bilaterally in the hypothalamus (Figure 8c; Hofmann and Meyer, 1991a, 1991b; 1992). The pathway that Schmidt and colleagues (Schmidt *et al.*, 1988; Schmidt and Wake, 1990) described as the terminal nerve in salamanders and caecilians is more likely the extrabulbar olfactory

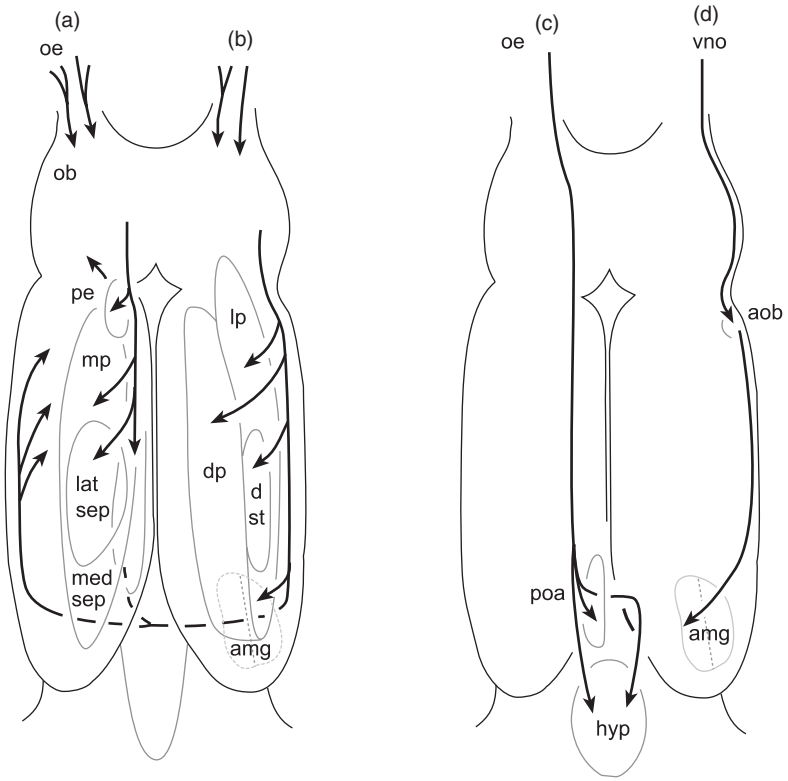

Figure 8 Schematic dorsal view of the forebrain in ranid frogs. a, Projections of the medial olfactory tract. b, Projections of the lateral olfactory tract. c, Projections of the extrabulbar olfactory pathway. d, Projections of the accessory olfactory tract. amg, amygdala; aob, accessory olfactory bulb; dp, dorsal pallium; d st, dorsal striatum; hyp, hypothalamus; lat sep, lateral septum; lp, lateral pallium; med sep, medium septum; mp, medial pallium; ob, olfactory bulb; oe, olfactory epithelium; pe, postolfactory eminence; poa, preoptic area; vno, vomeronasal organ. Based on Kemali and Guglielmotti (1987), Northcutt and Royce (1975), Scalia (1972), and Scalia *et al.* (1991a).

pathway, as it was labeled by injection of tracer into the nasal cavity but not vomeronasal organ, and ganglion cells were not found. If this interpretation is correct, the extrabulbar pathway may be more extensive in both salamanders and caecilians than in frogs (Hofmann and Meyer, 1989).

The terminal nerve of both frogs and salamanders has been examined in detail. The cells and fibers of the nerve are immunoreactive for a variety of compounds, including GnRH (D'Aniello *et al.*, 1994b, 1995; Muske and Moore, 1988; Northcutt and Muske, 1994; Sherwood *et al.*, 1986), NPY (D'Aniello *et al.*, 1996a; Lázár *et al.*, 1993; Mousley *et al.*, 2006; Tuinhof *et al.*, 1994), and FMRFamide (D'Aniello *et al.*, 1996b; Muske and Moore, 1988; Northcutt and Muske, 1994). In tiger salamanders (*Ambystoma tigrinum*), the terminal nerve also displays acetylcholinesterase activity, indicating that acetylcholine is present (Wirsig and Getchell, 1986). In frogs, the terminal nerve may send an additional projection to the retina (Uchiyama *et al.*, 1988; Wirsig-Wiechmann and Basinger, 1988).

In salamanders, terminal nerve-derived peptides alter both the odorant sensitivity and excitability of olfactory receptor neurons. The olfactory epithelium appears to contain GnRH receptors (Wirsig-Wiechmann and Jennes, 1993), and GnRH modulates both odorant responses and the voltage-dependent, tetrodotoxin-sensitive sodium current in olfactory receptor neurons (Eisthen *et al.*, 2000; Park and Eisthen, 2003). Interestingly, olfactory receptor neurons are more responsive to GnRH during the breeding season, suggesting that GnRH may play a role in regulating responses to odorants in a way that promotes reproduction (Eisthen *et al.*, 2000). Other studies show that estrogen affects GnRH concentrations in the terminal nerve in *Xenopus* (Wirsig-Wiechmann and Lee, 1999) and that concentrations are higher in courted female salamanders than in uncourted conspecifics (Propper and Moore, 1991), lending further support to the idea that the GnRH-containing fibers of the terminal nerve play a role in reproductive behavior. In addition, FMRFamide modulates both odorant responses and the sodium current in olfactory receptor neurons (Park *et al.*, 2003). Recently, we have also shown that the terminal nerve in axolotls (*Ambystoma mexicanum*) is NPY-immunoreactive, and that application of synthetic axolotl NPY modulates both odorant responses and the sodium current, but only in hungry animals (Mousley *et al.*, 2006). Taken together, these data suggest that terminal nerve modulates responsivity in the olfactory epithelium in concert with changes in internal state, perhaps to maximize sensitivity to odorants most relevant to the animal's condition.

17.2.6.2 Reptiles The class Reptilia includes rhynchocephalians, or tuatara; squamates, a group that consists of lizards, snakes, and amphisbaenians; crocodilians; and turtles. Because birds (Aves) form the sister group to extant crocodilians, we include birds in this group.

17.2.6.2.(i) Tuatara The rhynchocephalians, or tuatara, constitute a separate order of reptiles. The two living species of tuatara, *Sphenodon punctatus* and *S. guntheri*, are found only on small islands off the coast of New Zealand. Because of their phylogenetic importance as a sister group to squamate reptiles, their chemosensory systems have been studied to a limited extent.

Unlike snakes and some lizards, tuatara do not have a forked tongue (Schwenk, 1986), and they do not tongue-flick (Walls, 1981). The role of the olfactory and vomeronasal systems in tuatara feeding has been reviewed by Schwenk (2000). Much of the available evidence is anecdotal (e.g., Walls, 1981), but suggests that visual cues predominate and that chemoreception plays a secondary role in foraging and food choice (Schwenk, 2000). Tuatara will bite cotton that has been swabbed on prey, demonstrating that the animals will respond to chemical cues related to feeding (Cooper *et al.*, 2001). The role of chemical signals in social and reproductive behavior is unknown.

The nasal cavity of *Sphenodon* is fairly simple in organization, although some conchae are present. A long choana connects the ventral nasal cavity to the oral cavity. Olfactory epithelium lines the posterior portion of the dorsal half of the nasal cavity, and the vomeronasal sensory epithelium is confined to the dorsal portion of the small vomeronasal organ, which lies along the septum. The vomeronasal organ opens into the ventromedial portion of the nasal cavity, anterior to the choana. Unlike that of snakes and lizards, the vomeronasal organ has no direct connection to the oral cavity. The ultrastructure of the olfactory and vomeronasal epithelia has not been described. The axons of the vomeronasal receptor neurons join the olfactory nerve to project to a small accessory olfactory bulb dorsomedial to the much larger main olfactory bulb (Hoppe, 1934, in Parsons, 1959a; Parsons, 1967). Cairney (1926) described the olfactory projections in normal material without distinguishing among the tracts, deducing that the olfactory bulbs project to the medial pallium, septum, olfactory tubercle, olfactostriatum, lateral pallium (piriform cortex),

anterior nucleus of the amygdala, and a region he called the 'hypopallium posterius'. The nucleus sphericus of squamates has a roughly similar position and appearance, and receives a large projection from the accessory olfactory bulb. The nucleus sphericus has been thought to be present only in squamates (Northcutt, 1978), but without studying projections in tuatara using modern tract-tracing techniques, the uniqueness of this structure cannot be determined.

17.2.6.2.(ii) Squamates: Amphisbaenians, lizards, and snakes Squamate reptiles are diverse and widespread, and many species rely heavily on chemosensory input to mediate all aspects of their behavior. In many snakes and lizards the vomeronasal system is behaviorally more important than the olfactory system and, in some species, the neural structures that comprise the vomeronasal system are hypertrophied relative to those of the olfactory system. Although squamates are favored model animals for neurobiologists seeking to understand the structure and function of the vomeronasal system, this endeavor is paradoxically complicated by the dominance of the vomeronasal system: in many squamates, the behavioral relevance of olfactory information is not clear. This broad generalization does not apply to all squamates, however. For example, in geckos the olfactory system appears to be the dominant chemosensory system (Schwenk, 1993b).

The nasal chemosensory systems play a critical role in foraging and feeding in many squamates. For example, pygmy rattlesnakes (*Sistrurus miliarius*), which are sit-and-wait predators, aggregate in areas containing chemical cues from potential prey animals (Roth *et al.*, 1999). Snakes are well-known to follow chemical trails left by prey (Schwenk, 1994), as can some lizards, like Gould's monitor lizards, *Varanus gouldii*, and Gila monsters, *Heloderma suspectum* (Garrett *et al.*, 1996). Rattlesnakes, which strike and release their prey, use chemical cues to follow and identify envenomated animals. These cues derive from the venom itself as well as recognition of the envenomated individual (Chiszar *et al.*, 1999; Furry *et al.*, 1991; Lavin-Murcio and Kardong, 1995). Both snakes and lizards with experimentally impaired vomeronasal systems will attack but not consume prey, and garter snakes (*Thamnophis sirtalis*) with sectioned vomeronasal nerves stop following prey trails and eventually cease eating (Alving and Kardong, 1996; Graves and Halpern, 1990; Halpern *et al.*, 1997; Haverly and Kardong, 1996).

Chemical cues can facilitate predator avoidance. The Texas banded gecko, *Coleonyx brevis*, displays defensive behavior when presented with odorants from a predatory snake; changes in the rate of buccal pulsing in response to these cues suggest that the discrimination is based on input to the olfactory system (Dial and Schwenk, 1996). The ability to discriminate harmful from harmless species based solely on chemical cues has been demonstrated in lizards (Lacertidae, Iguanidae, and Anguidae) and in amphisbaenians, *Blanus cinereus* (Amo *et al.*, 2004; Bealor and Krekorian, 2002; Cabido *et al.*, 2004; Lopez and Martin, 1994, 2001; Van Damme and Quick, 2001). Vomeronasal input is critical to the ability of crotaline snakes to recognize predatory kingsnakes, *Lampropeltis getula* (Miller and Gutzke, 1999). Responsiveness to chemical cues from predators has been shown to increase the probability of surviving an encounter with a predator in garden skinks, *Lampropholis guichenoti* (Downes, 2002).

Chemical cues play a role in discrimination of the sex of conspecifics in amphisbaenians, *Blanus cinereus* (Cooper *et al.*, 1994), in recognition of familiar and unfamiliar conspecifics in lacertid lizards (Aragon *et al.*, 2003; Font and Desfilis, 2002), and in recognition of mates in snow skinks, *Niveoscincus microlepidotus* (Olsson and Shine, 1998). Male skinks (*Eulamprus heatwolei*) use chemosensory cues to assess female receptivity (Head *et al.*, 2005). Mate choice is influenced by chemical cues in snakes (*Thamnophis sirtalis*) and lizards (*Lacerta monticola*) (Lopez *et al.*, 2003; Shine *et al.*, 2003), and female Swedish sand lizards (*Lacerta agilis*) prefer odorants from males that differ from themselves at the MHC class 1 loci (Olsson *et al.*, 2003). Taken together, these observations indicate that squamates can use chemosensory information to discriminate sex, receptivity, quality as a potential mate, genotype, and individual identity of conspecifics. In squamates, pheromonal cues are not always attractants: female brown tree snakes, *Boiga irregularis*, produce a cloacal secretion that decreases courtship intensity and duration in males (Greene and Mason, 2003).

In general, squamates sniff to draw odorants across the olfactory epithelium and use their tongues to physically pick up chemical stimuli that will be deposited in the vomeronasal organ. Geckos, which depend more on olfactory than on vomeronasal input, appear to sniff via oscillations of the throat (Dial and Schwenk, 1996). Garter snakes (*Thamnophis sirtalis*) can detect prey odorants in air, without physical contact with the tongue, using the olfactory system (Halpern *et al.*, 1997).

Olfactory input appears to be important for elevating the rate of tongue-flicking, which then allows the animal to sample with the vomeronasal organ (Halpern *et al.*, 1997; Zuri and Halpern, 2003). Although tongue-flicking is widespread among squamates, not all have forked tongues (Schwenk, 1993a, 1994). Forked tongues have evolved repeatedly among squamates, particularly in groups that forage widely (Schwenk, 1994). This arrangement may be used to enhance two-point comparisons, for example to facilitate trail following by improving edge detection (Schwenk, 1994).

The vomeronasal organ of squamate reptiles opens directly into the oral cavity and has no connection with the nasal cavity, a condition that is different than in any other tetrapod (Schwenk, 1993a). Tongue-flicking brings molecules into the vomeronasal organ (reviewed in Schwenk, 1995; see also Graves and Halpern, 1989; Halpern and Kubie, 1980). Although the tongue tips are essential for vomeronasal sampling, the mechanism by which compounds move from the tongue to the vomeronasal ducts and into the organ is not understood (reviewed in Schwenk, 1994). The sublingual plicae have been suggested to play a key role, perhaps by creating suction within the vomeronasal organ or duct (Young, 1993), but snakes with cauterized plicae are able to find and consume food, and transport of molecules into the vomeronasal organs does not differ between lesioned and control animals (Halpern and Borghjid, 1997). Instead, the heavily vascularized vomeronasal organ may use a pumping mechanism to create suction, similar to a mechanism used by some mammals (Halpern and Martínez-Marcos, 2003).

The squamate nasal cavity has a relatively simple organization, and in most species is essentially an elongated sac, the posterior dorsal portion of which is lined with olfactory epithelium (Parsons, 1959b, 1967). The dorsal wall of the spherical vomeronasal organ is lined with sensory epithelium, and the lumen is made narrow by a ventral protruding structure called the mushroom body. In garter snakes (*Thamnophis sirtalis*), as in other squamates, fluid from Harderian glands flows into vomeronasal organ (Rehorek, 1997; Rehorek *et al.*, 2000a, 2000b). The products of the gland play a critical role in solubilizing a lipophilic pheromone in this species, allowing the pheromone to be detected by vomeronasal receptor neurons (Huang *et al.*, 2006). Animals from which the gland has been removed display impaired courtship behavior and prey capture, demonstrating the essential role of Harderian gland secretions in signal detection in the vomeronasal system in snakes (Mason *et al.*, 2006).

Both cilia and short microvilli are present together on the olfactory receptor cells of the blue-tongued lizard, *Tiliqua scincoides* (Kratzing, 1975), although only ciliated receptor cells were found in a scanning electron microscopic investigation of the olfactory epithelium in the garter snakes *Thamnophis sirtalis* and *T. radix* (Wang and Halpern, 1980b). The morphology of receptor neurons may vary between species, but it also seems possible that short microvilli could have been overlooked or difficult to see in the preparations from snakes. Both lizards and snakes possess only microvillar vomeronasal receptor neurons (Altner and Brachner, 1970; Altner and Müller, 1968; Bannister, 1968; Kratzing, 1975; Takami and Hirosawa, 1990; Wang and Halpern, 1980a, 1980b). The olfactory and vomeronasal receptor genes from squamates have not yet been sequenced. In snakes and lizards, the vomeronasal epithelium expresses $G\alpha_o$ and $G\alpha_{i2}$, which are also found in the vomeronasal epithelium of mammals, but lack $G\alpha_{olf}$, $G\alpha_{11}$, and $G\alpha_q$, which are found in the mammalian olfactory epithelium (Labra *et al.*, 2005; Luo *et al.*, 1994). The vomeronasal epithelium in garter snakes expresses type IV adenylyl cyclase (Liu *et al.*, 1998), as does that of rats (Rössler *et al.*, 2000).

The olfactory bulb of squamates is distinctly laminated, with both external and internal plexiform layers present. In adult lizards (*Podarcis hispanica*), periglomerular cells have a single dendrite, oriented parallel to the bulbar surface (García-Verdugo *et al.*, 1986). Each mitral cell has one primary dendrite that arborizes in a single glomerulus, as well as secondary dendrites that travel through the external plexiform layer. In contrast, in embryonic snakes (*Elaphe quadrivirgata*), the primary dendrites of mitral cells arborize in many glomeruli (Iwahori *et al.*, 1989a). In both animals, the spiny dendrites of the axonless granule cells extend into the plexiform layer, but do not arborize in the glomeruli (García-Verdugo *et al.*, 1986; Iwahori *et al.*, 1989a).

The accessory olfactory bulb lacks the clear laminar organization of the olfactory bulb, and plexiform layers are absent (Iwahori *et al.*, 1989b; Llahi and García-Verdugo, 1989). In *Podarcis*, incoming axons of vomeronasal receptor neurons branch and enter more than one glomerulus, which does not appear to be the case for olfactory receptor cell axons entering the main olfactory bulb (García-Verdugo *et al.*, 1986; Llahi and García-Verdugo, 1989). The dendrites of periglomerular cells arborize in more than glomerulus, as do those of the mitral cells (Iwahori *et al.*, 1989b; Llahi and

García-Verdugo, 1989). Mitral cells also possess secondary dendrites that extend through the external and internal plexiform layers (Iwahori *et al.*, 1989b; Llahi and García-Verdugo, 1989). A class of cells that Llahi and García-Verdugo call 'small mitral cells' have a soma in the outer mitral cell layer or external plexiform layer and a single dendrite that arborizes sparsely in the glomerular layers; these cells might be equivalent to the tufted cells present in the mammalian olfactory bulb (Llahi and García-Verdugo, 1989). The granule cells resemble those in the main olfactory bulb (Iwahori *et al.*, 1989b; Llahi and García-Verdugo, 1989). Stellate cells do not seem to be present in the main or accessory olfactory bulbs in either species.

Among squamates, the central olfactory projections have been described in most detail in garter snakes, *Thamnophis sirtalis* and *T. radix* (Halpern, 1976; Lanuza and Halpern, 1997, 1998). Three central olfactory projections are present in these animals, comprising the lateral, intermediate, and medial olfactory tracts, illustrated in Figure 9. The medial olfactory tract projects ipsilaterally to the anterior olfactory nucleus (Figure 9a). The lateral olfactory tract projects bilaterally to the lateral cortex, as well as to the external and ventral anterior amygdala (Figure 9b). The intermediate olfactory tract projects to the olfactory tubercle and olfactory gray, and joins the lateral olfactory tract in a projection to the contralateral hemisphere (Figure 9c). The accessory olfactory tract, carrying information from the vomeronasal organ, projects to three portions of the amygdala: the nucleus sphericus, medial amygdala, and nucleus of the accessory olfactory tract (Figure 9d). The homology of the nucleus sphericus with regions of the amygdala in other tetrapods is unclear, because it has been suggested to be the only amygdalar target of the accessory olfactory bulb that does not project to the hypothalamus (Bruce and Neary, 1995; Lanuza and Halpern, 1997). This interpretation is complicated by newer data demonstrating a small projection from the nucleus sphericus to the hypothalamus, and a much larger projection to the olfactostriatum (Martínez-Marcos *et al.*, 1999, 2002). The latter structure in turn projects to the lateral posterior hypothalamic nucleus, and may be homologous with the nucleus accumbens (Martínez-Marcos *et al.*, 2005a, 2005b). Thus, the nucleus sphericus may be unique to squamates, may be unique to lepidosaurs (squamates and tuatara), or may be homologous with amygdalar regions in other vertebrates but have reorganized connections in squamates.

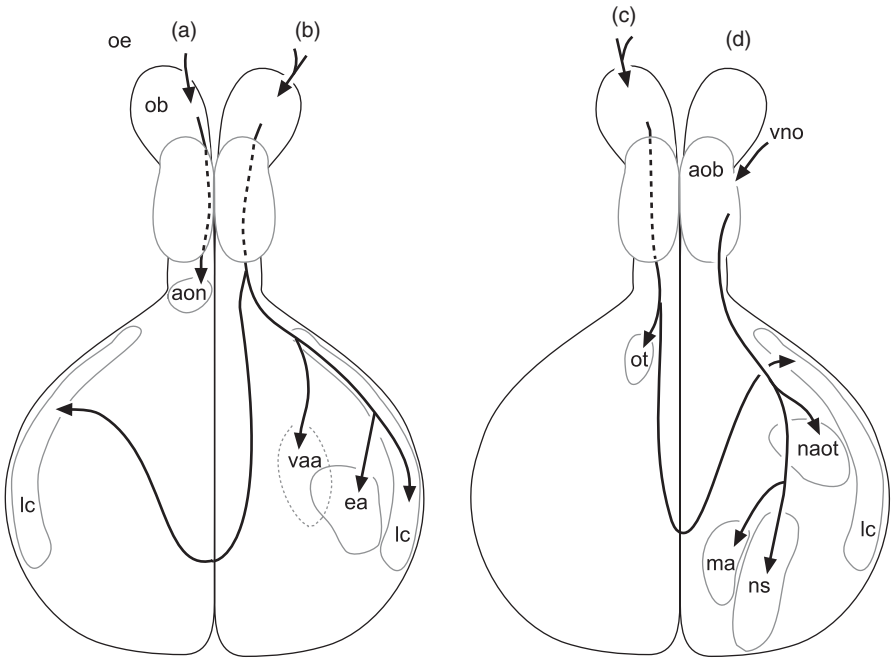

Figure 9 Schematic dorsal view of the forebrain in garter snakes, *Thamnophis sirtalis*. a, Projections of the medial olfactory tract. b, Projections of the lateral olfactory tract. c, Projections of the intermediate olfactory pathway. d, Projections of the accessory olfactory tract. aob, accessory olfactory bulb; aon, anterior olfactory nucleus; ea, external amygdala; lc, lateral cortex; ma, medial amygdala; naot, nucleus of the accessory olfactory tract; ns, nucleus sphericus; ob, olfactory bulb; oe, olfactory epithelium; ot, olfactory tubercle; vaa, ventral anterior amygdala. Based on Halpern (1976), Lanuza and Halpern (1997, 1998), and Lohman and Smeets (1993).

The medial amydala in garter snakes receives a direct projection from the accessory olfactory bulb, as well as indirect vomeronasal input via the nucleus sphericus. The olfactory bulb has an indirect projection as well, via the external and ventral anterior amygdaloid nuclei (Lanuza and Halpern, 1997, 1998; Martínez-Marcos *et al.*, 1999). Because the medial amygdala projects to the lateral posterior hypothalamic nucleus, which in turn projects to the hypoglossal nucleus, this pathway has been suggested to function in control of tongue-flicking in response to chemosensory input in snakes (Martínez-Marcos *et al.*, 2001).

Olfactory projections have also been examined in several lizards, including *Gekko gecko*. The lateral olfactory tract projects bilaterally to the entire length of the lateral cortex, as well as to the anterior olfactory nucleus, external amygdaloid nucleus, and perhaps the central amygdaloid nucleus. An intermediate olfactory tract projects to the olfactory tubercle and joins the lateral olfactory tract to project to the contralateral hemisphere. A short medial olfactory tract is present. Finally, an accessory olfactory tract projects ipsilaterally to the nucleus sphericus, external and central amygdaloid nuclei, and bed nucleus of the stria terminalis (Lohman and Smeets, 1993; Lohman *et al.*, 1988). In *Tupinambis teguixin*, the projections are similar, although the projections to the contralateral hemisphere differ somewhat between the two species (Lohman and Smeets, 1993). To our knowledge, an extrabulbar olfactory pathway has not been described in squamates.

The anti-peptide antisera often used to identify the terminal nerve in other vertebrates produce mixed results with squamates. In adult garter snakes (*Thamnophis sirtalis*), a GnRH-immunoreactive terminal nerve is present, with a discrete ganglion at the ventral border between the olfactory bulb and rostral telencephalon (Smith *et al.*, 1997). Intraventricular administration of a GnRH antagonist interferes with courtship behavior in these animals, although the relative contributions of the terminal nerve and central GnRH neurons to this effect cannot be determined (Smith and Mason, 1997). Among lizards, GnRH-immunoreactive cells and fibers are also present in the terminal nerve of adult *Eumeces laticeps* and *Sceloporus undulatus*, but not in adult *Anolis carolinensis* (Rosen *et al.*, 1997). In *Podarcis sicula*, GnRH-immunoreactive cells and fibers cannot be detected in a terminal nerve, nor in the olfactory epithelium and bulb, in either embryos or adults (D'Aniello *et al.*, 1994a; Masucci *et al.*, 1992). FMRFamide-immunoreactive cells and fibers are present in the terminal nerve during development in *Chalcides chalcides*, but not in adults (D'Aniello *et al.*, 2001). Overall, the available data indicate either that the immunoreactive characteristics of the terminal nerve in some squamate species differ from those in other jawed vertebrates, perhaps a way that varies with developmental stage, or that the nerve is not present at all developmental stages in all squamate species.

17.2.6.2.(iii) Crocodilians Crocodiles, alligators, caimans, and gharials are a relatively small group, comprising only 20–25 species. The nasal chemosensory systems have not been extensively studied in this group, and much of the information available concerning the role of chemoreception in behavior is reviewed by Weldon and Ferguson (1993).

The nares of crocodilians are closely situated on the dorsal portion of the snout and can protrude even while most of the animal is submerged under water, suggesting that olfactory cues may serve important functions in crocodilians. Crocodilians draw air through the nasal cavity through buccal oscillations that do not contribute to respiration (Gans and Clark, 1976). Electroencephalograph recordings from the olfactory bulb demonstrate activity coincident with buccal oscillations, demonstrating that this behavior is equivalent to sniffing in crocodilians (Huggins *et al.*, 1968; Naifeh *et al.*, 1970). Because of this demonstrated correlation, buccal pumping is used as a metric of olfactory sampling in some studies. For example, analysis of buccal pumping demonstrates that juvenile alligators (*Alligator mississippiensis*) respond to odorants from food (Weldon *et al.*, 1992). Other studies further demonstrate that olfactory cues play a role in localization of food (Scott and Weldon, 1990; Weldon *et al.*, 1990).

Crocodilians have prominent paracloacal and gular glands, which are suspected to play a role in territorial and sexual behavior (Weldon and Ferguson, 1993). Recent work has described the chemistry of the secretions from these glands (e.g., Garcia-Rubio *et al.*, 2002; Ibrahim *et al.*, 1998; Wheeler *et al.*, 1999; Yang *et al.*, 1999), but the behavioral significance of these compounds has not yet been elucidated. Behavioral observations suggest that crocodilians rub scent glands during courtship and nesting (Weldon and Ferguson (1993) and references therein), suggesting that pheromonal cues may play a role in crocodilian reproductive behavior.

The vomeronasal system has been lost in crocodilians. The vomeronasal organ begins to develop in the embryos of some alligators and crocodiles, but regresses by the time of hatching (Parsons, 1959a).

The accessory olfactory bulb is similarly present in crocodilian embryos but not adults (Parsons, 1967, 1970).

To our knowledge, the ultrastructure of the olfactory epithelium in crocodilians has not been described. The detailed anatomy of the olfactory bulb and tracts has been examined in young *A. mississippiensis* (Crosby, 1917). In these animals, clear laminae, including external and internal plexiform layers, are present. The glomeruli are surrounded by somata of periglomerular cells, which are clearly present. The mitral cells project primary dendrites into two or more glomeruli, but also possess secondary dendrites that extend laterally through the external plexiform layer. Somata in the external plexiform layer may belong to tufted cells, but could also belong to displaced mitral cells. The granule cell layer contains three classes of cells. The first are anaxonal intrinsic cells, with spiny dendrites that extend in all directions within the layer. The second are granule cells with spiny dendrites that project to the glomerular layer but do not seem to arborize inside glomeruli; Crosby (1917) called these 'stellate' cells. The axons of these cells enter the olfactory tracts and may project out of the olfactory bulb. The stellate cells have the smooth dendrites that arborize in glomeruli, and axons that project out of the olfactory bulb; Crosby (1917) called these cells 'goblet' cells.

The central projections of the olfactory bulb have been described in *A. mississippiensis* based on Golgi-stained material (Crosby, 1917) and in *Caiman sklerops* based on a degenerating fiber stain (Scalia *et al.*, 1969). In *Alligator*, as in garter snakes, three centripetal tracts emerge from the olfactory bulb. The individual tracts could not be visualized in *Caiman*, but where the same target was noted in both species we will assume that the same tract carries the fibers to it. The lateral tract projects bilaterally to the lateral cortex, amygdala, and lateral portion of the olfactory peduncle. The medial tract projects to the anterior hippocampus and medial septum. The intermediate tract projects to the nucleus of the diagonal band of Broca and bilaterally to the olfactory tubercle. An additional small projection to the internal plexiform layer of the contralateral olfactory bulb was observed in *Caiman*.

Medina *et al.* (2005) report that Nile crocodiles (*Crocodylus niloticus*) possess a GnRH-immunoreactive terminal nerve, but a detailed description of the pathway has not been published. The terminal nerve of the spectacled caiman (*Caiman crocodilus*) displays FMRFamide-like immunoreactivity in embryos, but not adults (D'Aniello *et al.*, 1999, 2001).

17.2.6.2.(iv) Birds Although olfaction used to be considered unimportant or even absent in birds, birds possess a robust olfactory system that mediates many types of behavior. Notably, olfaction has been shown to play a role in food finding in brown kiwis (*Apteryx australis*; Wenzel, 1968, 1971), and in some, but not all, species of vultures (Graves, 1992; Houston, 1984), as well as in ravens (*Corvus corax*; Harriman and Berger, 1986), parrots (*Strigops habroptilus*; Hagelin, 2004), and procellariiforms, the group of Antarctic seabirds that includes shearwaters, petrels, and albatrosses (Hutchison and Wenzel, 1980). Some procellariid species are highly sensitive to specific odorants such as dimethyl sulfide and 3-methyl pyrazine that are associated with krill, an important and patchily distributed food source (Nevitt, 2000; Nevitt and Haberman, 2003; Nevitt *et al.*, 1995, 2004). Olfaction plays a role in both feeding and predator avoidance in chickens (reviewed in Jones and Roper, 1997).

Olfaction has also been implicated in homing and navigation by passerines, which may use stable features such as the presence of airborne hydrocarbons to orient within landscapes (reviewed in Wallraff, 2003, 2004). Nevitt and Bonadonna (2005) suggest that Procellariiforms may use dimethyl sulfide in a similar fashion for navigating to small islands in large open ocean areas.

The role of olfaction in reproduction has been examined in only a handful of species. Crested auklets (*Aethia cristatella*) produce a scent that is attractive to conspecifics, which humans perceive as resembling tangerine odor (Hagelin *et al.*, 2003). Antarctic prions, *Pachiptila desolata*, can discriminate between chemical cues from their partners and from other birds in the breeding colony, demonstrating that this species is capable of individual recognition based on odorant cues (Bonadonna and Nevitt, 2004).

Many vertebrates show learned preferences for odorants experienced before birth or hatching, as do chicks, *G. domesticus*, which could use such cues to recognize and orient to the nest (Sneddon *et al.*, 1998). Prions and petrels (Procellariidae), that nest in burrows and return to them at night, use olfactory cues to find their own burrow, but olfaction appears to be unimportant for nest recognition in diurnal and surface-nesting species (Bonadonna and Bretagnolle, 2002; Bonadonna *et al.*, 2001, 2003a, 2003b, 2004). The use of acoustic cues to recognize partners and chicks might attract avian

predators; thus, the use of chemical cues by some species may represent an adaptation for avoiding predation by other birds (Bonadonna *et al.*, 2003a). In some species, olfactory cues play an additional role in nesting: both European starlings (*Sturnus vulgaris*) and blue tits (*Parus caeruleus*) use olfactory cues to select plant leaves that are used as nest fumigants (Clark, 1991; Clark and Mason, 1985, 1987; Petit *et al.*, 2002).

Physiological studies demonstrate that the components of the olfactory system function similarly in birds and other groups of vertebrates. At the level of the olfactory epithelium and nerve, both excitatory and inhibitory responses can be observed in a wide range of odorants (Jung *et al.*, 2005; Shibuya and Tucker, 1967; Tucker, 1965). Similarly, single-unit recordings from the olfactory bulb demonstrate both excitatory and inhibitory responses to odorants (McKeegan, 2002; and references therein), and an electroencephalography study with chickens found no significant differences in responses to odorants relative to those recorded in mammals using the same technique (Oosawa *et al.*, 2000).

Birds lack a vomeronasal organ and accessory olfactory bulb (Huffman, 1963; Parsons, 1959b). The olfactory epithelium is located on a single spiral-shaped turbinate bone inside the nasal cavity, and air is drawn across it as the animal breathes in and out (Bang and Wenzel, 1985). The anatomy of the olfactory epithelium has been examined in members of five orders of birds (Anseriformes, Charadriiformes, Ciconiiformes, Columbiformes, and Galliformes), and all possess unusual olfactory receptor neurons that are capped with cilia surrounded by short microvilli (Bedini *et al.*, 1976; Brown and Beidler, 1966; Drenckhahn, 1970; Graziadei and Bannister, 1967; Matsuzaki *et al.*, 1982; Müller *et al.*, 1979; Okano and Kasuga, 1980).

Of the 20 000–23 000 genes in the genome of jungle fowl (*Gallus gallus*), 283 odorant receptor genes and ~100 odorant receptor pseudogenes have been identified (Hillier *et al.*, 2004); however, a study of domesticated chickens (*Gallus domesticus*) reported only 12 odorant receptor genes (Nef and Nef, 1997). It is not clear whether this large discrepancy is due to methodological differences, or to loss of genes as a result of domestication. The odorant receptor genes in galliforms are evolutionarily more closely related to those found in mammals than to those found in aquatic vertebrates, like teleost fishes (Niimura and Nei, 2005).

The size of the olfactory bulb relative to the brain varies considerably across birds (Bang and Cobb, 1968), and some species have small, conjoined bulbs whereas others have large, obvious olfactory bulbs (Nieuwenhuys, 1967). Lamination appears to be highly variable across taxa, with small, indistinct layers in species that have tiny olfactory bulbs (Nieuwenhuys, 1967) but clear cellular laminae with internal and external plexiform layers visible even in species with moderately sized olfactory bulbs, such as chickens and pigeons (McKeegan, 2002; Rieke and Wenzel, 1978). We are unaware of any Golgi studies of the olfactory bulbs in birds; thus, the cell types present and the details of cellular morphology are largely unknown. Rieke and Wenzel (1978) speculate that somata visible in the external plexiform layer of pigeons may belong to tufted cells.

An early study with pigeons (*Columba livia*) using a combination of electrophysiology and degenerating fiber stains demonstrated ipsilateral projections to the piriform cortex, hyperstriatum ventrale and the medial striatum, as well as projections to the contralateral globus pallidus and caudal portion of the medial striatum (Rieke and Wenzel, 1978; terminology after Reiner *et al.*, 2004). The authors of this study did not distinguish among the different olfactory tracts. The results of later study with the same species indicate that the medial olfactory tract projects ipsilaterally to the septum and to a region dorsal to this, that an intermediate tract projects to the olfactory tubercle and medial striatum, and that the lateral olfactory tract projects bilaterally to the piriform cortex and nucleus taeniae of the amygdala (Reiner and Karten, 1985; terminology after Reiner *et al.*, 2004). The authors note that the projection to the amygdala in birds is more restricted than in some other groups, including turtles, which could be related to the lack of a vomeronasal system in birds. The differences in results obtained in the two studies are difficult to understand, as they do not appear to be attributable to simple differences in nomenclature or mistaken identification of cell groups. In young ducks (*Anas platyrhynchos*), the medial olfactory tract projects to the dorsomedial hippocampus and superior frontal lamina, whereas the lateral tract terminates in the pallial–subpallial lamina, medial striatum, dorsomedial telencephalic wall, and posterior pallial amygdala (Teuchert *et al.*, 1986; terminology after Reiner *et al.*, 2004). An intermediate olfactory tract has not been described in this species.

The existence of an extrabulbar olfactory pathway in birds has not been directly demonstrated, but a study in which horseradish peroxidase was injected into the nasal cavity in ducks (*Anas platyrhynchos*) labeled a pathway that proceeded through the ventral olfactory bulb (Meyer *et al.*, 1987; von

Bartheld *et al.*, 1987). The terminations of these fibers were not observed, but ganglion cell bodies were not found and the injection is likely to have labeled primary olfactory receptor neurons.

As in other jawed vertebrates, the terminal nerve in birds arises from the nasal placode and, during development, demonstrates immunoreactivity to both GnRH and FMRFamide (Norgren and Lehman, 1991; Norgren *et al.*, 1992; Wirsig-Wiechmann, 1990; Yamamoto *et al.*, 1996). A FMRFamide-immunoreactive terminal nerve has been described in adult Japanese quail (*Coturnix japonica*), but the anatomy and chemical characteristics of the terminal nerve have not been studied in detail in adult birds (Fujii and Kobayashi, 1992).

17.2.6.2.(v) Turtles Turtles live in diverse habitats, ranging from completely terrestrial tortoises to fully aquatic sea turtles. Many freshwater species are semi-aquatic. Turtles sample the olfactory environment both on land and under water, and in both cases, sniffing involves throat movements that resemble those used by crocodilians and amphibians (Belkin, 1968; McCutcheon, 1943; Root, 1949). As an adaptation for prolonged diving, many species are remarkably tolerant of anoxia (Lutz and Milton, 2004). This trait makes turtles well suited for electrophysiological experiments, and many studies have used turtles as model animals for understanding general principles of olfactory system function in vertebrates. These studies will not be comprehensively reviewed here.

Sea turtles can swim thousands of miles to particular nesting beaches, and olfactory cues have been suggested to play a key role in this behavior. The available data indicate that olfactory-based homing cannot account for long-distance migration in turtles (reviewed in Lohmann *et al.*, 1999). However, some studies indicate that hatchlings may imprint on odorants specific to their local environment, suggesting that such cues could play a role in short-range orientation or selection of nesting sites (Grassman, 1993; Grassman and Owens, 1987; Grassman *et al.*, 1984). In addition, freshwater turtles (*Chrysemys picta*) can use chemical cues to discriminate water from home ponds versus that from ponds with and without conspecifics (Quinn and Graves, 1998).

The importance of chemical cues in foraging and feeding has not the subject of extensive study in turtles. Nevertheless, Honigmann (1921) showed that both aquatic and semi-aquatic species will bite at a bag filled with fish, but not at one filled with sand. Olfaction has also been shown to play a role in foraging in leatherback turtles, *Dermochelys coriacea*, in a laboratory setting (Constantino and Salmon, 2003).

Olfactory cues play a role in courtship and reproduction in turtles. Many turtles are endowed with secretory glands (Ehrenfeld and Ehrenfeld, 1973), although the behavioral significance of these secretions has not been thoroughly studied. Males of many species sniff or bob their heads when stimulated by odorants from female scent glands (e.g., Auffenberg, 1978; Kaufmann, 1992; Rose, 1970). Some species, such as the musk turtle *Sternotherus odoratus*, produce chemicals that probably serve as a deterrent or aposematic signal to predators, but could also function in intraspecific communication (Eisner *et al.*, 1977). The size of chin glands in male desert tortoises (*Gopherus agassizii*) is testosterone dependent, varying seasonally and with dominance status (Alberts *et al.*, 1994). Both males and females can discriminate individuals based on secretions from these glands (Alberts *et al.*, 1994). In male Berlandier's tortoise, *Gopherus berlandieri*, fatty acids from chin glands elicit aggressive behavior in males and cause females to approach and bob their heads at a model painted with these compounds (Rose, 1970; Rose *et al.*, 1969). In contrast, in desert tortoises (*Gopherus agassizii*), males prefer to use burrows scented with chin gland rubbings from conspecific males compared with untreated burrows (Bulova, 1997). Male stripe-necked terrapin (*Mauremys leprosa*) have been shown to avoid water conditioned by other males and prefer water conditioned by conspecific females, but only during the breeding season (Muñoz, 2004). The results of these studies clearly indicate that chemical signals play a role in social and reproductive behavior in some turtles.

The organization of the nasal cavity and vomeronasal organ varies considerably among species. In some, such as *Testudo* or *Emys*, the vomeronasal epithelium is not contained in a separate organ, but lies along the ventromedial wall or in grooves in the floor of the nasal cavity; the olfactory and vomeronasal regions of the nasal cavity are separated only by a slight horizontal ridge (Parsons, 1959a, 1959b). In loggerhead turtles, *Caretta caretta*, the vomeronasal epithelium is more widely distributed in the nasal cavity than is the olfactory epithelium (Saito *et al.*, 2000). On the other hand, in *Dipsochelys* and *Dermochelys coriacea*, the vomeronasal organ is a discrete structure that is encapsulated in bone and opens into the oral cavity (Gerlach, 2005). A comparison of axon counts indicates that olfactory receptor neurons outnumber vomeronasal receptor neurons in the Russian tortoise (*Testudo horsfieldii*), which is terrestrial, but

that two semi-aquatic species (*Chinemys reevesii* and *Mauremys japonica*) have more vomeronasal than olfactory receptor neurons (Hatanaka and Matsuzaki, 1993).

As in some other reptiles, the dendrites of the olfactory receptor neurons in Hermann's tortoise, *Testudo hermanni*, bear both cilia and numerous short microvilli (Delfino *et al.*, 1990). Like other tetrapods, turtles possess only microvillar vomeronasal receptor neurons (Graziadei and Tucker, 1970; Hatanaka *et al.*, 1982). Electrophysiological recordings from both the vomeronasal epithelium and accessory olfactory bulb in *Geoclemys reevesii* demonstrate that the vomeronasal system in turtles responds to general odorants with no inherent behavioral significance, such as amyl acetate, geraniol, cineole, and citral (Hatanaka and Matsuzaki, 1993; Hatanaka and Shibuya, 1989; Shoji *et al.*, 1993; Shoji and Kurihara, 1991). Recordings from dissociated vomeronasal receptor neurons in stinkpot turtles, *Sternotherus odoratus*, demonstrate that turtle vomeronasal cells also respond to a variety of complex natural odorants, including urine and musk from both males and females, as well as odorants derived from food pellets (Fadool *et al.*, 2001). $G\alpha_o$ is expressed in different vomeronasal receptor neurons than is $G\alpha_{i1-3}$, although the zonal segregation of expression seen in mammals does not occur in *Sternotherus* (Murphy *et al.*, 2001). In the same species, females show higher levels of $G\alpha_{i1-3}$ expression and lower levels of TRP2 immunoreactivity than do males (Murphy *et al.*, 2001), and odorant responses recorded from vomeronasal receptor neurons from females are larger than those from males (Fadool *et al.*, 2001). Taken together, these data demonstrate that the vomeronasal system in turtles responds to a wide range of chemicals, including cues that may be involved in intraspecific communication, and that the functioning of the system may be sexually dimorphic.

The turtle olfactory bulb is highly laminar, with both external and internal plexiform layers separating the cell layers (Johnston, 1915; Orrego, 1961). The glomeruli are surrounded by periglomerular cells (Orrego, 1961). Mitral cells usually extend primary dendrites into two glomeruli, and possess long secondary dendrites that project through the external plexiform layer (Mori *et al.*, 1981; Orrego, 1961). Tufted cells may be present; Johnston (1915) called these 'brush cells'. Two classes of granule cells are present, one of which has processes that arborize in glomeruli (Johnston, 1915; Orrego, 1961). As in frogs, the olfactory bulbs are fused in some species, with continuous layers and some neurites interacting across the midline (Skeen and Rolon, 1982).

As in other reptiles, three central olfactory tracts have been described in turtles. The medial olfactory tract projects ipsilaterally to the septum and to a medial cortical area that may be the homologue of the medial pallium/hippocampus of other vertebrates (Johnston, 1915; Reiner and Karten, 1985). The lateral olfactory tract has a massive bilateral projection to a lateral cortical area, and an intermediate olfactory tract that may be a subdivision of the lateral tract projects to the olfactory tubercle and the basal amygdaloid nucleus (Gamble, 1956; Reiner and Karten, 1985). In addition, a large olfactory projection to the entire pial surface of the amygdala was described in *Trachemys scripta* (Reiner and Karten, 1985). Given that the accessory olfactory bulb in turtles is sometimes included in injections or lesions of the main olfactory bulbs (Chkheidze and Belekhova, 2005; Gamble, 1956), the description of the large amygdalar projection in *Trachemys* may be due in part to inclusion of projections from the accessory olfactory bulb (see discussion in Eisthen, 1997). Because the separate projections of the accessory olfactory bulb in turtles have not been described, it is not clear whether the main and accessory olfactory systems project to different portions of the amygdala, as in other tetrapods (Chkheidze and Belekhova, 2005).

The development of the terminal nerve in turtles has been described by Johnston (1913) and Larsell (1917), who were able to visualize the nerve as it courses over the surface of the olfactory nerve due to its several conspicuous ganglia. To our knowledge, the histochemical characteristics of the nerve have not been examined in detail in turtles, although FMRFamide-immunoreactive cells and fibers do not appear to be present in or around peripheral olfactory structures in adult *Trachemys scripta* (D'Aniello *et al.*, 2001, 1999).

17.2.6.3 Mammals The comparative neurobiology of the olfactory system in mammals will not be described in detail here. Nevertheless, the textbook view of the organization of the vertebrate olfactory system typically includes many features that are unique to mammals, which should be noted by those interested in understanding the structure and function of the olfactory system in vertebrates in general. For example, the olfactory epithelium in mammals contains only ciliated olfactory receptor neurons (reviewed in Eisthen, 1992), whereas in other vertebrates, the morphology of olfactory receptor neurons varies considerably. As in other tetrapods, the vomeronasal receptor neurons in mammals are microvillar (Eisthen, 1992).

The organization of the olfactory bulb of mammals is similar to that of other tetrapods, with a distinct laminar organization that includes large plexiform layers. In mammals, the mitral cells possess a single primary dendrite that arborizes in one glomerulus, and prominent secondary dendrites that extend orthogonal to the primary dendrite through the external plexiform layer (reviewed in Eisthen, 1997; Nieuwenhuys, 1967). In addition to mitral cells, mammals possess tufted cells, a second class of output cell (Nieuwenhuys, 1967; Pinching and Powell, 1971). Although the somata of most tufted cells are found in the external plexiform layers, another group, the external tufted cells, have cell bodies in the glomerular layer; the glomerular layer also contains short-axon cells with no clear counterpart among nonmammalian vertebrates (Pinching and Powell, 1971). The lateral olfactory tract projects ipsilaterally, and not bilaterally as in other vertebrates (Skeen *et al.*, 1984). Mammals may lack a true medial olfactory tract that arises from the olfactory bulb; instead, the tract of the same name in mammals appears to arise from the anterior olfactory nucleus (Lohman and Lammers, 1967; Nieuwenhuys, 1967). The vomeronasal system is generally present in mammals, but has been lost in cetaceans as well as in some bats and primates (reviewed in The Vomeronasal Organ and Its Evolutionary Loss in Catarrhine Primates).

17.3 Conclusions

In this section, we integrate the preceding information, both to describe patterns of change and to consider the functional implications of these changes. In general, we will not cite references for information presented earlier, as details are provided in the sections pertaining to each taxonomic group.

17.3.1 Evolutionary Changes in the Organization of the Olfactory Epithelium

Before odorants contact the sensory receptor cells, they pass through a mucous layer. This mucus contains odorant binding proteins, which are known to be present in terrestrial mammals and in frogs. Interestingly, in *Xenopus*, the binding proteins are expressed in the principal cavity, which is used for detecting odorants in air, but not in the middle cavity, which is used for detecting odorants in water. Odorant binding proteins have been suggested to represent an adaptation for detecting odorants in air, to transport hydrophobic molecules through the mucous layer to the receptor neurons. Another possibility is that odorant binding proteins are too energetically expensive for use by aquatic vertebrates, as the hydrophilic proteins could easily dissolve in the water flowing over through the nasal sac (discussed in Eisthen, 2002). Although their presence in *Xenopus* and mammals suggests that odorant binding proteins should be broadly present in terrestrial tetrapods, Baldaccini *et al.* (1986) were unable to find binding proteins in birds (*Columba livia* and *Cairina moschata*) and turtles (*Testudo hermanni*), despite being able to sequence them from several mammalian species. Given that the odorant binding proteins in both *Xenopus* and mammals are lipocalins, it is surprising that these proteins have not been found in other classes of vertebrates. Perhaps different types of molecules are used as binding proteins in other groups of vertebrates, or perhaps the presence of these molecules in both *Xenopus* and mammals is convergent and not informative about tetrapods generally.

Although vomeronasal receptor neurons invariably terminate in microvilli, vertebrate olfactory receptor neurons are morphologically diverse; their phylogenetic distribution is shown in Figure 10. Researchers have long considered the possibility that the microvillar olfactory receptor

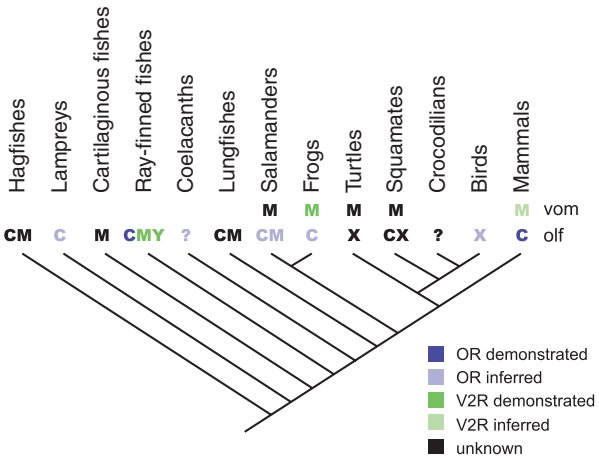

Figure 10 Phylogenetic distribution of receptor cell types and correlation with expression of olfactory (OR) and vomeronasal (V2R) receptor genes in the olfactory (olf) and vomeronasal (vom) epithelia. The expression of genes is demonstrated with electron microscopy; otherwise, it is inferred. Boldface letters indicate cell types: C, ciliated receptor neuron; M, microvillar receptor neuron; X, receptor neuron with both cilia and microvilli; Y, crypt-type receptor neuron. ? indicates that the morphology of the receptor neurons is unknown. Salamanders possess both ciliated and microvillar receptor neurons, but we do not yet know whether one or both express olfactory-type odorant receptor genes. Crocodilians, birds, and all fishes lack a vomeronasal organ. This diagram is simplified, as it assumes homogeneity within large taxonomic groups, such as teleost fishes. See text for references.

neurons in teleosts could be homologous with mammalian vomeronasal receptor neurons (discussed in Dulka, 1993; Eisthen, 1992). Recent data demonstrating that the olfactory epithelium of teleost fishes contains ciliated receptor neurons that express genes typical of olfactory receptor neurons, and microvillar neurons that express genes typical of vomeronasal receptor neurons may bolster this impression. Nevertheless, the expression of olfactory- or vomeronasal-typical genes may not correlate tightly with cell morphology (Figure 10). Further, if one assumes that the correlation applies to all craniates, then unlikely patterns are predicted to emerge. For example, although hagfish have both ciliated and microvillar olfactory receptor neurons, lampreys possess only ciliated olfactory receptor neurons, and those of sharks and skates terminate in microvilli. If the association between receptor cell morphology and gene expression arose with the earliest craniates, we would expect to see only olfactory-type genes expressed in lampreys, and only vomeronasal-type genes expressed in sharks and skates. Thus, the apparent correlation between receptor cell morphology and gene expression observed in teleosts and mammals may be a coincidence, may represent examples of convergent evolution, or may represent a derived condition that pertains only to bony vertebrates.

Alternatively, the morphological categories we are using may be too crude. For example, distinct subsets of microvillar and ciliated olfactory receptor neurons project to different regions of the olfactory bulb in channel catfish, *Ictalurus punctatus* (Morita and Finger, 1998). Similarly, in goldfish (*Carassius auratus*), the microvillar receptor neurons that are immunoreactive for $G\alpha_q$ are shorter and have more stiff microvilli than other microvillar receptor neurons (Hansen *et al.*, 2004). If 'ciliated' and 'microvillar' receptor neurons in vertebrates actually comprise several subclasses of cells, cell morphology and receptor gene expression may be tightly correlated within specific subclasses of receptor neurons. If so, some of the vomeronasal-type microvillar receptor neurons in goldfish could be homologous with vomeronasal receptor neurons in mammals. On the other hand, microvillar olfactory and vomeronasal receptor neurons often contain centrioles and basal bodies, suggesting that they derive evolutionarily from ciliated cells (Delfino *et al.*, 1990). If ciliogenesis is suppressed in microvillar cells (Kolnberger and Altner, 1971; Pyatkina, 1976), then receptor cell morphology might be evolutionarily labile, with no fixed relationship between receptor cell morphology and receptor gene expression.

17.3.2 Evolutionary Changes in the Organization of the Olfactory Bulbs

The organization of olfactory bulb circuits differs among vertebrate groups. Figure 11 illustrates the phylogenetic distribution of the cellular elements of the olfactory bulb. In interpreting the significance of these patterns, it is important to note that we assigned names to the different cell types based on their morphology alone, and did so independently of the names used by the authors of the source material. Thus, we use the term 'stellate cell' for any cells

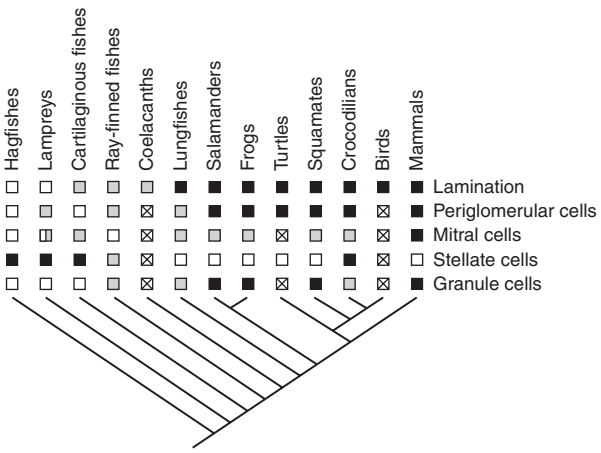

Figure 11 Phylogenetic distribution of cellular elements of olfactory bulb circuitry. The hypothesized ancestral condition, illustrated by hagfish, includes a low degree of lamination, with cell bodies frequently located across laminar boundaries and a lack of plexiform layers; periglomerular cells absent; mitral cells with primary dendrites that arborize in more than one glomerulus and no secondary (basal) dendrites; stellate cells present, with smooth dendrites that arborize in glomeruli; and granule cells bearing smooth dendrites, with an axon present. Boxes with crosses indicate cases for which information is unavailable. Lamination: white box low degree of lamination, with cell bodies frequently located across laminar boundaries and a lack of plexiform layers; gray moderate degree of lamination, with cell bodies confined to clear layers, and a lack of plexiform layers; black high degree of lamination, with cell bodies confined to clear layers, separated by one or more plexiform layers. Periglomerular cells: white absent; gray ambiguous condition, in which candidate periglomerular cells have been described by some authors; black periglomerular cells present. Mitral cells: white primary dendrites arborize in more than one glomerulus, with no secondary dendrites; white/gray primary dendrites arborize in only one glomerulus, with no secondary dendrites; gray primary dendrites arborize in more than one glomerulus, with secondary dendrites that extend laterally; black primary dendrites arborize in only one glomerulus, with secondary dendrites that extend laterally. Stellate cells: white absent; gray stellate cells present, with spiny dendrites that arborize in glomeruli; black stellate cells present, with smooth dendrites that arborize in glomeruli. Granule cells: white smooth dendrites, with an axon present; gray spiny dendrites, with axon present; black spiny dendrites, with no axon. See text for references.

with a soma in the deep layers of the bulb, and with multiple dendrites that arborize in glomeruli. The somata of stellate cells are generally star-shaped, as the name implies. A 'granule cell' is a cell with an oval-shaped soma located in a deep layer of the bulb, and with dendrites that project upward but that do not enter or arborize in glomeruli. The dendrites of granule cells generally bear spines, whereas those of stellate cells are generally smooth. In many groups, both types of cells have a long axon. 'Periglomerular' cells have a soma in the glomerular layer, with dendrites that arborize in several glomeruli. In many groups, these cells have an axon that projects at least to deeper layers of the bulb. Ideally, additional criteria would be used to recognize cell types, such as data concerning the neurotransmitter characteristics of the cells. Unfortunately, such data are available for an extremely limited set of species, and cannot be used for broad phylogenetic comparisons. Thus, the categories we are using are quite broad, and in at least some groups probably encompass several recognizably different types of cells; for example, Golgi data indicate that multiple classes of mitral cells are present in some animals, and histochemical data indicate that several types of periglomerular cells are present in some species.

With these caveats in mind, the pattern depicted in Figure 11 indicates that the organization of the olfactory bulb did not undergo any sudden, dramatic shifts over the course of vertebrate evolution. Rather, changes in organization occurred as a series of steps. Laminar organization of brain structures is generally regarded as indicative of multiple stages of integration, with cells in one layer receiving processed input from cells in other layers and then processing these signals in more extensive ways. What are the functional consequences of the almost complete lack of lamination observed in hagfish or lampreys, or the high degree found in squamates or mammals? It is difficult to make predictions based on this feature alone, particularly because the numbers of cell types with axons that project to secondary processing areas differs considerably among these groups. Perhaps more processing occurs in the olfactory bulbs in some animals, and in secondary olfactory regions in others.

The textbook view of a mitral cell is one with a single primary dendrite that extends to one glomerulus, and prominent secondary dendrites that extend laterally to interact with dendrites of granule cells as well as secondary dendrites of other mitral cells. Nevertheless, this type of mitral cell is present only in mammals, and in most vertebrates, mitral cells have multiple primary and secondary dendrites. The breadth of inputs to mitral cells therefore differs considerably among groups. In mammals, each olfactory receptor neuron is believed to express only one odorant receptor gene, and receptor neurons that express the same gene project to the same glomerulus (reviewed in Mombaerts, 2004). Thus, mitral cells with dendrites in a single glomerulus receive inputs from a homogeneous population of receptor neurons. In contrast, olfactory receptor neurons in teleost fishes may express more than one receptor gene (Ngai *et al.*, 1993a; Speca *et al.*, 1999), and mitral cells in these animals extend dendrites to several glomeruli. Perhaps these glomeruli receive inputs from receptor neurons that express one receptor gene in common, in which case the type of coding occurring in the olfactory bulbs of teleosts and mammals may be similar. If not, the nature or location of odorant information processing in teleosts may differ considerably from that in mammals, which seems quite possible given that teleosts possess stellate cells, which mammals lack, and that the stellate and granule cells in teleosts have long axons that may project to secondary olfactory regions in the telencephalon.

Other aspects of bulbar circuitry differ among groups, although the functional consequences are not easy to predict. For example, morphologically distinct periglomerular cells are present only in tetrapods, but other cells, such as the displaced mitral cells in the glomerular layer of hagfish and lampreys, or even stellate cells, may serve similar functions. Similarly, a dorsal commissure connecting the two olfactory bulbs is unique to hagfish and lampreys, but other types of connections between the bulbs exist in other groups. For example, in frogs and some turtles and birds, the two olfactory bulbs are fused across the midline, with mitral and granule cell dendrites apparently integrating inputs from both sides. In other animals, such as the hedgehog *Erinaceus europaeus*, the olfactory bulb projects ipsilaterally to the anterior olfactory nucleus, which sends fibers to the mitral cell layer of the contralateral olfactory bulb (De Carlos *et al.*, 1989). These different anatomical arrangements may facilitate integration of inputs to the two nares, or perhaps bilateral comparison of inputs to enhance localization of odorant sources.

Granule cells lack dendritic spines in hagfish, lampreys, and cartilaginous fishes, and functionally equivalent circuitry is probably lacking. The presence of spines varies even within groups: for example, although granule cell dendrites are spiny in most amphibians, Herrick's (1931) studies indicate that the dendrites are smooth in mudpuppies

(*Necturus*). What are the functional consequences of this diversity? Given that dendritic spines are often involved in plasticity in other regions of the central nervous system, does this suggest that olfactory bulb circuits in animals lacking spines are more hard-wired? Alternatively, or in addition, perhaps less compartmentalization of processing occurs in the olfactory bulbs of animals with granule cells that lack dendritic spines (e.g., Woolf *et al.*, 1991). Finally, additional types of output cells have been described in some animals, such as the ruffed cells present in teleost olfactory bulbs, or the tufted cells present in reptiles and mammals. Without intensive electrophysiological and neurochemical studies of the olfactory bulb circuits in diverse vertebrates, the functional significance of these differences in organization are likely to remain mysterious. Given that we also lack detailed psychophysical data concerning the relative capabilities of the olfactory system in diverse vertebrates, the behavioral consequences of differences in circuitry cannot be predicted.

17.3.3 Evolutionary Changes in the Organization of Central Olfactory Projections

Broad patterns of change in the organization of central olfactory pathways are illustrated in Figure 12. Most vertebrates possess a medial olfactory tract that projects to the septum. The tract has been lost in mammals and may also be lost in cartilaginous fishes, although the central projections in this group must be examined using modern tract-tract methods before strong conclusions can be drawn. In tetrapods, the tract may acquire a projection to the medial pallium/hippocampus. Such a projection may also be present in ray-finned fishes, but given the confusion concerning pallial homologies in this group, we cannot reach a conclusion concerning this matter at present. Nevertheless, the medial pallium/hippocampus is generally involved in memory and spatial perception, and one might expect that olfactory input to this region would be behaviorally important for many vertebrates.

In general, a lateral olfactory tract is present and projects bilaterally to lateral and dorsal pallial or cortical areas. Although it is tempting to interpret this connectivity as a conserved feature that must be critical for processing olfactory information in all vertebrates, in many animals the homologies of pallial areas are based largely on their receipt of olfactory input. Thus, it would be circular to argue that this projection is conserved or that the lateral olfactory tract projects to homologous areas in diverse vertebrates. One clear trend is that the lateral olfactory tract has extensive bilateral projections to both dorsal and ventral telencephalic regions in hagfish and lampreys, and that these projections are more restricted in jawed vertebrates. In addition, the lateral olfactory tract projects to the striatum in most groups discussed in this review, but not in reptiles; instead, an intermediate olfactory tract projects to the olfactory tubercle in the ventral striatum. This shift in connectivity suggests that the intermediate olfactory tract may be a branch of the lateral olfactory tract, or may have arisen from it evolutionarily. Some support for this idea comes from data demonstrating that the intermediate and lateral olfactory tracts project together to the contralateral hemisphere in snakes (Lanuza and Halpern, 1998), and that the two tracts run in partial continuity with each other through the rostral forebrain in turtles (Reiner and Karten, 1985).

A discrete vomeronasal system, including an accessory olfactory bulb and tract, is present only in tetrapods. The main central target of the accessory olfactory tract is the amygdala. The lateral

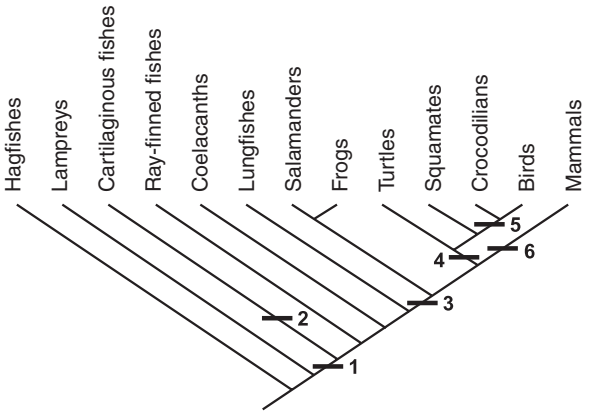

Figure 12 Evolutionary changes in the central projections of the olfactory bulbs. The hypothesized ancestral condition includes the presence of a lateral olfactory tract that projects bilaterally to lateral pallial/cortical areas and the striatum; the presence of a medial olfactory tract that projects ipsilaterally to the septum; and an extrabulbar olfactory pathway. Numbers indicate hypothesized changes in connectivity. 1, Reduction in the extent of projections of the lateral olfactory tracts. 2, Possible loss of the medial olfactory tract and bilateral projections of the lateral tract. 3, Origin of a distinct vomeronasal system, including an accessory olfactory tract that projects to the amygdala. Origin of an olfactory projection to separate portions of the amygdala via the lateral olfactory tract. Origin of an olfactory projection to the medial pallium / hippocampus via the medial olfactory tract. 4, Origin of an intermediate olfactory tract that projects to the olfactory tubercle, and loss of olfactory projection to the striatum via the lateral olfactory tract. 5, Loss of the vomeronasal system. 6, Loss of the medial olfactory tract and the contralateral projection of the lateral olfactory tract. See text for references.

olfactory tract also projects to the amygdala, but the specific regions of the amygdala that receive input from the lateral and accessory olfactory tracts show little or no overlap in the species studied to date. Clear olfactory projections to the amygdala have been recognized only in tetrapods; thus, the origin of an olfactory bulb projection to the amygdala correlates roughly with the origin of the vomeronasal system. Given that the portions of the amygdala that receive olfactory and vomeronasal input are interconnected (reviewed in Halpern and Martínez-Marcos, 2003), perhaps this olfactory projection arose to facilitate integration of information from the two systems. If so, integration of chemosensory inputs cannot be the only current function of the olfactory projection to the amygdala, as this projection is retained in crocodilians and birds, which have lost the vomeronasal system. An olfactory projection to the amygdala may also exist in ray-finned fishes, but the homologies of possible amygdala-equivalent areas in ray-finned fishes are controversial (reviewed in Northcutt, 2006). Given that the vomeronasal-like and olfactory-like receptor neurons in teleosts project to different portions of the olfactory bulb, perhaps tracing the central projections of these two regions would provide new insight into the organization of the amygdala in teleosts.

17.3.4 Evolution of Vertebrate Olfactory Subsystems

A common assertion is that the olfactory system is phylogenetically ancient, or that olfaction is the oldest sensory system. The basis of such statements is unclear. Olfactory systems in animals in several phyla, including nematodes, mollusks, arthropods, and vertebrates, possess olfactory systems with similar features, but these features probably arose independently in each group, in response to similar constraints and as adaptations for similar tasks (Eisthen, 2002). Perhaps such statements simply indicate that the ability to sense chemicals in the external environment is widespread among animals, although this ability is by no means restricted to metazoans. A third possibility is that such statements are an oblique reference to a long-standing idea that among vertebrates, the forebrain was originally an olfactory structure, and that inputs from other sensory systems 'invaded' the telencephalon via thalamus over the course of vertebrate evolution (Ariëns-Kappers *et al.*, 1936; Edinger, 1904; Herrick, 1948). As described above, the olfactory projections in hagfish and lampreys distribute to larger portions of the telencephalon than do those in jawed vertebrates (Northcutt and Puzdrowski, 1988; Wicht and Northcutt, 1993). Overall, however, the available data clearly demonstrate that the invasion scenario is incorrect (reviewed in Northcutt, 1981).

Two large-scale changes in the organization of the olfactory system have occurred over the course of vertebrate evolution: the origin of the terminal nerve, and the origin of the vomeronasal system. Both hagfish and lampreys appear to lack a terminal nerve, as no projection has been described that comprises a peripheral ganglion and fibers that display the types of immunoreactivity that characterize the terminal nerve (reviewed in Wirsig-Wiechmann *et al.*, 2002a). If so, then the terminal nerve arose in jawed vertebrates. As described above, studies of the terminal nerve ganglion and retina in teleost fishes strongly indicate that the terminal nerve serves a modulatory function, and studies with salamanders demonstrate that terminal nerve-derived peptides modulate activity in the olfactory epithelium. In teleost fishes, the terminal nerve ganglion receives input from the olfactory, visual, and somatosensory systems. In amphibians, the extent to which terminal nerve peptides modulate olfactory epithelial activity depends on the animal's physiological or behavioral state, as both hunger and reproductive condition appear to play a role. Similar centrifugal modulation occurs in the retina and cochlea of many vertebrates (reviewed in Akopian, 2000; Manley, 2000, 2001). Do hagfish and lampreys lack this modulation, or do they possess alternate mechanisms for regulating olfactory responses with regard to their physiological needs? What are the overall functional consequences for animals that possess or lack such mechanisms? Perhaps the more active foraging and courting behaviors of jawed vertebrates benefit from more central control of olfactory epithelial function, which is unnecessary in hagfishes and lampreys.

A discrete vomeronasal system is present only in tetrapods, but recent work, described above, clearly indicates that the elements of the vomeronasal system are present in teleost fishes: the olfactory and vomeronasal receptor genes, as well as their associated G-proteins and ion channels, are expressed in different receptor neurons in the olfactory epithelium. In goldfish (*Carassius auratus*), the vomeronasal-type elements are expressed in microvillar olfactory receptor neurons, whereas the olfactory-type elements are expressed in ciliated receptor neurons (Hansen *et al.*, 2004). Although these morphological cell types are superficially similar to the vomeronasal and olfactory receptor neurons in mammals, as discussed above, it appears

unlikely that the two cell types in teleosts simply segregated into two epithelia to give rise to a separate vomeronasal organ (Eisthen, 2004). In zebra fish (*Danio rerio*), the two classes of receptor neurons send their axons to different portions of the olfactory bulb (Sato *et al.*, 2005), an arrangement similar to that in tetrapods, in which the olfactory and vomeronasal receptor neurons send axons to distinct olfactory and accessory olfactory bulbs. In tetrapods, the projections of the olfactory and accessory olfactory bulb differ, and the next step is to determine whether the portions of the olfactory bulb that receive input from the two cell types in teleosts also have distinct projections. If so, it would appear that teleost fishes have a complete vomeronasal system intermingled with the olfactory system. It would be interesting to know whether the same is true of other classes of fishes; perhaps the vomeronasal system has been present since the origin of vertebrates, and only became separate from the olfactory system in tetrapods. Because the functional differences between the olfactory and vomeronasal systems are unclear (reviewed in Baxi *et al.*, 2006; Halpern and Martínez-Marcos, 2003), it is difficult to speculate about the causes or consequences of the origin of a separate vomeronasal system in tetrapods.

Acknowledgments

The authors thank Anne Hansen for providing them with the lovely electron micrographs in Figure 6. Their research is supported by a grant from the National Institutes of Health (DC05366).

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18 The Evolution of Taste Systems

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Glossary

amphid A paired chemosensory organ at the anterior end of nematodes including *Caenorhabditis elegans*. The amphid is innervated by 11 chemo sensory neurons and one associated thermosensory neuron.

auxiliary cells Nonsensory cells of invertebrate sensilla. This class of cells includes socket cells, sheath cells, tormogen cells, and thecogen cells.

Ecdysozoa One of two major groups of the protostome division of the animal kingdom (cf. Lophotrochozoa). Members of this group, including arthropods and nematodes, pos sess an outer cuticle rather than an internal skeleton.

ecto ATPase An enzyme that breaks down extracellular ATP. The ecto ATPases can be divided into sev eral molecular families.

inner labial sensillum One of eight chemosensory organs around the mouth (stoma) of nematodes. This organ contains only two sensory cells only one of which has free access to the exter nal surface of the worm.

labellum A fleshy ovoid pad at the end of the probiscus of a fly used as a taste organ. The labellum houses numer ous taste and tactile sensilla.

labrum A chemosensory organ in flies situ ated at the anterior end of the oral cavity.

Lophotrochozoa One of two major groups of the protostome divisions of the animal kingdom (cf. Ecdysozoa). Members of this group, including annelids and mollusks, share common devel opmental forms although the adults appear quite diverse.

Merkel cell A specialized epithelial cell found in vertebrates which participates in mechanoreception. Upon stimula tion, Merkel cells release serotonin and ATP to activate a closely asso ciated sensory nerve fiber.

Merkel like basal cell A basal cell of nonmammalian taste buds named because of their similarity with epithelial Merkel cells in terms of structure and neu rotransmitter contents.

Schreiner organ A presumed epithelial chemosensory organ of hagfish. This multicellular end organ is superficially similar, but not homologous, to taste buds. Unlike taste buds, Schreiner organs can be innervated by any epithelial nerve.

sensillum A sensory organ of invertebrates in which the sensory cells extend a hair like process out of the cuticle.

sheath cells The inner non neuronal cell of a sensory end organ of invertebrates, especially *C. elegans*, closely sur rounding the sensory neurons.

socket cells The outer non neuronal cell of an invertebrate sensory organ.

solitary chemoreceptor cell (SCC)	Scattered specialized chemosensory cells in the epidermis of aquatic vertebrates also found in the gut and airways of terrestrial vertebrates.
T1R	A family of mammalian taste receptors that includes three members which heterodimerize to form either sweet or umami receptors.
T2R	A large family of mammalian taste receptors that form bitter sensitive taste receptor molecules.
taste cell	An elongate specialized epithelial cell of vertebrate taste buds.
thecogen cell	The inner auxiliary (nonsensory) cell of a sensillum, cf. sheath cell.
tormogen cell	The outer auxiliary (nonsensory) cell of a sensillum, cf. socket cell.

18.1 Introduction

We humans recognize taste as sensation arising from the oral cavity and indicating information about the chemical quality of potential foodstuffs. The sense of taste uniquely arises from the specialized sensory end organs for this system: taste buds. In humans, taste sensations are only those that we describe as salty, sweet, sour, bitter, and 'umami' (the taste of glutamate). All other oral sensations, for example, the coolness of mint, the smoothness of fats or the hotness of pepper, arise from the general cutaneous innervation of the epithelium and should not be considered to be 'taste'. So, for humans and other vertebrates, 'taste' is a system defined by the sensory end organs mediating the sensibility. For humans, and by extension, other vertebrates, taste can be defined by the use of taste buds in the context of food selection.

When examining the evolution of the sense of taste, we are met with several difficulties. First, how can the sense of taste be defined for organisms lacking taste buds and, second, is the sense of taste evolutionarily conserved across species, and if so, across what range of species? This second question leads directly to the issue of where and at what point in phylogeny did taste buds evolve, and what tissues they may have evolved from. I will consider each of these points in the following sections leading to the conclusion that taste is a well-defined sense only in vertebrates, where taste buds are a clearly recognizable feature. Related chemical senses in other taxa may have arisen independently and are not 'taste' other than by having an analogous function.

18.1.1 What is Taste?

For humans and other vertebrates, taste is a sensory system that starts with taste buds as the specialized sensory end organ and deals with information concerning the chemical composition of food in contact with mouthparts. Primarily, the sense of taste is used across taxa to distinguish the edible from the inedible (Glendinning *et al.*, 2000). The important features in the above definition are that taste is a chemical sense associated with mouthparts and utilized in control of feeding. Note that the definition says nothing about the medium conveying the stimulus (e.g., air vs. water), nor does it include any description of molecular features or transduction mechanisms – neither is a defining feature of the taste system. For the vertebrate clade, taste is defined by the sensory end organ; for invertebrates, this definition fails since taste buds exist only in vertebrates.

Single-cell organisms may show positive chemotaxis toward a food source by following a concentration gradient of an attractive substance (Van Houten, 2000). Although this behavior shares some aspects of taste-mediated behaviors in more complex organisms, it is not taste. Single-cell organisms have no oral cavity and have no specialized sensory end organs. To include the positive chemotaxis of single-cell organisms under the rubric of 'taste' would necessitate extending the abilities of taste and smell to plants which exhibit positive and negative growth in response to chemical signals in the environment (Filleur *et al.*, 2005).

A more difficult situation arises when examining the invertebrates. Complex invertebrates such as crustaceans and mollusks (Ache, 1987) have specialized chemoreceptors associated with well-defined mouth parts. These chemoreceptive end organs are not homologous to taste buds although they share several features with taste buds, for example, multicellular aggregates specialized for the detection of a limited variety of chemical substances. The presence of such specialized chemoreceptor organs on mouthparts certainly makes these end organs similar to taste buds in terms of function and behavior; yet, are they taste? The difficulty in drawing a conclusion about this depends on the context in which one wishes to use the comparison. For example, if one wishes to compare the behavior of a fly with that of a rat, then referring to the feeding-related perioral chemosensors of these animals as 'taste' has some utility. However, calling both of these systems 'taste' is misleading when considering the detailed molecular or cellular features of the sensory end organs, that is, the labellar sensillae of a fly are

entirely different from the taste bud of a rat. The sensory cells in flies are bipolar neurons extending an axon into the central nervous system (CNS); the sensory cells of taste buds are axonless, modified epithelial cells that synapse onto the peripheral process of a cranial nerve ganglion cell. These systems are analogous, but clearly not derived from a common ancestral condition, that is, they are not homologous.

Even for vertebrates, including humans, the word 'taste' is confounded by common usage meaning sensations arising from the mouth. Conversationally, we use the word 'taste' to include many aspects of flavor other than salty, sweet, sour, bitter, and umami. The confusion arises because of the nasopharynx connecting our oral and nasal cavities. Vapors from food in the oral cavity pass retronasally through the nasopharynx to reach the olfactory epithelium. Thus, food in our mouth stimulates not only taste buds, but also chemoreceptors of the olfactory and trigeminal systems. A further confusion is that, even among vertebrates, taste buds are not always confined to the oral cavity. Catfish, for example, have plentiful taste buds scattered across the body surface, being especially densely distributed on the barbels and leading edges of the fins. Despite their location, these oddly situated taste buds are innervated by a gustatory nerve (facial N.) and are used in the context of finding foods (Bardach *et al.*, 1967).

18.2 Taste in Invertebrates

As mentioned above, taste, when applied to invertebrates, is not as clearly defined as for vertebrates. Following the definition above, I will consider the sensory end organs used by different invertebrates in detecting nutritive substances and toxins in potential food items. By definition, the sensory end organs for taste must be associated with mouthparts or other appendages used in feeding. However, in many segmentally organized invertebrates, similar end organs often occur on mouthparts and legs. This may, in part, be due to the fact that mouthparts and legs are serial homologues in many segmented invertebrates. Even in nonsegmented invertebrates, for example, octopus, apparent taste end organs occur on the legs as well as mouthparts. In these cases, the anatomical distinctions are blurred and one must rely more on the context in which the end organ is used to define the system. By analogy to vertebrates, for invertebrates, we can then extend the definition of taste to include contact, or near-range (i.e., high-threshold) chemoreceptors used in a feeding context and which are similar to the chemoreceptors of the mouthparts.

The invertebrate clade includes relatively primitive, radially symmetric groups, for example, Cnidaria and Porifera, and the Bilateria including the Protostomes (Holland, 2000). While the more basal group of animals clearly respond to a variety of chemicals (presumably via specialized chemoreceptors), it is difficult to draw distinctions between various modes of chemoreception. Also, comparatively little is known about the nature of chemosensory cells in these basal forms.

The Protostomes fall into two large groups: Ecdysozoa (including nematodes and arthropods) and Lophotrochozoa (including flatworms, annelids, and mollusks; Holland, 2000). In both groups, taste as a feeding-related sense, can be distinguished from other well-developed chemosensory modalities. This article will describe aspects of the 'taste' systems in representatives of each of these major groups; it is not meant to be a comprehensive review.

18.2.1 Ecdysozoa

Many Ecdysozoa have a relatively impermeable cuticle covering the outside of the body. Hence, exteroceptive end organs including chemoreceptors must have sensory processes extending beyond the cuticle or else have openings in the cuticle to permit access to the external stimuli. Sensory end organs of this clade have a common general structure in which the cell bodies of the sensory neurons lie beneath the surface cuticle and extend dendrites to reach through or near the cuticle. The apical dendrites of the sensory cells are usually associated with one or more non-neuronal accessory cells designated by a variety of names, for example, sheath, socket, auxiliary, tormogen, and thecogen cells.

The overall organizational scheme of taste-like sensory organs in Ecdysozoa is similar in many respects to vertebrate taste systems. Yet, any similarity must be attributed to convergence rather than common origin. In both major groups, each taste organ comprises a variety of sensory cells 'tuned' to different chemical stimuli. That is, although each end organ responds to many different chemical cues, the individual sensory cells within the end organ are tuned fairly narrowly. In the Ecdysozoa, each receptor cell responds either to appetitive or to aversive substances, but never both. This dichotomy is reflected in the nonoverlapping central connectivity of the receptor cells and the behaviors driven by their stimulation (Wang *et al.*, 2004).

18.2.1.1 Nematodes Chemoreceptor cells and their molecular receptors are well studied in *Caenorhabditis elegans*. Unfortunately, the literature in this field is confounded by the tendency to refer to chemoresponses to water-soluble compounds as 'taste' while chemoresponses to volatiles is termed 'olfaction' although the same end organ (amphid chemoreceptors) is used to mediate both responses. As discussed above, the separation of taste and smell according to chemical nature of the stimulus is not generally useful (e.g., compare taste and olfaction in catfish). The well-studied chemoreceptor of the nematode *C. elegans* consists of paired amphid organs each innervated by 12 neurons (Figure 1; Ward *et al.*, 1975). Of these, 11 are chemosensory, the other being thermoceptive (Bargmann and Mori, 1997). Eight of the chemosensory neurons (ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK) extend dendrites through the amphid pore in the cuticle to be in fairly direct contact

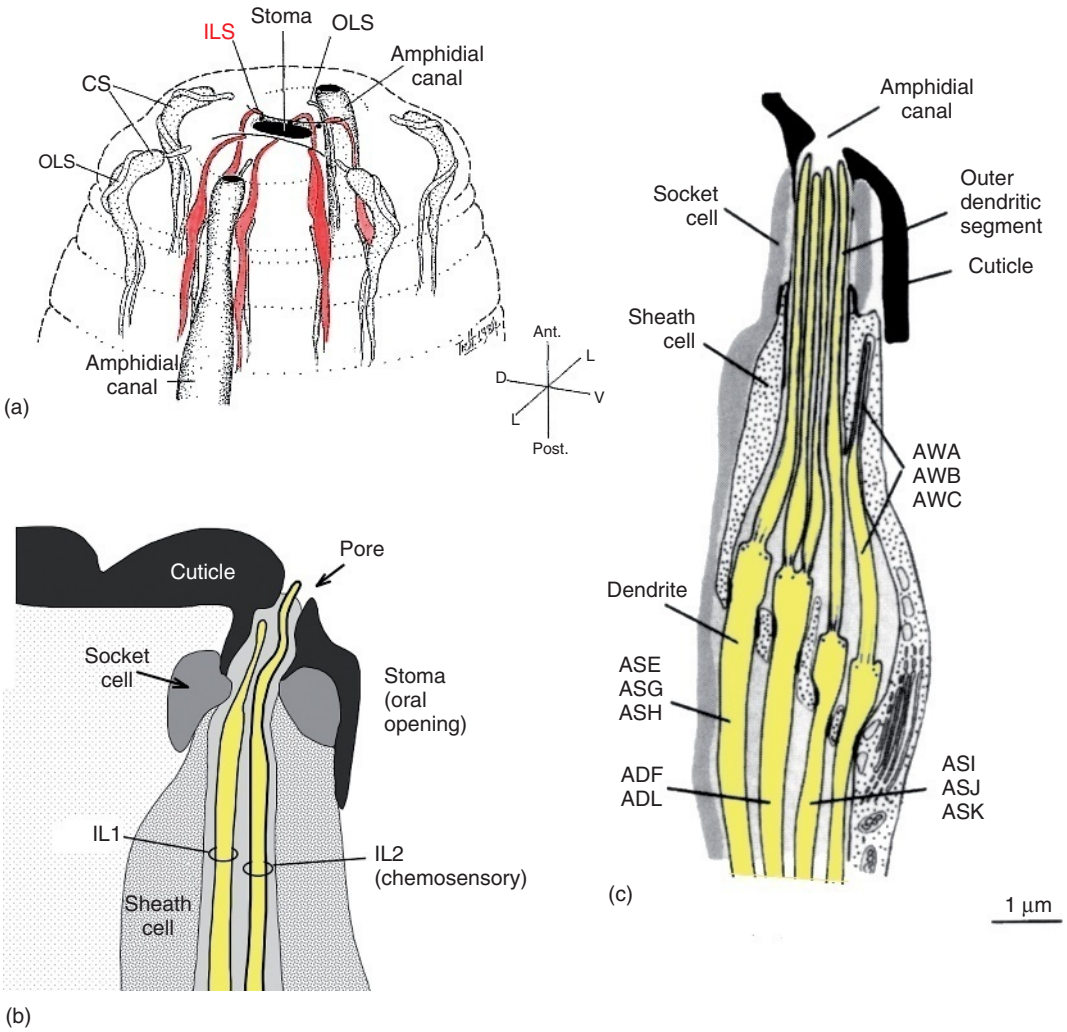

Figure 1 Chemoreceptor organs on the head of nematodes. a, Diagram showing the location of the head sensilla of *Pratylenchus* sp. CS, cephalic sensillum; ILS, inner labial sensillum; OLS, outer labial sensillum. b, Diagram of an inner labial sensillum (rendered in red in panel a). IL2, whose dendritic tip is exposed to the outside milieu, is a chemosensory neuron while IL1, whose tip is not exposed, is reported to be mechanosensory. c, The amphid contains numerous chemosensory neurons which detect either soluble (ASE, ASG, ASH, ADF, ADL, ASI, ASJ, ASK) or volatile (AWA, AWB, AWC) substances. It is not clear whether the amphid chemoreceptors should be considered 'taste' according to the definition in this chapter since stimulation of these receptor cells results in chemotaxis rather than feeding. a, Reproduced from Trett, M. W. and Perry, R. N. 1985. Functional and evolutionary implications of the anterior sensory anatomy of species of root-lesion nematode (genus *Pratylenchus*). *Revue Nematol.* 8(4), 341 355, with permission from IRD. b, Drawing based on Ward, S., Thomson, N., White, J. G., and Brenner, S. 1975. Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans. J. Comp. Neurol.* 160(3), 313 337. c, Reproduced from Cell biology of olfactory epithelium. In: Neurobiology of Taste & Smell; Farbman, A.; *eds.* T. E. Finger, W. L. Silver, and D. Restrepo; Copyright © 2000, Wiley. Reprinted with permission of John Wiley & Sons, Inc.

with the environment. These cells respond to water-soluble substances. Three other amphid chemosensory neurons (AWA, AWB, AWC) have dendrites extending near the amphid pore, but are encapsulated by the 'sheath' or 'wing' cell and thus do not have direct contact with the environment. The AWA, AWB, and AWC cells respond to volatile substances, presumably those capable of diffusing through or being transported across the sheath cell. The commonly studied chemotaxic behaviors are driven almost entirely by these amphid chemoreceptors (Bargmann and Mori, 1997). As described above, the nematode chemotaxic behaviors are commonly divided into 'taste' and 'smell' according to the nature of the chemical stimulus (water soluble or volatile, respectively). I suggest that all behaviors mediated by the amphids should more properly be considered 'smell' since none of the measured behaviors is concerned with palatability of a suspected food object. Rather the amphid drives locomotor behaviors, just as the olfactory sense in vertebrates drives approach/avoidance locomotor responses.

Nematodes, including *C. elegans*, have a set of lesser-known chemoreceptors, the inner labial neurons, which are situated more within the oral cavity and appear likely to mediate taste-like behaviors (Tabish *et al.*, 1995). Yet, little is known of the function or responses of these perioral presumed chemoreceptors. Trett and Perry (1985) suggest, on the basis of structure, that the IL2 neuron of inner labial sensilla serve as contact chemoreceptors (just as taste buds have been described) but this speculation has yet to be confirmed by functional or behavioral studies. Nematodes studied to date possess six radially symmetric paired inner labial sensilla with cuticular openings facing the inner side of the rostral end of the oral cavity in many species (see Figure 1). Each sensillum is innervated by two neurons (IL1 and IL2) one of which (IL2) extends a process to reach the outer environment; the other sensory dendrite terminates just below the surface beneath the opening in the cuticle (Ward *et al.*, 1975). In parasitic species, however, the inner labial sensilla may be purely mechanosensory in that their sensory processes do not have access to the surface (Fine *et al.*, 1997) but this arrangement would not preclude detection of volatile substances like the AWA, AWB, and AWC amphid neurons.

18.2.1.2 Arthropods Arthropods, including insects, arachnids, and crustaceans, rely on chemosensory sensilla to detect chemicals in the environment. The best-characterized system is that of the fruit fly *Drosophila* (see Figure 2) but other arthropods appear to have receptors of similar ilk. Chemosensory sensilla are present not only on the mouthparts but also on the wing margins, tarsi (feet) and some other appendages likely to contact potential foodstuffs (e.g., Dethier, 1962). The chemosensory sensilla in the perioral region and upper alimentary canal apparently mediate feeding behavior and therefore fit into the definition of a sense of taste. The chemosensory sensilla on the other appendages are structurally and molecularly similar to the oral ones and are usually used in the context of food detection. So it is not reasonable to exclude these from the taste system merely because of their location on the body. The end organ structure is right and the behavioral context is right. Including the tarsal chemoreceptors as part of the taste system is analogous to including the taste buds on the barbels and body of fishes in their taste system. In the case of the external taste buds of fishes, they are clearly part of the taste system (based on the end organ structure, innervation, and behavioral context). By analogy, we should then accept the tarsal chemoreceptors of arthropods as being part of the taste system. A similar argument can be made for the chemoreceptors on the wing margins, although their behavioral context is less well studied. In contrast, the chemosensory sensilla of other body parts, for example, antenna or ovipositor, should not be included in the taste system, regardless of expression of common receptor molecules, since they are used in other behavioral contexts, for example, navigation or egg-laying.

The basic structure of the chemosensory sensilla is similar whether the end organ be on a mouthpart, wing, or leg. These end organs contain one mechanoreceptor cell and several (2–4), physiologically distinct chemosensory cells with an apical process (outer dendritic segment) extending into the sensilla proper which is a thin, hair-like protrusion of the cuticle (Shanbhag *et al.*, 2001). One or more pores lies at the apex of the sensilla thereby permitting substances in the outside medium to come into contact with the fluid (sensillum lymph) filling the space around the dendrites within the sensillum. Potential tastants must then traverse the fluid-filled space to activate receptors on the dendrites of the sensory cells. As is typical of invertebrate sensory cells, each receptor cell of the chemosensory sensilla contributes an axon to the peripheral nerves which then enter the CNS. In addition, there are numerous taste 'pegs', which are smaller sensory sensilla that protrude little from the surface of the epithelium and which bear only one chemosensory cell along with a mechanosensory cell (Shanbhag *et al.*, 2001).

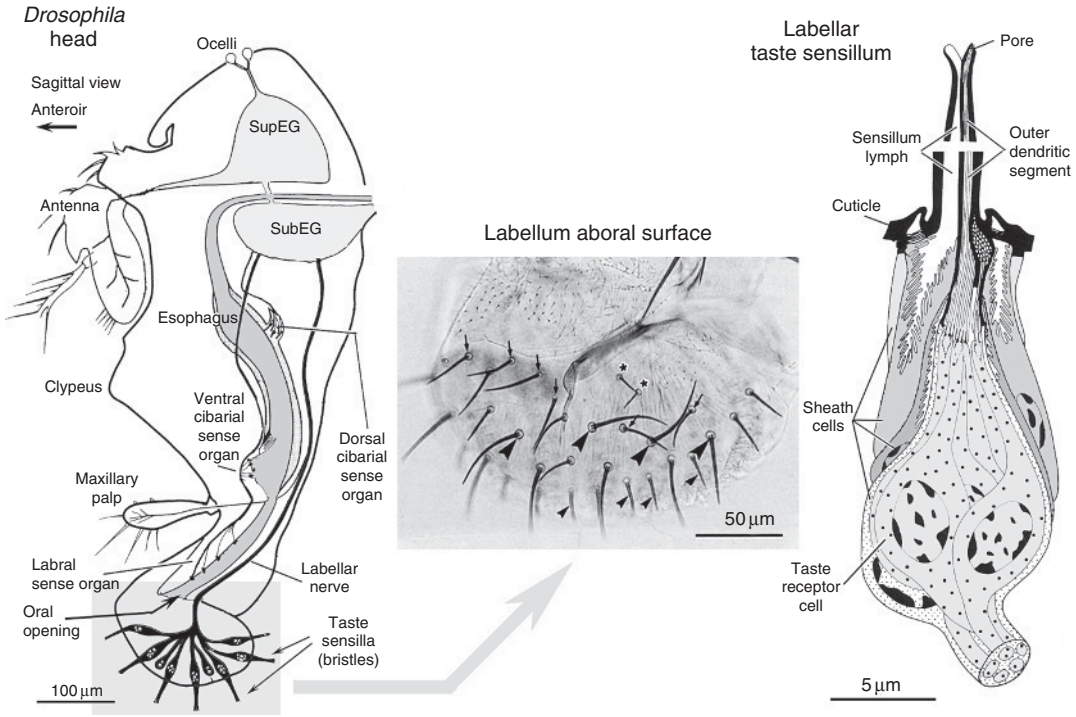

Figure 2 Taste receptors on the fly *Drosophila.* Left: Drawing of a sagittal section through the head of the fly showing the location of the major taste organs: labellum, labral sense organ, and cibarial sense organs. Center: Labial palp whole-mount preparation showing the aboral surface of left palp. Anterior is left and dorsal top. Sensilla marked with stars are purely mechanosensory and the remaining are taste bristles. Taste sensilla are divided into three sub-types: short (small arrowheads), intermediate (arrows), and large (large arrowheads). Only some sensilla of each sub-type are marked. Right: drawing of a single sensillum showing the receptor and auxiliary cells. Center panel, reproduced from *Cell Tissue Res.*, vol. 304(3), 2001, pp. 423 437, Gustatory Organs of *Drosophila melanogaster*: Fine Structure and expression of the putative odorant-binding protein PBPRP2, Shanbhag, S. R., Park, S. K., Pikielny, C. W., and Steinbrecht, R. A., Figure 1b, with kind permission of Springer Science and Business Media. Left and right panels, reproduced from cell biology of taste epithelium. In: Neurobiology of Taste & Smell; Finger, T. E. and Simon, S. A.; *eds.* T. E. Finger, W. L. Silver, and D. Restrepo; Copyright © 2000, Wiley, and modified from the original work of Singh (1997). Reprinted with permission of John Wiley & Sons, Inc.

In *Drosophila*, chemosensory sensilla are especially dense on the labellum and to a lesser extent on the labrum, which sits at the entrance to the oral cavity. Intraoral chemosensory sensilla are also present in the cibarial sense organs. Both the intraoral and oral chemosensory end organs form nerves that terminate within the subesophageal ganglion, in contradistinction to the olfactory (antennal) receptors that project to the antennal lobes of the supraesophageal ganglion.

The labellar chemosensory sensilla are divisible into three morphological types according to the length of the sensillum: short (s-type), intermediate (i-type), and long (l-type) (Shanbhag *et al.*, 2001). The i-type sensilla possess only two chemosensory cells, whereas the s- and l-types have four chemosensory cells. The chemosensory cells fall into four broad functional classes according to chemoresponsiveness. The w-cells respond to water, s-cells respond to sugars, L1-cells respond to low concentrations of salt, and L2-cells to high concentrations of salt and to various bitter substances. But this formulation may be overly simple (e.g., see Hiroi *et al.*, 2002). The two chemosensory cells of the i-type sensilla consist of one cell with L2-type responses (bitter, high salt) and the other cell with a combination of S and L1 properties (Hiroi *et al.*, 2004). Water-responsive units are present only in the s-type and l-type sensilla. In summary, the sensory cells of *Drosophila* gustatory sensilla fall into one of two groups according to the behavior elicited by their activation: one group (e.g., s-units, w-units, and L1-units) drives appetitive behaviors under the right motivational conditions, while the other group (L2-units responsive to high salt and bitter substances) drives aversive behaviors.

The dichotomy in driven behaviors of the different types of receptor cells coupled with the presence of an axon extending directly from the receptor cell to the CNS, permits direct assessment of the pattern of projection into the brain of these functionally different types of receptor cells (Inoshita and

Tanimura, 2006; Wang *et al.*, 2004). Gustatory information in the CNS of *Drosophila* is organized first, according to gustatory end organ, and second, according to driven behaviors – appetitive or aversive. Thus, the taste sensilla on the labellum project to a different part of the subesophageal ganglion than do the taste organs within the oral cavity proper (Stocker and Schorderet, 1981; Wang *et al.*, 2004). Within the subesophageal ganglion, bitter-responsive cells (L2-type) map dorsomedial to the sugar-responsive (L1-type) neurons. Water-responsive receptor cells also project to the lateral neuropil of the subesophageal ganglion, perhaps overlapping or slightly lateral to the sugar-responsive group (Inoshita and Tanimura, 2006).

Some interesting similarities exist between the insect and mammalian gustatory systems. First, the gustatory end organs comprise multiple sensory cells exhibiting a limited range of chemoresponsiveness. That is, each end organ responds to a spectrum of tastants, although each sensory cell within that end organ is more limited. Second, the fundamental organizational plan in the CNS is one of organotopy, that is, each part of the body is represented in a unique part of the CNS, suggesting that the location of a chemical cue is key to gustatory-mediated behavior. Finally, within each organ-specific zone of the CNS, quality may be encoded by position within the somatotopically delineated field of neuropil. Just as different areas of neuropil are implicated in appetitive versus aversive cues in the subesophageal ganglion of the fly, different areas of neuropil appear activated by different tastants in the gustatory centers of mammals (Harrer and Travers, 1996; Sugita and Shiba, 2005).

18.2.2 Lophotrochozoa

18.2.2.1 Annelids The annelids, as represented by earthworms and leeches, have widespread chemoreceptors scattered across their body surface, but a set of these, associated with the lips (labia) control feeding behavior (Elliott, 1987). These are relatively poorly characterized, except for the labial chemoreceptors of leech which were studied both anatomically and physiologically by Elliot (1986, 1987).

The medicinal leech, *Hirudo medicinalis*, will initiate a full sequence of feeding behavior in response to human blood or plasma whether presented at room or body temperature (Elliott, 1986). The essential components of blood appear to be NaCl and arginine, which together provoke the full feeding behavior. The sensory region crucial to this behavior is the dorsal lip whose ablation results in loss of the feeding sequence in response to chemical stimulation. Likewise, in *Haemopsis marmorata*, a carnivorous leech that eats and trails earthworms, ablation of the dorsal lip abolishes their ability to track earthworm trails (Simon and Barnes, 1996).

The dorsal lips of leeches contain large and small sensilla containing unique ciliated sensory cells. As is typical of invertebrates, the sensory cells are bipolar neurons with a centrally directed axon and a dendritic process that extends to the surface of the epithelium. The sensory cells are grouped together into sensilla of two different sizes. The approximately 150 larger sensilla are arrayed ~125 μm apart in a band across the dorsal lip. Each sensillum forms a raised papilla of ~35 μm in diameter with an apical opening of ~20 μm through which extend the cilia of the underlying sensory cells (Elliott, 1987). Each sensillum contains multiple sensory cells, but the number is not specified. The 250 small lip sensilla are 8–10 μm in diameter and lie along the edges of the stripe of large sensilla. The smaller sensilla which sit flush to the surface of the surrounding epithelium can be recognized by the collection of cilia protruding from the surface. Since each sensory cell possesses a small number of cilia, a small sensillum is likely to comprise a dozen or so sensory cells (Elliott, 1987).

The nerves formed by the axons of the sensory cells assemble mostly into the dorsal cephalic nerves (Perruccio and Kleinhaus, 1996) to reach the cerebral ganglia. Stimulation of the lip region with either NaCl or arginine evokes robust neural activity (Li *et al.*, 2001). Interestingly, simultaneous stimulation with quinine or denatonium, both of which are feeding deterrents in these animals, reduces peripheral afferent activity. These findings suggest that feeding deterrents may act, at least in part, by inhibiting the neural response to appetitive cues (Li *et al.*, 2001).

18.2.2.2 Mollusks The taste-related chemoreceptors of mollusks have been characterized in both gastropods and cephalopods. In gastropods, as typified by *Aplysia*, feeding-related chemoreceptors are present on the lips and anterior tentacles (Jahan-Parwar, 1972). Likewise, cephalopods, especially well studied in octopus, have chemoreceptors on the tentacles as well as in the perioral region. Those associated with the suckers on the tentacles were well described in an elegant series of papers by Graziadei (Graziadei, 1964a, 1964b, 1965; Graziadei and Gagne, 1976) following studies by Emery (1975a, 1975b) on ciliated sensory cells (assumed to be chemoreceptors) on the lips of

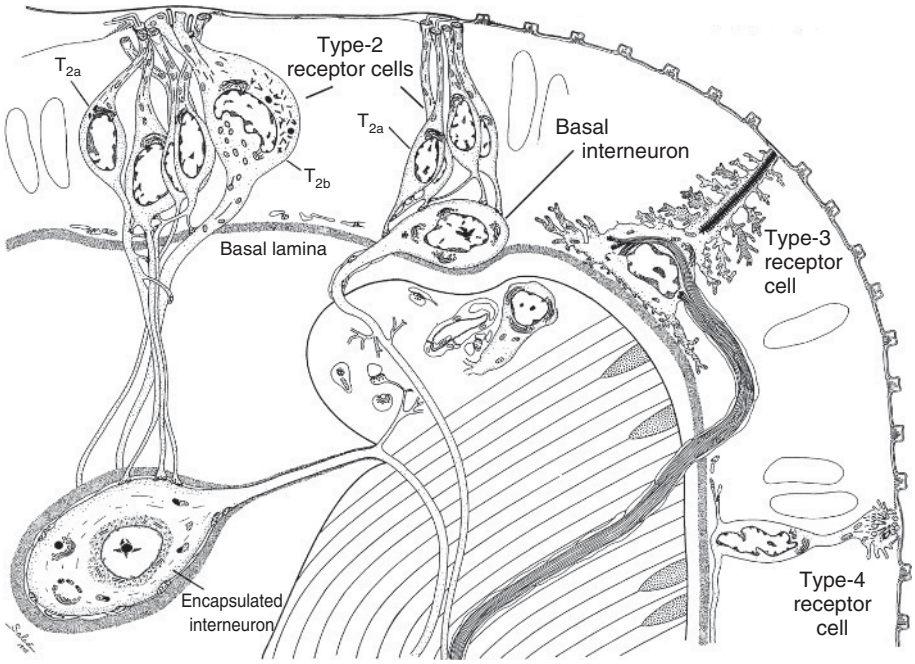

Figure 3 Diagram of sensory neurons in the rim of a sucker on the arm of an octopus. Based on structural considerations, type-2 receptors are likely to be chemoreceptors as are the type-4 cells which look similar to olfactory receptor cells in squid. Type-3 cells appear to be mechanoreceptors. The clusters of type-2 cells superficially resemble vertebrate taste buds, but obvious structural differences exist. The occasional contacts between some type-2 cells and basal interneurons is reminiscent of the relationship between elongate taste cells and Merkel-like basal cells in nonmammalian vertebrates. Reproduced from 'Sensory innervation in the rim of the octopus sucker', *J. Morphol*.; Graziadei, P. P. and Gagne, H. T.; Copyright © 1976, Wiley-Liss. Reprinted with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc.

squid and octopus. A brief summary of Graziadei's findings follows, but the reader should refer to the original papers for a complete description of the sensory apparatus of the tentacles.

Sensilla of the mollusks are similar in many ways to the sensilla of leeches. The sensory cells are bipolar neurons with an apical dendrite that extends to the surface of the epithelium, and a basal axonal process that contributes to nerves coursing to the CNS. The likely chemoreceptors of the sucker are elongate epithelial cells (termed type-2 cells by Graziadei and Gagne, 1976) which are collected into small 'apical clusters' (5–10 cells), superficially similar to taste buds in vertebrates (see Figure 3). The most common form of sensory cell in the apical cluster is a narrow elongate cell (type 2a of Graziadei and Gagne) which extends a small number (e.g., 3–8) cilia above the surface of the surrounding epithelium. The apical clusters may contain a second elongate cell type (type 2b) which is larger than the 2a cells and has somewhat different cytological features. The apical clusters also may be associated with a horizontally oriented 'basal interneuron' lying between the apical cluster and the basal lamina of the epithelium. These basal interneurons as well as 'encapsulated' interneurons apparently receive synaptic contacts from the type-2 cells of the apical cluster. The situation is reminiscent of the organization of taste buds in bony fishes where the elongate sensory cells synapse onto a Merkel-like basal cell (see below). Of course, the tentacle sensilla of octopus are not homologous to taste buds in vertebrates, hence similarities in organization must be due to convergence rather than phyletic continuity.

18.3 Taste in Vertebrates and Chordates

Taste buds are recognizable throughout the vertebrate lineage – from lampreys to teleosts to mammals. Although structural details can be quite varied across species, taste buds retain a host of key features that distinguish them from other end organs. The common features of taste buds include: (1) aggregates of specialized epithelial cells including both receptor and supporting cells (since the cells are epithelial, they have a limited life span and are continuously replaced through out the life span of the animal); (2) more than one type of sensory cell reaching the epithelial surface via an

opening (taste pore) in the surrounding epithelial covering; and (3) sensory (afferent) innervation from facial, glossopharyngeal, or vagus nerves which project to the viscerosensory column of the medulla. Taste buds in diverse vertebrates share other features but it is unclear whether such features are necessary as defining features, or are rather elements in common to a subset of vertebrates. Such common features include: (1) a cell type capable of concentrating and releasing serotonin (Kim and Roper, 1995; Nada and Hirata, 1977); (2) one or more cells that manifest a neuron-like phenotype (e.g., expressing NCAM: Nelson and Finger, 1993; Smith *et al.*, 1993), neuron-specific enolase (NSE) (Toyoshima *et al.*, 1991; Yoshie *et al.*, 1989), or neural differentiation markers such as Mash-1 (Kusakabe *et al.*, 2002); and (3) strong ecto-ATPase activity (Iwayama and Nada, 1967; Barry, 1992) perhaps because ATP is a requisite neurotransmitter in this system (Finger *et al.*, 2005).

18.3.1 Epithelial Chemoreceptors in Chordates

The chordate lineage includes the invertebrate cephalochordates (e.g., *Amphioxus*) and craniates. The craniates can be subdivided into two groups: (1) hagfish and their relatives, and (2) true vertebrates, including both agnathan (lamprey) and gnathostome lineages. All extant vertebrates, from lampreys to amniotes, have clearly recognizable taste buds innervated by branches of the facial (CN VII), glossopharyngeal (CN IX), or vagus (CN X) nerves. The cells of taste buds are modified epithelial cells and, unlike most invertebrate receptors, do not possess an axon or any process extending below the basal lamina. While taste buds are clear in all vertebrates, the evolutionary origins of these end organs is obscure.

Sensory cells in nearly all invertebrates are primary sensory neurons, also called type I receptors, complete with both a sensory dendrite extending to the epithelial surface and an axon connecting to the CNS (see above). The amphioxus has many such epithelial sensory cells including type I cells of Lacalli (Lacalli and Hou, 1999). But secondary sensory neurons first make a substantial appearance in this group of organisms. The epithelial secondary sensory cells of *Amphioxus* (type II receptors; Holland and Yu, 2002) extend immotile cilia to the epithelial surface. These cilia contain numerous microtubules (Lacalli and Hou, 1999) rather than the more standard 9+2 arrangement for cilia. The apical morphology of the type II receptors is striking in that a ruff or collar of microvilli surround a central elongate cilium (Figure 4). This feature is commensurate with a chemosensory rather than mechanosensory function. The type II epithelial receptors extend two or three basal processes a short distance within the epithelium to synapse onto neural processes (Lacalli and Hou, 1999).

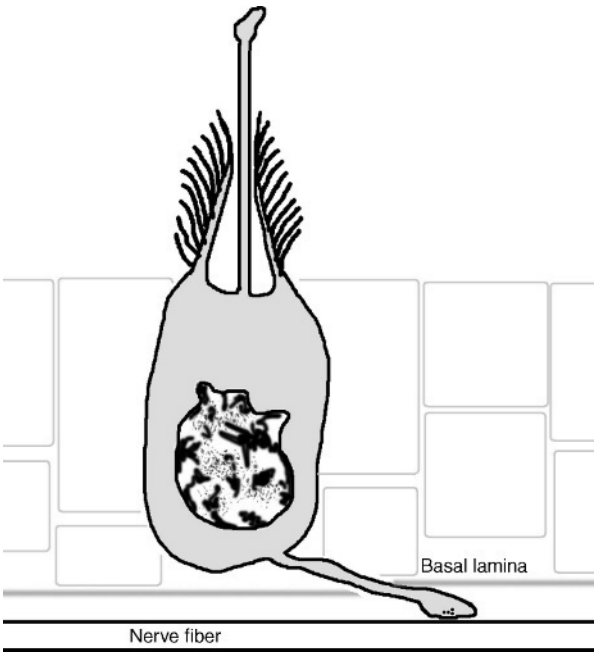

Figure 4 Schematic drawing of a type II sensory cell from *Amphioxus*. These receptor cells bear a long central cilium surrounded by a ruffle of microvilli. The numerous microvilli, which serve to expand the surface area of the cell, coupled with the lack of a 9+2 microtubule arrangement in the cilium are consonant with a chemosensory function. These sensory cells often extend a short process, sometimes through the basal lamina, to synapse on nearby nerve fibers. Based on descriptions and figures in Lacalli, T. C. and Hou, S. 1999. A re-examination of the epithelial sensory cells of amphioxus. *Acta Zool.* 80, 125 134.

18.3.2 Solitary Chemoreceptor Cells and Schreiner Organs

All craniates, including hagfishes, possess solitary chemoreceptor cells (SCCs) scattered within the epithelium of the gut, respiratory tract and even across the body surface (Whitear, 1992; Finger, 1997; Sbarbati and Osculati, 2003). SCCs resemble the type II sensory cells of amphioxus as well as the individual cells of taste buds in terms of being elongate, columnar epithelial cells which synapse onto cranial nerve sensory processes. The SCCs differ from taste buds in that they can be innervated by any cutaneous or visceral nerve. For example, SCCs scattered across the surface of the body of fishes are innervated by the local cutaneous nerve – either

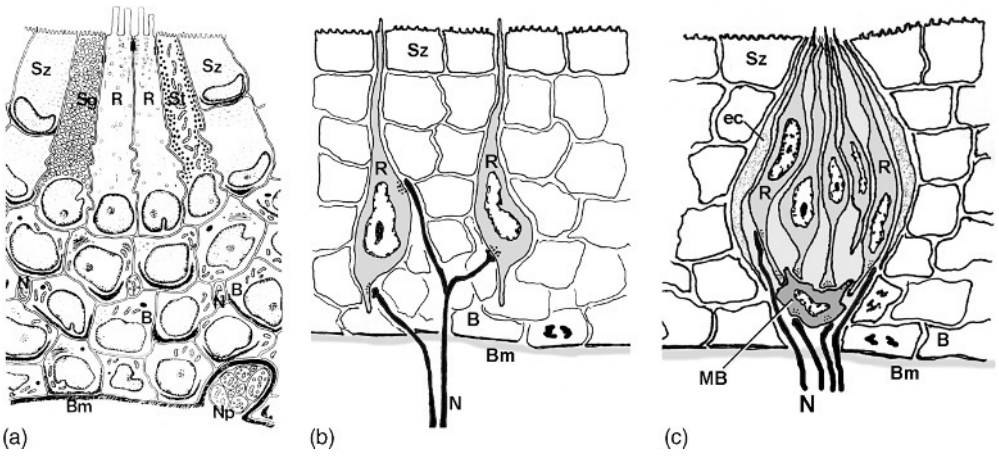

Figure 5 Schematic drawing comparing a Schreiner organ in a hagfish (a), with SCCs (b), and a taste bud in a typical teleost fish (c). In (a), the receptor cells (R) do not extend to the basal lamina and are flanked by various supporting or secretory cells. Part b is a schematic diagram of SCCs in a typical teleost. The SCCs are isolated in the epithelium appearing without associated supporting or secretory cells. Part c is a schematic diagram of a taste bud from a typical teleost. Multiple types of receptor cells are surrounded by flattened edge cells. The receptor cells reach nearly to the basal lamina where they form synapses with both nerve processes and Merkel-like basal cells. B, basal cell; Bm, basal lamina (basement membrane); ec, edge cell; MB, Merkel-like basal cell; N, nerve fiber; Np, nerve plexus; R, receptor cell; Sz, mucous cell; Sg, glandular supporting cell; St, type II supporting cell. a, Reproduced from Georgieva, V., Patzner, R., and Adam, H. 1979. Transmissions- und rasterelektronenmikroskopische Untersuchungen an den Sinnesknospen der Tentakel von *Myxine glutinosa* L. (Cyclostomata). *Zool. Scripta* 8, 61 67, with permission from Blackwell Publishing.

spinal or trigeminal according to location. In contrast, taste buds on the body are innervated by a recurrent branch of the facial nerve, not by the local spinal nerve (Herrick, 1901). Hence, taste buds always have a unique relationship with the cranial nerves associated with epibranchial placodes (Northcutt and Barlow, 1998).

Hagfish (chordates, but perhaps not vertebrates) lack taste buds as defined above, although they do possess Schreiner organs, which are multicellular aggregates of presumed chemoreceptor cells. These may simply be aggregations of SCCs, but the cells of the Schreiner organ are not identical to SCCs. Schreiner organs also have several features similar to taste buds, but do not share all of the features of taste buds, for example, Schreiner organs do not span the full thickness of the epithelium and do not possess three cytologically distinct cell types. The relationship between Schreiner organs, SCCs, and taste buds remains enigmatic (see Braun, 1998, for a nice discussion of this issue).

The ultrastructure of the Schreiner organs has been described by Georgieva *et al.* (1979), who found there to be one type of sensory cell (type I) replete with microvilli, a likely supporting cell and an associated secretory cell similar to mucus cells elsewhere in the epithelium (see Figures 5 and 6). The sensory cells of Schreiner organs appear identical to the SCCs in the same species. Further ultrastructural studies are necessary in order to determine the degree of similarity between the Schreiner organ cell types and those of taste buds. For example, the supporting cells (type II cells) of Schreiner organs are similar to type I (glial-like) cells of taste buds in that they wrap around the sensory cells. Our preliminary data indicate that Schreiner organs are not associated with high levels of ecto-ATPase, which is a key feature of the type

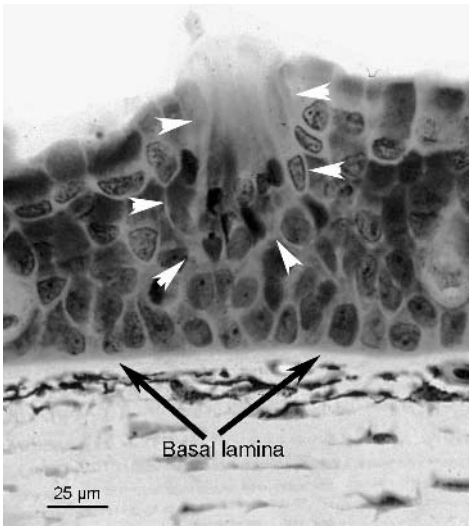

Figure 6 Photomicrograph of a Schreiner organ for the hagfish, *Eptatretus*. Note that the sensory organ (arrowheads) lies well above the basal lamina. Photomicrograph courtesy of Dr. C. Braun, Hunter College, New York, NY, USA.

I (glial-like) cells of vertebrate taste buds in which ATP serves as a neurotransmitter (Finger *et al.*, 2005; Kirino *et al.*, 2006). Thus, Schreiner organs and tastes buds are further distinguished in terms of utilizing different neurotransmitter systems.

Whatever the similarities of Schreiner organs and taste buds, it is noteworthy that SCCs themselves and taste buds share several features. Both comprise modified epithelial cells that undergo continuous replacement during the life of the animal. In the catfish, *Ictalurus punctatus*, the SCCs and taste buds react similarly to the PHA-E lectin (*Phaseolus vulgaris* agglutinin) which reacts with the arginine-binding taste receptor protein (Finger *et al.*, 1996). Thus, in these fish, it appears that SCCs and taste buds may utilize a common receptor mechanism. Similarly, in mammals, nasal and gut SCCs, like taste buds, express T2R (bitter) and T1R receptors and their associated downstream signaling components (Finger *et al.*, 2003; Sbarbati and Osculati, 2003). Thus, in both teleosts and mammals, SCCs and taste buds may utilize common receptor mechanisms. Nonetheless, differences do exist. Whereas SCCs form clear synapses with nerve fibers, the cells of taste buds that share biochemical features with SCCs (type II cells – see Section 18.4.3.1.(*ii*)) do not. Further studies are needed to understand the evolutionary relationships between these cutaneous chemoreceptor systems.

18.4 Taste Buds in Vertebrates

In this article, I present an overview of some of the different appearances of taste buds, but this is not meant to be comprehensive. An excellent comparative view of taste buds can be found in the work of Reutter and Witt (1993).

The structure of taste buds varies considerably across vertebrates (see Figure 7) but several consistent features emerge when comparing across species, as described above. These include: (1) aggregates of 50–150 specialized epithelial cells including both receptor cells and glial-like supporting cells, (2) multiple types of elongate cells reaching an opening in the epithelial surface, and (3) innervation by one of the three gustatory nerves: facial, glossopharyngeal, or vagus. Categorization of cell types within taste buds is complicated by the fact that taste buds consist not only of different functional types of cells, but also cells of different ages within each functional class. Taste buds are surrounded by specialized epithelial cells, 'edge', 'marginal', or ciliated cells (in frog), which form the outer boundary of the taste bud proper. In addition, all taste buds are closely associated with proliferative basal cells which divide to replace the aging and apoptotic cells of the taste bud. The literature on the types of cells in taste buds is extensive and complex (reviewed in Yee *et al.*, 2001). Rather than reviewing the vagaries of this literature, I will present a summary of our current understanding of the organization and structure of taste buds.

Some groups, such as frogs, have distinctive apomorphic characteristics, where taste buds take on a broad cylindrical form of large taste 'disks' spanning 100 μm. Most vertebrates have more compact taste buds organized in an onion-like configuration with an apical pore only tens of micra across. These more compact taste buds, found in all vertebrate groups, have two different plans of organization, typified in the descriptions below as the nonmammalian and mammalian schemes (the situation in birds is not clear). Whether these differences in morphology are more related to phylogeny or to habitat is unknown.

Historically, elongate taste cells in taste buds have been categorized according to their propensity to stain with acidophilic dyes or degree of osmiophilia in preparations for electron microscopy. This has led to descriptions of cells as being either 'light' or 'dark' but these descriptors may vary according to preparatory technique and particular stain utilized. Some authors have extended this classification system to imply function, characterizing the elongate taste cells as being 'sustentacular' (or 'supporting') versus 'sensory' ('receptor') cells. More careful ultrastructural analysis leads to a characterization according to structural features such as size and shape of apical specialization, presence of distinctive granules, or size and shape of the nucleus. Nonetheless, the mixed nomenclature remains in the current literature.

18.4.1 Taste Buds in Nonmammalian Vertebrates

Taste buds in these aquatic forms have been described in many teleosts, a few elasmobranchs, and urodeles (reviewed in Reutter and Witt, 1993) as well as in a lamprey, where the end organs have been called 'terminal buds' (Baatrup, 1983). The detailed structure of taste buds can vary substantially between species, or even within a species, between taste buds situated in different locations, for example, oral compared to extraoral (Reutter and Witt, 1993). Nonetheless, a common organizational plan can be abstracted.

18.4.1.1 Cell types Taste buds in this group are distinguished by containing not only elongate

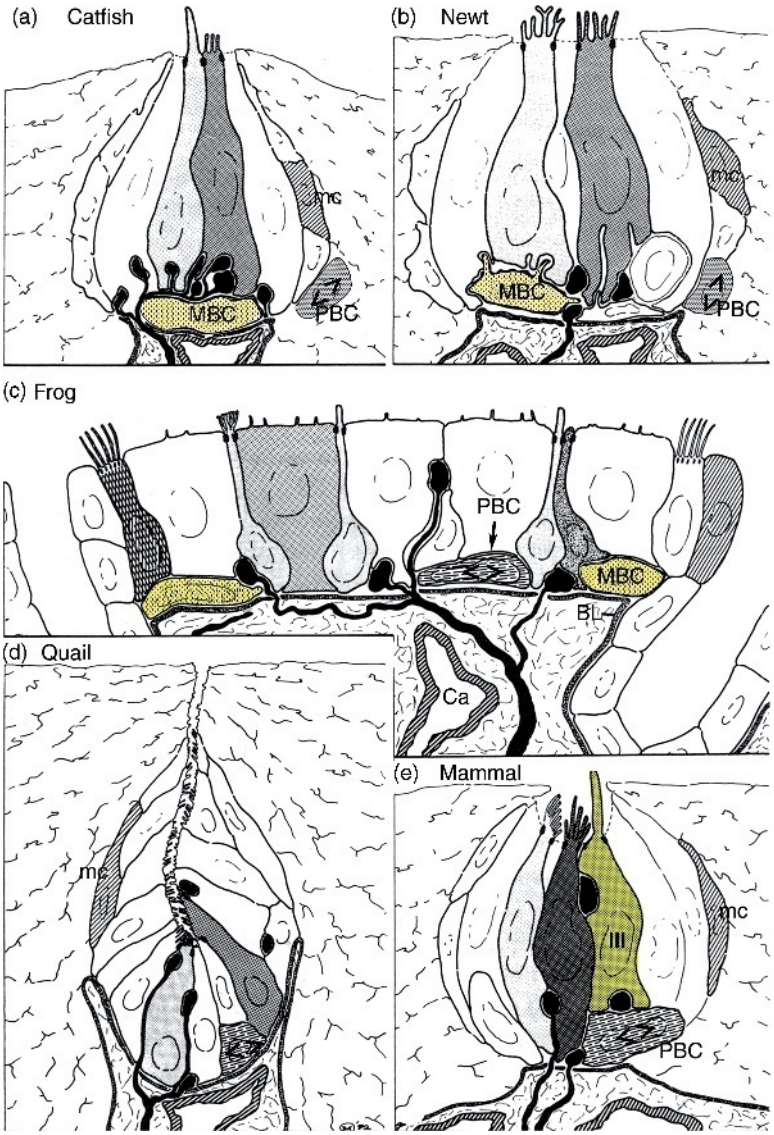

Figure 7 Schematic drawings of taste buds from various vertebrates. The area over which receptor cells gain access to taste substances (receptor area) is relatively broad in aquatic species, but narrows to a 'taste pore' in mammals and birds. Taste buds in all species contain different types of elongate cells indicated by the varied shading. Also, in all species, taste buds are bounded by specialized epithelial cells termed 'edge' cells or 'marginal' cells (mc). In all species, taste buds contain a serotonergic cell type Merkel-like basal cells (MBCs) in nonmammalian forms, and type III taste cells (III) in mammals. All taste buds are also associated with a population of proliferative basal cells (PBCs) which undergo continuing cell division to replace the taste bud cells throughout the life span of the animal. BL, basal lamina; Ca, capillary. Copyright © 1993; From 'Morphology of vertebrate taste organs and their nerve supply'. In: Mechanisms of Taste Transduction by Reutter, K. and Witt, M.; *eds*. S. A. Simon and S. D. Roper. Reproduced by permission of Routledge/Taylor & Francis Group, LLC.

(columnar) spindle-shaped cells, but also a small number (e.g., five) of nonproliferative, 'Merkel-like' basal cells, lying in the lower half of the taste bud and which do not extend to the apical surface of the epithelium. Like cutaneous Merkel cells, the Merkel-like basal cells of taste buds concentrate biogenic amines including serotonin and are immunoreactive for NSE (Reutter and Witt, 1993). Also, like cutaneous Merkel cells, the Merkel-like cells of taste buds extend numerous spine-like processes from their cell body to form synapses on nerve fibers as well as on the elongate taste cells in the taste bud. It is likely that these Merkel-like basal cells, like cutaneous Merkel cells, serve as mechanoreceptors or perhaps in the taste bud, as integrative elements (Ewald and Roper, 1994). It is unfortunate that these Merkel-like basal cells are sometimes referred to simply as 'basal cells' in that this causes confusion with the proliferative basal cells associated with taste buds of both aquatic and terrestrial species.

The nonmammalian type of taste bud also possesses several types of elongate modified epithelial cells that extend an apical process into the region of the taste pore. In aquatic forms, including fishes and aquatic amphibians, the apex of the taste bud is a substantial opening – 10–20 μm or larger – in the surrounding epithelium through which extend the apices of the elongate taste cells (Figure 8). This opening in the epithelium is much larger than the equivalent 'taste pore' present in mammals or birds. Whether this difference in the size of the taste pore is characteristic of the clade of vertebrates (e.g., poikilothermic vs. homothermic) or of the habitat (aquatic–terrestrial) is unclear.

Elongate cells in fish and amphibia usually are characterized as being 'light' or 'dark'. These two descriptors are undoubtedly inadequate to fully characterize all of the different types of elongate cells present in these taste buds. The light cells are spindle-shaped cells with a single, large apical microvillous extending into the taste pore. Light cells extend short branches from their base to synapse with the Merkel-like basal cells and with nerve fibers. Dark taste cells are irregular in cross-sectional form and may envelop or extend interdigitating processes between the light cells (Reutter and Witt, 1993). At its apex, a dark cell extends numerous (10–25) small microvilli into the taste pore. Although dark cells apparently form synaptic contacts with the Merkel-like basal cells, they rarely do so with nerve fibers. In *Necturus*, light cells constitute only ~25% of the elongate cells within the taste bud, the remainder being dark cells. Taste buds in fish and *Necturus* also contain a less common, third cell type with a brush-like or bushy microvillous apex.

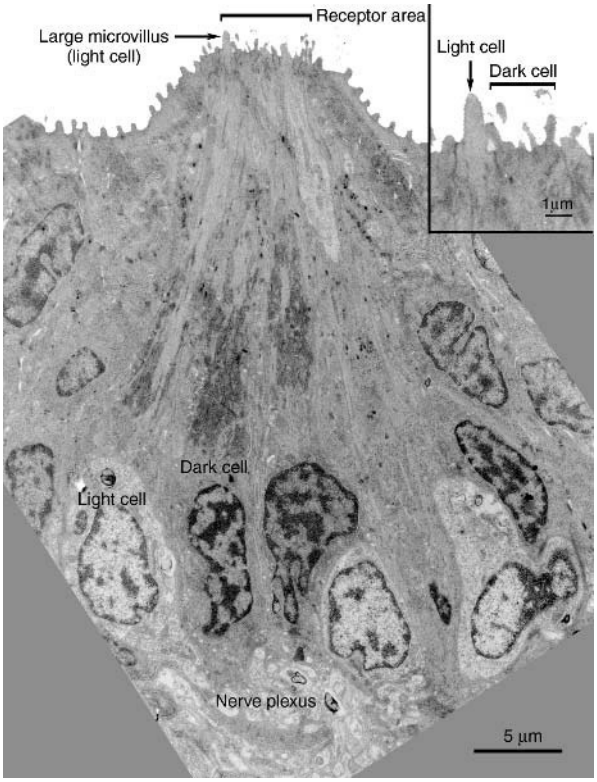

Figure 8 Electron micrograph of a taste bud from a zebra fish. Even at this low magnification, the different sizes of microvilli within the receptor area (taste pore) are evident. The large microvillus belongs to a 'light cell' while the smaller microvilli originate from a 'dark cell'. Inset (upper right) shows an enlargement of the receptor area. Courtesy of Dr. Anne Hansen, University of Colorado.

18.4.1.2 Proliferative cells In nonmammalian vertebrates, the taste bud is closely associated with a small number (e.g., 5) of proliferative basal or marginal cells that apparently generate daughter cells which enter into the taste bud and differentiate into the various mature cell types. These proliferative cells do not sit directly below the taste bud, where the Merkel-like basal cells reside, but rather around the basal circumference of the bud (Raderman-Little, 1979).

18.4.2 The Specialized Taste Organ of Frogs

The taste organs of frogs, called 'taste disks' are highly derived compared to other anamniote vertebrates (Osculati and Sbarbati, 1995) although many commonalities can be observed. In frogs, the apical opening is an expansive disk over 100 μm in diameter (see Figure 8). The taste disk is surrounded by specialized, ciliated cells. Inside this ring is a floor largely consisting of short, broad mucous cells each surrounded by the apical processes of 'wing' cells, thought to be supporting cells (Figure 9). The elongate taste (sensory) cells have their nucleus situated deeper in the taste disk than the wing and mucous cells, but extend a thin apical process to the surface of the taste organ. These cells are divided into two forms: type II cells and type III cells. Although type II cells have substantial contacts with basally situated axons, no obvious synaptic junctions occur. This situation appears similar to the type II taste cells of mammals (see below). The type III cells of the frog taste disk do exhibit clear synaptic contacts with nerve and are similar in that respect to type III cells of mammals. Glial-like sustentacular cells embrace and separate the different cells and nerve fibers in the lower half of the taste disk. This relationship is similar to the type I cells in mammalian taste buds. In addition, frogs have serotonergic Merkel-like basal cells characteristic of

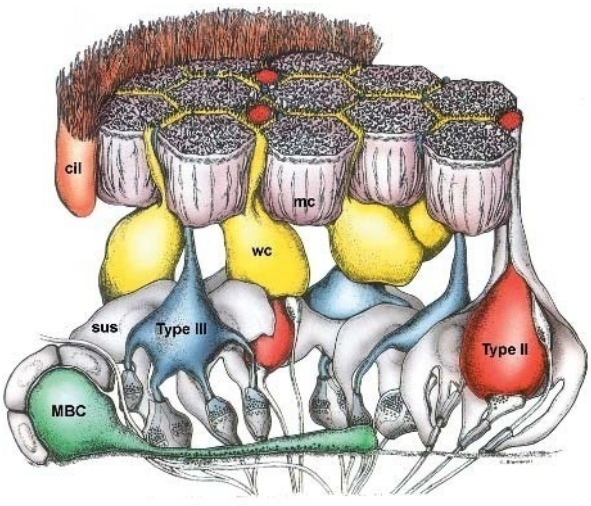

Figure 9 Drawing of the principal cell types of a frog taste disk. Ciliated cells (cil) surround the receptor surface of the taste organ. The superficial third of the organ is occupied by mucus (mc) and wing cells (wc), which are probably involved in maintenance of the mucus layer covering the taste disk. In the middle layer of the disk lie the cell bodies of the elongate receptor (type II and type III) cells which extend an apical process penetrating the surface layer. Both type II and type III cells contact afferent nerve fibers, although distinct synaptic complexes occur only between the type III cells and the nerve fibers. Sustentacular (sus type Ic) cells wrap the other cell types and nerve fibers. Finally, MBCs lie in the deepest portion of the taste disk. Reproduced from Osculati, F. and Sbarbati, A. 1995. The frog taste disc: A prototype of the vertebrate gustatory organ. *Prog. Neurobiol.* (*Oxford*) 46(4), 351 399, with permission from Elsevier.

nonmammalian taste buds. The presence of both these Merkel-like basal cells and the type III sensory cells suggests that the transition from a nonmammalian type of taste bud to a mammalian type of taste bud is not simply the migration and transformation of the Merkel-like basal cells to an elongate morphology.

18.4.3 Mammalian Taste Buds

Taste buds in amniotes differ from anamniote taste buds in two respects. First, the taste pore is considerably narrower (~10 μm or less). Whether this is attributable to a drier, terrestrial lifestyle, or to phylogenetic factors is unclear. Second, mammalian taste buds lack the Merkel-like basal cell characteristic of nonmammalian taste buds. The taste buds of mammals do, however, possess a type of elongate cell which, like the Merkel-like basal cells, concentrates serotonin and forms distinctive synapses with the afferent nerve fibers. This has led many authors to speculate that the serotonin-containing elongate cells of amniote taste buds are homologous, if not functionally equivalent, to the Merkel-like basal cells (e.g., Ewald and Roper, 1994; see Evolution of Gustation).

18.4.3.1 Taste cells Taste buds in mammals comprise three distinct morphological types of elongate cells (type I, II, and III taste cells). These are defined according to ultrastructural criteria following the original descriptions of taste cells in rabbit foliate papillae by Murray (1986). Although the different types of taste cells are fairly distinct in rabbit foliate papillae, the morphological distinctions are less clear in other species. This has led to a great deal of confusion in the literature as to the equivalencies and distinctions between taste cell types in various mammals, especially rats and mice. In reviewing past literature on this subject, it is important to keep in mind that one author's 'type II' cell may not be the same as another author's cell of the same name. To further complicate matters, some authors have retained the older light microscopic terms: dark cell and light cell (originally based on staining properties of aniline dyes). The light–dark cell descriptors are only loosely equivalent to the morphological types as defined by electron microscopy. That is, type I cells nearly always have an electron dense cytoplasm and thus are called dark cells. Unfortunately, type III cells are more variable in staining characteristics and have been grouped by various authors into the category of 'light cell', 'dark cell', or 'intermediate cell', thereby seriously confusing the literature. With the advent of immunocytochemistry, it is possible to recognize the three distinct cytological and functional classes as originally defined by Murray.

18.4.3.1.(i) Type I taste cell The type I taste cells constitute over 50% of the total cells within a mature taste bud. As described by Murray and others, this cell often wraps around other taste cell types and nerve fibers. The cytoplasm is electron dense and stains heavily with acidophilic dyes, giving the cell a dark appearance in both light and electron microscopy. The nucleus is elongate with an irregular, indented nuclear membrane and substantial amounts of heterochromatin along the inner leaflet. These cells usually contain large apical granules ~100 nm in diameter and extend long, slender microvilli into the taste pore.

In many ways, the type I cells are similar to glia of the CNS. They express GLAST, a glial glutamate transporter (Lawton *et al.*, 2000) and NTPDase2, an astrocytic ecto-ATPase (Bartel *et al.*, 2006; Wink *et al.*, 2006). The processes of type I cells insinuate themselves between the other cell types and often cover a point of contact between other taste cells

and nerve fibers, just as astrocyte processes embrace synapses in the CNS. Since ATP is a crucial neurotransmitter between taste cells and the afferent nerve fibers (Finger *et al.*, 2005), these type I cell processes may serve to restrict cross-talk between cells within the taste bud by diffusion of ATP away from points of functional contact between taste cells and nerve fibers.

18.4.3.1.(ii) Type II taste cell Type II cells represent ~25–30% of the cells in each taste bud and are responsible for transduction of many tastants. These cells are elongate, spindle-shaped cells with short and thick apical microvilli. The cell is typified by a large, round, clear nucleus, and pale cytoplasm (Figure 10a). Type II cells express the bevy of receptor and second-messenger proteins implicated in transduction of bitter, sweet, or umami stimuli. These include the known T1R and T2R families of taste receptors, gustducin (G-protein), PLCβ2, and IP3R3 (Yang *et al.*, 2000b; Miyoshi *et al.*, 2001; Kusakabe *et al.*, 2002; Clapp *et al.*, 2004). Thus, type II cells mediate detection of these classes of tastants. Curiously, although type II cells closely contact afferent nerve fibers within taste buds (e.g., Kinnamon *et al.*, 1985; Yang *et al.*, 2000a), synapses between these elements are rare. Rather, subsurface cisternae appear at points of contact between afferent fibers and type II cells (Clapp *et al.*, 2004).

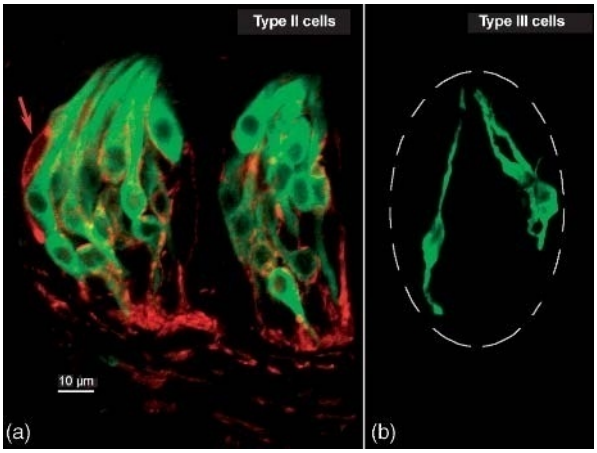

Figure 10 Fluorescence micrographs of immunocytochemically reacted taste buds of the circumvallate papilla from rodents. a, Reactivity for the inositol-trisphosphate receptor 3 (IP3R3) in a rat circumvallate papilla shows the morphology of typical type II taste receptor cells: broad, triangular cell body with a prominent, large, round nucleus. This section is also reacted for synaptobrevin revealing the numerous afferent nerve fibers and a rare taste cell (arrow), most likely a type III taste cell near the edge of the taste bud. b, A taste bud in the circumvallate papilla of a mouse, reacted for serotonin to reveal a population of type III taste cells. Note that they are more slender and less regular in shape than the type II cells illustrated in the left-hand panel. The approximate boundary of the taste bud is indicated by the dashed line. Photo courtesy of Drs. J. C. Kinnamon and R. Yang, Denver University, Denver, CO, USA.

Each type II taste cell is specified for detection of one class of taste substance and, therefore, expresses only one class of taste receptor, although multiple members of a class may be expressed in a single taste cell (Chandrashekar *et al.*, 2000; Zhang *et al.*, 2003). For example, a taste cell that expresses one member of the T2R family of receptors (for detecting bitter substances) will express several members of this same family (Adler *et al.*, 2000). Since each receptor molecule is responsive to only a small set of bitter substances, by expressing multiple members of the T2R family, a taste cell then exhibits broader responsiveness to many different bitter compounds. Whether type II cells also are the transduction elements for detection of sour and salty stimuli is unclear. At least some evidence implicates type III cells in these processes.

18.4.3.1.(iii) Type III taste cell The type III cell is relatively scarce in taste buds, comprising only 10–15% of the total population. Type III cells are sometimes called 'intermediate cells' since they share features with both type I and type II cells. What distinguishes type III cells from the others is the presence of well-formed synapses from the taste cells onto the afferent nerve fibers. Type III cells also exhibit some distinctive histochemical features which can be used to distinguish them from the other cell types. A subset of type III cells concentrates biogenic amines, including serotonin (Figure 10a). This property has led to their being likened to the Merkel-like basal cells of anamniote vertebrates (see the discussion above).

Type III taste cells are narrow, spindle-shaped cells that extend a single, thick apical process into the taste pore. Their nuclei are more elongate than those of type II cells and exhibit some degree of indentation. The cytoplasm is variable in staining density, ranging from light to dark. These features then are intermediate between the type I and II cells and have led some authors to consider type III cells to be a stage in the maturation of type II cells or to combine these cells into a single class (e.g., Delay *et al.*, 1986; Pumplin *et al.*, 1997). Recent studies suggest that type II and type III cells may arise from a common lineage (Finger, 2005; Kusakabe *et al.*, 2002; Miura *et al.*, 2005).

The function of type III cells is not established, but two possibilities are clear. First, the type III cells, being the only taste cells with prominent synapses, may serve as the only output cells of the taste bud,

receiving input from the transducing, type II taste cells and integrating this information before transmitting a signal to the afferent nerve fibers (Roper, 1992). The other possibility is that both type II and type III cells transmit information to nerve fibers, but that type II cells do so using a mechanism that does not require a conventional-looking synapse. Since type III cells exhibit voltage-gated ion channels (Medler *et al.*, 2003) and such cells respond to acidification (Richter *et al.*, 2003), then it is likely that type III cells are capable of directly transducing sour information and also passing this along the nerve fibers.

18.4.3.1.(iv) Other cells In addition to the three elongate types of taste cells described above, the taste buds of mammals, like those of nonmammalian vertebrates, are associated with proliferative basal cells and edge or marginal cells. Indirect evidence indicates that the taste bud progenitor cells of the basal epithelium are different than the basal cells of the general epithelium. When gustatory nerves are directed to grow into lingual epithelium that does not normally produce taste buds, the taste buds do not form despite the abundance of gustatory nerve fibers (Krimm *et al.*, 2001). The fact that taste nerves are unable to induce taste buds in anything but taste epithelium suggests that the epithelial cells in taste-bud bearing regions have a special capacity to generate these end organs. Conversely, when taste epithelia are innervated only by nongustatory nerves, then production of taste buds is limited at best (Farbman, 1971). Together, these studies indicate that basal cells of taste epithelia have a unique capacity to produce taste buds under the influence of gustatory innervation.

18.5 Detection and Representation of Different Tastes

The taste systems in vertebrates have the ability to respond to a variety of stimuli according to the habitat and nutritional needs of the organism. These taste stimuli are varied in chemical properties, including size, charge, hydrophobicity, and pH. Despite the diverse array of vertebrates and habitats, the taste system has a remarkable consistency in the types of compounds it can respond to. This may be due to the fact that many substances, for example, plant alkaloids, are toxic to most vertebrates and therefore all vertebrates require a food monitoring system capable of detecting potential toxins in the food supply. Conversely, different vertebrates have different nutritional needs and drives, so somewhat more divergence exists in terms of what substances can drive appetitive behaviors. Looking across all organisms, the taste system serves two primary functions: avoiding toxins and driving ingestion for nutritive substances. This means that the responses of the taste system should vary according to the diet of the particular organism. For example, the taste system of carnivores should not be driven by sugars, whereas the taste system of herbivores should be highly responsive to sugar. In contrast, most species should respond to amino acids.

Different cells in taste buds respond optimally to different taste qualities. It is interesting to note that this principle of cellular coding also occurs in taste organs of invertebrates. Since taste buds and 'taste' organs of invertebrates are not homologous, this property of encoding taste information should be viewed as convergent rather than evolutionarily conserved. Indeed, the chemosensory cells of the invertebrates seem more organized according to the behaviors they induce rather than the nature of the chemical stimulus detected. For example, a single chemosensory cell (ASE) in the amphid of *C. elegans* may respond to cAMP, biotin, and lysine despite their diverse chemical structures. But all of these substances are attractants. So, stimulation of the ASE cell will produce attraction. The taste systems in more complex organisms, for example, flies, fish, or mammals, have more complexity. Several substances may drive ingestion, but may be detected by different receptor cells. For example, both alanine and arginine drive appetitive behavior in catfish, but these amino acids appear to be detected by different receptors expressed in different taste cells (Finger *et al.*, 1996). Similarly, different taste cells in mice express receptors for glutamate and sweeteners although both drive food intake (Zhang *et al.*, 2003).

18.6 Evolution of Taste Preference and Taste Receptors

In order for a species to adapt to a new habitat or feeding strategy, the spectrum of substances to which its taste organs respond must change. For example, for terrestrial animals, especially those with a purely vegetarian diet, sodium is a crucial nutrient. Salt-deprived amniotes have a drive to seek out and ingest salt (Schulkin, 1991). Their taste system carries unique information about the sodium content of potential foodstuffs and detection of the sodium is regulated in part by

circulating hormones that alter the sensitivity of the sodium-detecting channels of the taste buds (Herness, 1992; Lin *et al.*, 1999). Yet, sodium is not a crucial nutrient for aquatic anamniotes, so their taste systems are not particularly responsive to sodium content of food (Caprio *et al.*, 1993). Thus, the responsiveness of taste buds had to change when vertebrates made the transition from water to land. In frogs, the entire epithelium is sensitive to sodium levels, perhaps via the same ion channel (AsNaC) used in sodium detection in many mammals (Nagai *et al.*, 1999). Thus transduction of sodium by taste may have evolved from a general epithelial property of regulated sodium transport.

The receptors for tastes can be ion channels themselves (as in the case of sodium (salty) or protons (sour), may be ligand-gated ion channels (e.g., for arginine-detection in catfish; Brand *et al.*, 1991), or can be G-protein-coupled receptors (Brand *et al.*, 1991; Adler *et al.*, 2000; Chandrashekar *et al.*, 2000; Zhang *et al.*, 2003; Ishimaru *et al.*, 2005). The G-protein-coupled receptors, T1R and T2R families, are phylogenetically old since family members have been identified in fish as well as in mammals. Yet in mammals, some of these receptors respond to sweeteners whereas in fish, sweeteners are not effective taste stimuli. Accordingly, evolutionary change of the receptor molecules is likely to correspond to evolutionary changes in the spectrum of substances to which the taste system can respond. This can be seen in the evolution of felines which are insensitive to sugars and other sweeteners unlike other carnivores. The taste system of basal carnivores most likely responds well to sweet substances since many contemporary carnivores, for example, dogs and bears, are strongly attracted to sweets, whereas cats are not (Li *et al.*, 2005). One of the genes encoding the sweet taste receptor is nonfunctional in cats, thereby rendering them insensitive to sugar and other sweeteners. Thus, a simple mutation in a single taste receptor gene is capable of altering the diet of a species.

Acknowledgments

This article is dedicated to the memory of Theodore H. Bullock, whose boundless enthusiasm and insights served as an inspiration to us all. The author thanks Drs. Anne Hansen and Linda Barlow for their comments and improvements on various drafts of this work. This work was supported by NIH Grants to T. Finger and D. Restrepo.

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19 Shared and Convergent Features of the Auditory System of Vertebrates

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Glossary

interaural time difference Birds and mammals use the interaural time difference (ITD) for localization in the horizontal plane. When sound comes from one side of the body, it reaches one ear before the other, which creates an ITD. These ITDs depend upon head size, and in some cases on an interaural canal. In general, animals with large heads have larger time differences available to them.

phase locking The auditory system uses phase locked spikes to encode the timing or phase of the auditory signal. Phase locked neurons fire spikes at or near particular phase angles of sinusoidal waveform. Physiological experiments measure this spike phase with respect to the stimulus period. Spike phase is plotted in a period histogram, and is used to calculate the statistic vector strength (r). Each spike defines a vector of unit length with a measured phase angle. The vectors characterizing the spikes are plotted on a unit circle and the mean vector calculated. The length of the mean vector provides a measure of the degree of synchronization.

19.1 Introduction

Grothe *et al.* (2005) have pointed out that we cannot construct a comprehensive description of the evolution of the vertebrate central auditory system because we do not know what the common ancestors of terrestrial vertebrates could hear or how their ears worked. It seems likely, however, that tympanic ears have evolved independently in synapsids, lepidosauromorph diapsids, archosaurs, turtles, and amphibians (Clack, 1997). These new ears could convey responses to both vibration and airborne sound to brainstem auditory neurons, leading to the parallel evolution of the central targets of the auditory nerve. Further developments in ancestral mammals, such as moveable ears and multiple ossicles, may have had additional reorganizing effects. In this review we will argue that the elaborated central auditory systems in the different clades of recent land vertebrates have evolved in parallel.

With the transition to airborne sound, selection might be expected to improve the auditory system's ability to encode the more rapid changes associated with higher frequency sounds. For example, precise encoding of temporal information at best frequencies above 1–2 kHz is biophysically demanding (see Koppl, 1997), but also has direct behavioral

relevance for sound localization and communication (Hafter and Trahiotis, 1997; Heffner and Heffner, 1992) and should be selected for.

We will discuss the appearance of similar physiological and morphological adaptations for encoding both time and level information in the auditory brainstem of birds and mammals, and draw two major conclusions. First, the similarities among the brainstem circuits that encode sound in birds and mammals appear to be the result of parallel evolution. Second, the existence of these similar circuits allows us to identify algorithms shared by the auditory system of birds and mammals, and to argue that these are suited to extracting the stimulus variables relevant for auditory coding; thus, studies of evolution can inform computational neurobiology. For further discussion of this topic, the reader is directed to recent reviews including Grothe *et al*. (2005) and Carr and Soares (2002).

Animals with tympanic ears should experience similar constraints in detecting sounds. The essential features of auditory coding are very similar in birds and mammals. Understanding the evolutionary and developmental events behind the similar form and function of temporal coding cells in birds and mammals will require a detailed knowledge of multiple species under study, and of their phylogenetic relationships. This requires a deliberate concentration upon comparative neurobiology, and the differences among animals. In this review we compare similar coding strategies in the auditory systems of a few species of birds and mammals in order to identify shared features (Section 19.2). We then examine the special case of temporal coding in birds and mammals, and use these comparative studies to argue that natural selection has produced suitable solutions to the problems of temporal coding (Sections 19.3 and 19.4).

19.2 Encoding Sound: Similar Strategies in Birds and Mammals

Both birds and mammals use similar strategies to encode various aspects of the auditory scene. In fact, all animals may use similar auditory codes (Fay, 1988; Manley, 2005). Vertebrate auditory systems exhibit a similar basic Bauplan, in which the auditory nerve enters the hindbrain and bifurcates to contact different subdivisions of the cochlear nucleus (Ryugo and Parks, 2003). The cochlear nuclei give rise to different connections within the lower auditory system, including projections to groups of neurons in the superior olive and the lateral lemniscus before these pathways reunite at the level of the auditory midbrain. Despite these large-scale similarities, the details of the connections and cell types show substantial diversity between the major vertebrate clades (reviews in Cant, 1992; Carr and Code, 2000; McCormick, 1999).

In both birds and mammals, the cochlear nuclei encode parallel ascending streams of auditory information. In birds, the auditory nerve projects to nucleus magnocellularis and nucleus angularis in the pattern described for the bird and reptile morphotype. The nucleus magnocellularis is the origin of a neural pathway that encodes timing information, while a parallel pathway for encoding sound level originates with nucleus angularis (Takahashi and Konishi, 1988a). In mammals, auditory nerve afferents send an ascending branch to the anterior ventral cochlear nucleus and a descending branch to both the posterior ventral cochlear nucleus and the new dorsal cochlear nucleus. This division may also support parallel encoding of sound level and time, although evidence for this simple division is not strong. Instead, it appears that the cells in the mammalian cochlear nucleus form many parallel ascending streams (Cant and Benson, 2003; see the discussion below). In both birds and mammals, the auditory nerve forms different types of terminals onto different cell types in the cochlear nucleus (Ryugo and Parks, 2003). Endbulbs of Held terminals are formed on bushy cells (see Section 19.3), and bouton-like terminals on the other cell types in the cochlear nuclei. The auditory nerve appears to use glutamate as a transmitter, often with the postsynaptic cell expressing 'fast' AMPA type glutamate receptors that can mediate precise temporal coding (Parks, 2000).

The avian and mammalian cochlear nuclei both contain heterogeneous cell populations, and exhibit similar responses to sound (Koppl and Carr, 2003; Soares *et al.*, 2002; for review of the mammalian cochlear nuclei, see Romand and Avan, 1997; Cant and Benson, 2003). We do not know if these similar features evolved as a response to selective pressures to encode airborne sound. There are two reasons why similarities might be due to parallel evolution. First, true tympanic ears arose independently in birds and mammals (Clack, 1997). These peripheral changes would have had different reorganizing effects upon the ancestral population of brainstem auditory neurons. Second, the cell types of the avian and mammalian cochlear nuclei are similar but not identical. We describe the similarities and differences between birds and mammals in this section. A satisfactory study will, however, require detailed analyses of the development, morphology and

physiology of cell types in the cochlear nucleus in all amniote groups, including turtles, basal lizards, and crocodilians.

19.2.1 Organization of the Cochlear Nuclei in Mammals and Birds

It has been historically difficult to compare the mammalian and avian cochlear nuclei. To do so in this article, we will first describe the cochlear nuclei in mammals and birds, then discuss their similarities and differences. The mammalian cochlear nuclear complex is divided into dorsal and ventral nuclei that contain separate, well-defined populations of cells. The large projection cells are distinguished on the basis of morphology, projections, and physiological responses to sound (Cant and Benson, 2003; Rhode and Greenberg, 1992; Rouiller, 1997; Young, 1998). The anterior part of the ventral cochlear nucleus contains bushy cells that respond in a primary or auditory nerve-like fashion to the auditory stimulus. The posterior part of the ventral cochlear nucleus contains octopus cells that respond to onsets or stimulus transients and two classes of multipolar neurons that respond principally with 'chopper' firing patterns.

Bushy cells receive endbulb inputs from the auditory nerve and exhibit accurate temporal coding. There are two forms of bushy cells, spherical and globular. Spherical cells dominate the anterior ventral cochlear nucleus, respond to lower best frequencies and project to the medial superior olive, which is sensitive to interaural time differences (ITDs). Globular bushy cells by comparison sometimes chop or exhibit onset responses to the stimulus, respond to higher frequencies, and project to the lateral superior olive and the medial nucleus of the trapezoid body. These projections may mediate detection of interaural level differences. Octopus cells in the posterior ventral cochlear nucleus are multipolar, with thick dendrites that extend across the nerve root (Oertel *et al*., 2000). This morphology enables them to integrate auditory nerve inputs across a range of frequencies. Octopus cells encode the time structure of stimuli with great precision and exhibit onset responses to tonal stimuli. Onsets play an important role in theories of speech perception, and segregation and grouping of sound sources (Bregman, 1990). Cochlear root neurons send widespread projections to areas of the reticular formation involved in startle reflexes and autonomic functions. Type I multipolar cells may encode complex features of natural stimuli and send excitatory projections directly to the inferior colliculus. Type II multipolar cells send inhibitory projections to the contralateral cochlear nuclei (Cant and Benson, 2003).

The dorsal cochlear nucleus (DCN) appears for the first time in mammals, perhaps associated with the development of high-frequency hearing and motile external ears. DCN cells exhibit wide variety of response types, with one theory of function relating to echo suppression. The DCN is composed of a cerebellar-like circuit in the superficial layers, with projection cells in the deep layers that receive auditory nerve inputs (Young, 1998). The granule cells in the superficial layers receive sensory input that may convey information about head and ear position. The deep portion of the DCN contains fusiform and giant cells. Fusiform cells exhibit complex (Type IV) frequency-tuning curves, with small areas of excitation at best frequency and at the edges of the response curves. Type IV responses appear well suited to detecting the notches in sound level created by the pinna that provide cues for locating sound in elevation (May, 2000). Fusiform cell responses may mediate localization of sounds based on spectral cues and send direct excitatory projections to the inferior colliculus. Giant cells in the DCN also project directly to the inferior colliculus; some of them may convey inhibitory inputs to the contralateral cochlear nucleus as well (Cant and Benson, 2003).

In birds the auditory nerve projects to two cochlear nuclei, the nucleus magnocellularis and the nucleus angularis (Carr and Code, 2000). The nucleus magnocellularis principal cells dominate all but the low best-frequency region of the nucleus, and project to the nucleus laminaris, which is sensitive to ITDs. The nucleus angularis contains 4–5 cell types that project to the superior olive, to the lemniscal nuclei, and to the central nucleus of the auditory midbrain. The parallel ascending projections of angularis and laminaris may or may not overlap with one another, and probably do overlap in the primitive condition.

The mammalian bushy cells are very similar to the avian magnocellular neurons (see Sections 19.3 and 19.4) and originally nucleus angularis was thought to be similar to the mammalian DCN (Boord, 1969; Sachs and Sinnott, 1978; Sachs and Young, 1980). Closer examination has, however, shown that there are no deep morphological correspondences between nucleus angularis (NA) and the DCN (Soares and Carr, 2001) although there are physiological similarities. Both nuclei contain a cell type that exhibits type IV (complex nonmonotonic) physiological responses (Koppl and Carr, 2003; Sachs and Sinnott, 1978; for review of DCN see Young *et al*., 1988). Parsimony would suggest that the type

IV responses observed in the redwing blackbird by Sachs and Sinnot and in the barn owl by Koppl and Carr may have emerged in parallel with similar responses in mammalian DCN. The DCN appears to be a unique feature of the mammalian auditory system. Furthermore, unlike the case with the NA, the DCN shares many common features with the cerebellum, including unique cell types and cortical circuitry (Berrebi *et al*., 1990; Oertel and Young, 2004; Wright and Ryugo, 1996).

19.2.2 Morphology of Cell Types in Birds and Mammals

The morphological characteristics of neurons contribute to the input–output functions of neural circuits. We suggest that similar rules of dendritic organization apply to the cochlear nuclei of both mammals and birds. Furthermore, examination of similar cell types in birds and mammals may reveal shared computational strategies.

Neurons of the rat ventral cochlear nucleus that project to the DCN have been divided into two main groups: radiate and planar (Doucet and Ryugo, 1997). Radiate neurons have long dendrites perpendicular to isofrequency contours and are sensitive to a broad range of frequencies. Planar neurons, on the other hand, have dendrites that are confined to an isofrequency plane, therefore more sensitive to a narrow range of frequencies.

At a first approximation, the avian NA has an organization similar to that seen in the rat ventral cochlear nucleus. In both the barn owl (Soares and Carr, 2001) and the chicken (Fukui and Ohmori, 2003; Soares *et al*., 2002), NA contains several major morphological classes of neurons. In the barn owl, these are classified as planar, radiate, vertical, and stubby. Planar neurons are confined to an isofrequency band, whereas radiate neurons have dendrites that could extend across an isofrequency band. Vertical cells have long dendrites oriented perpendicularly to isofrequency bands. Stubby cells are confined to an isofrequency band because of their short dendrites. Representatives of all cell classes can be found throughout NA of the chicken (Fukui and Ohmori, 2003; Soares *et al*., 2002). Thus, a similar pattern of organization appears to have evolved in parallel in the cochlear nuclei of both birds and mammals, in which one population (planar, stubby, and bushy) remains within an isofrequency band, another (radiate) extends across the isofrequency axis, and a third (vertical, marginal, and octopus) has a dendritic orientation orthogonal to the isofrequency axis.

Although there are shared morphological characteristics among individual neurons of both clades, other organizational rules differ. First, there are many cell types within the mammalian ventral cochlear nucleus that are not included in Doucet and Ryugo's classification scheme, principally bushy cells, octopus cells, and small cell types (for reviews, see Cant and Benson, 2003). Bushy cells appear to be similar to nucleus magnocellularis (NM) neurons, but there are no obvious morphological counterparts to octopus cells in the avian cochlear nuclei. This is significant because both octopus cells and cells in NA respond to sound with onset responses (Sullivan, 1985; Warchol and Dallos, 1990). Nevertheless, Golgi analyses of barn owl NA neurons and intracellular labeling of cells in chicken NA have not revealed cells with the characteristic octopus cell morphology – thick dendrites that extend across the incoming auditory nerve inputs (Soares and Carr, 2001; Soares *et al*., 2002). Thus, it appears that the evolution may not necessarily have produced identical solutions for encoding onset of sounds. Second, the majority of NA cells are stubby neurons that have no obvious counterpart within the multipolar cell types of the mammalian ventral cochlear nucleus. Instead, they most closely resemble NM neurons and bushy cells. Third, small cells rarely appear in NA (Soares and Carr, 2001), and it seems that they are not as various or numerous in NA as in the mammalian cochlear nucleus. Finally, the avian cochlear nuclei have neither the granule cell layer that characterizes mammalian cochlear nucleus, nor a DCN.

19.2.3 Intracellular Physiological Responses of Cochlear Nucleus Neurons in Birds and Mammals

Descriptions of neural circuitry are based on both dendritic morphology and physiological characteristics. Descriptions of both *in vivo* and *in vitro* responses complement the morphological studies, and responses of cochlear nucleus neurons in brain slices from both birds (chicken) and mammals (rat, mouse) can be compared.

There appear to be no direct one-to-one physiological correspondences between neurons in birds and mammals, with the exception of neurons that are specialized for temporal coding, such as mammalian bushy cells and avian magnocellular neurons. Even these very similar responses may have evolved in parallel: the suite of features that distinguish temporal coding neurons in auditory nuclei, including the nuclei of the lateral lemniscus (Wu, 1999a), is also found in temporal coding

neurons in electric fish (Carr, 1986; Rashid *et al*., 2001). One cannot therefore use shared features to argue for homology among cochlear nucleus neurons in birds and mammals. Instead, shared features may be used to identify common computational strategies.

Avian NM neurons and NA stubby neurons respond with only one spike when depolarized. The responses of NA stubby neurons are similar to those of both bushy and octopus cells in the mammalian VCN (Golding *et al*., 1999; Manis and Marx, 1991; Wu and Oertel, 1984). These mammalian cell types exhibit the depolarization-activated, dendrotoxin-sensitive, low-threshold K^+ conductance that is activated at rest (see Section 19.2; Manis and Marx, 1991; Bal and Oertel, 2000; Brew and Forsythe, 1995). A similar dendrotoxin-sensitive conductance underlies the responses of NA one spike neurons (Fukui and Ohmori, 2003), NM and nucleus laminaris (NL) neurons (Rathouz and Trussell, 1998; Reyes *et al*., 1994, 1996) and the irregularly firing principal cells of the tangential nucleus (Gamkrelidze *et al*., 1998, 2000). These biophysical similarities suggest that stubby neurons, like bushy, octopus, NM and NL neurons, may mediate accurate transmission of temporal information.

There are additional shared physiological characteristics between the cochlear nuclei of both groups. Despite the multiplicity of cochlear nucleus cell types, auditory nerve synapse kinetics are similar in all. In avian NA, spontaneous excitatory post synaptic current (EPSC) receptor kinetics are the same for all cell types (MacLeod and Carr, 2005). This is also the case for bushy, T-stellate, tuberculoventral and octopus cells in the mammalian VCN (Gardner *et al*., 1999). Other NA cell types share biophysical features with mammalian VCN neurons (Soares and Carr, 2001).

19.2.4 Ascending Lemniscal Projections

Avian and mammalian cochlear nuclei both share ascending lemniscal projections, but these differ in many respects (Figure 1). The greatest difference may be the comparative lack of descending projections in birds when compared with mammals (Carr and Code, 2000).

In birds and crocodilians, the NA projects to the superior olive, the contralateral posterior portion of the dorsal nucleus of the lateral lemniscus (Figure 1a; Takahashi and Konishi, 1988a; Wild *et al*., 2001) and the inferior colliculus (Conlee and Parks, 1986; Yang *et al*., 1999). The posterior division of the dorsal nucleus of the lateral lemniscus is the first site of binaural interactions in the intensity pathway of the barn owl and is where sensitivity to interaural level differences first appears (Manley *et al*., 1988; Moiseff and Konishi, 1983). The pathways encoding ITDs and interaural level differences ultimately converge in the external nucleus of the inferior colliculus, where neurons are selective for combination of interaural time and level differences (Figure 1a, for review, see Konishi, 2000). The projections of the mammalian cochlear nucleus are more elaborate than those in birds (Figure 1b; for reviews see Cant and Benson, 2003; Cant and Hyson, 1992; Romand and Avan, 1997).

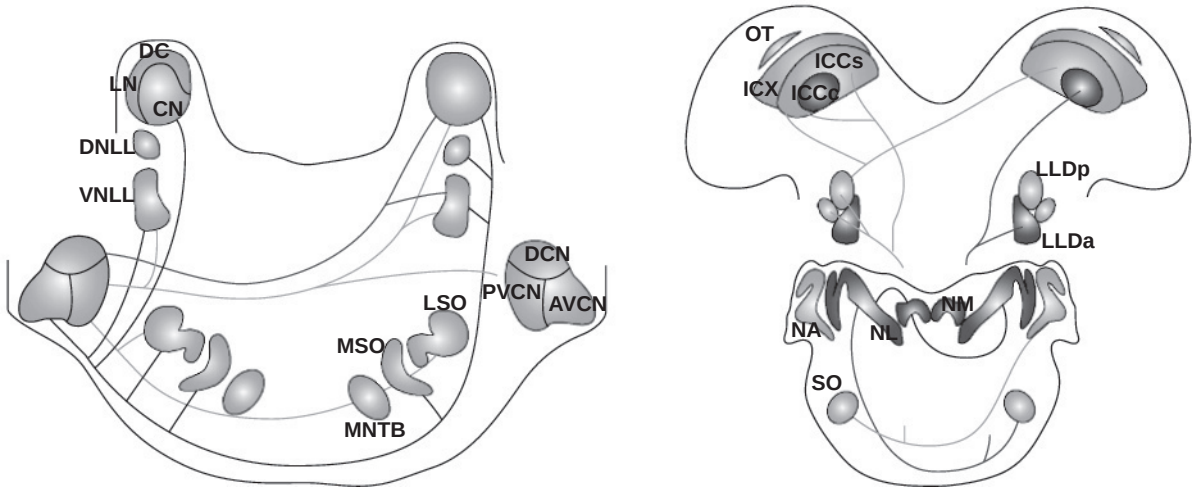
19.3 Encoding Temporal Information

Auditory nerve fibers encode temporal information by phase-locking to the waveform of the acoustic stimulus, and preserve this temporal information in projections to NM and NA. Three lines of evidence show that accurate temporal coding is important for sound localization. First, measurements of the vector strength of the auditory nerve signal, calculated from the variability in the timing of spikes with respect to the phase of the acoustic stimulus, show an improvement in high frequency phase-locking in the owl as compared to other animals by an octave or more (Koppl, 1997). Second, models of coincidence detection perform better when the vector strength of the inputs improves (Colburn *et al*., 1990; Simon *et al*., 1999). Third, inactivation of NM neurons with lidocaine removes sensitivity to ITDs from the responses of midbrain space-mapped neurons (Takahashi *et al*., 1984).

We will review the features associated with preserving temporal cues up to the point where ITDs are detected. There are several shared features of temporal coding circuits in the auditory systems of birds and mammals. These include high-quality inputs, presynaptic specializations to make neurotransmitter release precise, and postsynaptic specializations, including specific glutamate receptors, potassium conductances, and characteristic neuronal morphology. These features have been reviewed in Oertel (1999), Trussell (1999), and Carr and Friedman (1999).

19.3.1 Precise Synaptic Transmission

The task of accurately representing the stimulus phase becomes more difficult with increasing stimulus frequency (Hill *et al*., 1989). This is because the absolute temporal precision required for phase-locking to high frequencies is greater than that needed for low frequencies, that is, the same



(a) (b)

Figure 1 Schematic showing the connections in the mammalian and avian auditory brainstem. a, Mammals: the auditory nerve bifurcates to give rise to an ascending and a descending branch. The ascending branch innervates the AVCN, and the descending branch innervates first the PVCN and then the DCN. The projections of the cochlear nuclei are denoted as different lines (AVCN dark lines, PVCN light lines, and DCN dotted lines). The cochlear nuclei send ascending projections to the olivary and periolivary nuclei, which include the MNTB, MSO, and LSO. The IC in mammals is subdivided into a central nucleus, an external cortex, and a dorsal cortex. Stellate cells from VCN and fusiform and giant cells from DCN project to the contralateral central nucleus of the IC giving rise to banded inputs. The central nucleus receives bilateral input from LSO and a mostly ispilateral input from MSO, also forming banded, tonotopically organized projections. It also receives projections from the nuclei of the lateral lemniscus (DNLL and VNLL). b, Birds: in barn owls, separation into time and sound level pathways (dark lines and light lines, respectively) begins with the cochlear nuclei. Eighth nerve afferents divide to innervate both the level-coding NA and the time-coding NM. NM projects bilaterally to NL, which in turn projects to superior olive, nucleus of the lateral lemniscus, and to the central nucleus of the inferior colliculus. The superior olive projects back to NA, NM, and NL (projections are not drawn). In birds, the IC is subdivided into two principal subnuclei, the external nucleus (ICX) and the more medially located central nucleus (ICC). a, Reproduced from Sound Source Localization, 2005. Development of sound localization, Kubke, M. F. and Carr, C. E. (*eds.* A. N. Popper and R. R. Fay), Springer. With kind permission of Springer Science and Business Media. b, Reproduced from Kubke, M. F., Gauger, B., Basu, L., Wagner, H., and Carr, C. E. 1999. Development of calretinin immunoreactivity in the brainstem auditory nuclei of the barn owl (*Tyto alba*). *J. Comp. Neurol.* 415, 189 203.

variation in temporal jitter of spikes translates to greater variation in phase for high frequencies. Hill *et al*. (1989) estimated phase-locking in the auditory fibers of the pigeon in terms of the commonly used synchronicity index (vector strength) as well as by measuring temporal dispersion. Vector strength of phase-locking decreases for frequencies above 1 kHz. Temporal dispersion, however, also decreases with frequency, indicating enhanced temporal synchrony as frequency increased (Koppl, 1997). The upper frequency limit of phase-locking therefore appears to depend upon the ability of hair cells to phase-lock and upon an irreducible jitter in the timing of spikes. It is about 8–9 kHz for barn owls, and between 4 and 6 kHz for most other birds and mammals studied (Koppl, 1997).

Endbulb or caliciform synapses mediate the transmission of phase-locked spikes. These synapses form a fenestrated cup that envelopes most or part of the cell body, and contains large numbers of active zones (Nicol and Walmsley, 2002). The invasion of the presynaptic spike into the calyx leads to the synchronous release of quanta at these sites, endowing the synapse with a high safety factor (Sabatini and Regehr, 1999). Studies of endbulbs in both the avian NM and the medial nucleus of the trapezoid body (MNTB) have shown that the invading presynaptic spike is extremely narrow (Turecek and Trussell, 2001), probably due to rapid repolarization mediated by specific potassium conductances. Calcium influx into the presynaptic terminal is also brief and occurs only during the falling phase of the presynaptic spike (Turecek and Trussell, 2001). Because the spike is narrow, its downstroke occurs quickly, as does calcium influx, reducing the synaptic delay. The brief period of calcium influx produces a confined and phasic period of neurotransmitter release, increasing the temporal precision of transmission across the synapse (Brenowitz and Trussell, 2001b).

Endbulb terminals may have emerged as an adaptation for accurate transmission of phase

information for frequencies above ~500 Hz, perhaps also associated with the development of hearing in land vertebrates. Large endbulb-like terminals have been found in all amniote groups examined. There are no data on turtles, but in the alligator lizard the auditory nerve forms both large somatic terminals and smaller boutons in NM (Szpir *et al.*, 1990). In crocodilian NM, the rostral high best frequency NM neurons receive endbulb-like projections, while lower best frequency NM neurons receive bouton terminals (Soares, unpublished). Large endbulb terminals are found in both birds and mammals (Ryugo and Sento, 1991), and may be developed in parallel in archosaurs and mammals to mediate accurate transmission of temporal information at higher sound frequencies (Carr and Soares, 2002). Evidence for the parallel evolution of endbulbs in birds and mammals comes from studies of their development, which follows different trajectories (Rubel and Fritzsch, 2002), and from studies of transmitter release modulation (Trussell, 2002).

Support for the hypothesis that endbulbs evolved to facilitate transmission of high best frequency phase-locking comes from comparisons of low and high best frequency regions of NM. Endbulb terminals do not appear to be essential for transmission of phase-locked spikes at low frequencies, because they are not found in low best frequency regions. The very low best frequency cells of the NM receive large bouton terminals from the auditory nerve and phase-lock to frequencies below ~1 kHz (Koppl, 1997), while in crocodilian NM, only the rostral high best frequency NM neurons receive endbulb-like projections (Soares, unpublished).

19.3.2 Glutamate Receptors

Activation of AMPA type glutamate receptors at endbulb synapses generates brief, large synaptic currents that are suited to the transfer of temporally precise information from pre- to postsynaptic cell (Raman and Trussell, 1995; Zhang and Trussell, 1994). The brevity of EPSCs in these neurons depends not only on the time course of release but also on the specific properties of the postsynaptic receptors. AMPA receptors are made up of glutamate receptor subunit (GluR) splice variants, and the GluR3 and 4_{flop} isoforms found in auditory neurons have fast kinetics and rapid desensitization rates, such that the duration of miniature EPSCs in auditory neurons are among the shortest recorded for any neuron (Gardner *et al.*, 1999; Trussell, 1999). These rapid kinetics are due to a characteristic 'auditory' pattern of expression (Parks, 2000). In the chicken NM, where a homogeneous population of neurons makes mRNA analysis possible, the relative abundance of the four AMPA receptor subunits reveal very low levels of GluR2, and higher levels of the 'fast' flop splice variants of GluR3 and 4 (Parks, 2000; Ravindranathan *et al.*, 1996). Similar splice variants characterize mammalian bushy cells (Gardner *et al.*, 2001). Further support for the role of GluR4 has emerged from developmental studies. AMPA receptor-mediated EPSCs in NM and MNTB become faster in decay time as animals mature (Brenowitz and Trussell, 2001a; Joshi *et al.*, 2004; Koike-Tani *et al.*, 2005). In parallel with the increase in kinetics, GluR4 flop increases from P7 to P14 and changes little thereafter (Koike-Tani *et al.*, 2005).

19.3.3 Potassium Conductances

Although brief EPSCs underlie the precisely timed responses of neurons that phase-lock to the auditory stimulus, the intrinsic electrical properties of these neurons also shape the synaptic response as well as the temporal firing pattern. Two K^+ conductances are important for phase-locked responses in auditory neurons: a low-threshold conductance and a high-threshold conductance (Brew and Forsythe, 1995; Manis and Marx, 1991; Rathouz and Trussell, 1998; Reyes *et al.*, 1994; Wang and Kaczmarek, 1998).

The low-threshold conductance activates at potentials near rest and is largely responsible for the outward rectification and nonlinear current–voltage relationship around the resting potential seen in a number of auditory neurons (for review, see Oertel, 1999). Activation of the low-threshold conductance leads to a short active time constant so that the effects of excitation are brief and do not summate in time (Wu and Oertel, 1984). Only large excitatory post synaptic potentials (EPSPs) reaching threshold before significant activation of the low-threshold conductance would produce spikes with short latencies, whereas small EPSPs that depolarize the membrane more slowly would allow time for low threshold conductance activation to shunt the synaptic current and prevent spike generation and thus long latency spikes. Blocking the low-threshold conductance elicits multiple spiking in response to depolarizing current injection (Manis and Marx, 1991; Rathouz and Trussell, 1998) or synaptic activation (Brew and Forsythe, 1995). The K^+ channels underlying this conductance appear to be composed of Kv1.1 and Kv1.2 subunits. Both subunits are

expressed in auditory neurons, although the subcellular distribution is unknown (Grigg *et al*., 2000). In NM, neurons express Kv1.1 potassium channel mRNA and protein, in a gradient that is highest in the high-BF region of NM (Fukui and Ohmori, 2004; Lu *et al*., 2004).

The high-threshold conductance is characterized by an activation threshold around –20 mV and by fast kinetics (Brew and Forsythe, 1995; Rathouz and Trussell, 1998; Wang and Kaczmarek, 1998). These features of the high-threshold conductance result in fast spike repolarization and a large but brief afterhyperpolarization without influencing input resistance, threshold, or spike rise time. Thus, the high-threshold conductance can keep spikes brief without affecting spike generation. In addition, the high-threshold conductance minimizes Na^+ channel inactivation, allowing cells to reach firing threshold sooner and thereby facilitating high-frequency firing. In the MNTB, blockade of this conductance diminishes the ability to follow high-frequency stimuli in the range of 300–400 Hz, but has little effect on responses to low-frequency (< 200 Hz) stimulation (Wang and Kaczmarek, 1998). Also in MNTB, elimination of the Kv3.1 gene in mice results in the loss of a high-threshold component of potassium current and failure of the neurons to follow high-frequency stimulation (Macica *et al*., 2003).

Similar potassium conductances characterize other time-coding cells. There are numerous examples, many discussed in Oertel's (1999) review. In addition to the NM and mammalian MNTB neurons discussed above, the coincidence detectors in the avian NL and mammalian medial superior olive also express similar conductances and respond with temporal precision to the auditory stimulus (see the discussion below). The reasons for temporal precision are clear for the circuit that detects ITDs. There are also other aspects of the auditory stimulus that require temporal precision. In particular, the mammalian cochlear nucleus octopus cells form the origin of a circuit that encode timing of events, especially broadband transients. Octopus cells produce the briefest, most sharply timed synaptic responses in mouse cochlear nucleus (Golding *et al*., 1995). Octopus cells are characterized by both a large low-threshold conductance and a high-threshold conductance (Bal and Oertel, 2000). Type II cells in the ventral nucleus of the lateral lemniscus produce sharply timed responses and receive endbulb input from octopus cells (Wu, 1999b). Thus, selection for temporal accuracy may in each case drive expression of conductances that improve neuronal performance and behavioral accuracy.

19.3.4 Large Neurons

Selective pressure for temporal accuracy at the synapse, particularly for high-frequency inputs, may have driven the evolution of larger cell size. Larger somata and axons are less vulnerable to noise caused by stray currents, since their low input resistance keeps the influence of voltage fluctuations to a minimum. Many of the known time-coding pathways include large cells (Carr and Amagai, 1996). Enlarged size must be accompanied by an increase in synaptic current. Fast, large synaptic currents minimize the influence of ambient voltage fluctuations on the timing of spikes. Reducing the electrotonic distance between the synapse and the site of integration can enhance these effects. This occurs in electric fish neurons that encode the phase of the electric organ signal and in the cells of the nucleus magnocellularis and the nucleus laminaris in birds (Jhaveri and Morest, 1982; Smith and Rubel, 1979; Carr and Boudreau, 1993) and in electric fish (Kawasaki and Guo, 1996). In the mammalian auditory system, both bushy and MNTB neurons are large, and characterized by calciform synapses and large brief synaptic currents (see Cant and Benson, 2003).

19.4 Coincidence Detection and Coding of ITDs

Behavioral experiments have shown that most animals use ITDs to localize sound (Fay, 1988). These time differences depend upon head size, and in some cases also upon an interaural canal. In general, animals with large heads have larger time differences available to them. Thus, animals with smaller heads have to achieve much greater resolution of binaural time differences than a large animal in order to obtain the same degree of accuracy. For example, barn owls and humans have very similar abilities to localize sound, since psychophysical studies have shown that human subjects are able to localize a frontal tone with an accuracy of about 2°, while owls have a best accuracy of 3° (Bala and Takahashi, 2000; Middlebrooks and Green, 1991). The human discrimination task is easier than the barn owl's because the human head is larger, but both humans and barn owls are extremely accurate. Heffner and Heffner (1992) have suggested that a major selective pressure on localization comes when animals with narrow fields of best visual acuity such as a fovea use accurate sound localization to direct their gaze. Animals with smaller heads or animals that do not look directly at a sound source tend to have poor localization ability.

How good is an ability to discriminate targets 3° apart? Given the barn owl's head size, 1.5° separation is equivalent to an ITD of about 3 μs (Moiseff and Konishi, 1981). Discrimination of these microsecond ITDs requires accurate transduction and processing of the original stimulus, followed by detection of ITDs. The preferred model for detecting temporal disparities was first proposed by Jeffress with his place theory (Figure 2; Jeffress, 1948). The model circuit is composed of two elements, delay lines, and coincidence detectors. The delay lines are created by varying axonal path lengths, and the coincidence detectors are neurons that respond maximally when they receive simultaneous inputs, that is, when the time difference is exactly compensated for by the delay introduced by the inputs. The Jeffress model explains not only how ITDs are measured but also how they are encoded. The circuit contains an array of coincidence detectors receiving input from afferent axons serving as delay lines. Because of its position in the array, each neuron responds only to sound coming from a particular direction, and thus the anatomical place of the neuron encodes the location of the sound. These neurons compute a new variable, time difference, and in the process, transform the time code into a place code. The selectivity of all higher-order auditory neurons to time difference derives from the 'labeled-line' output of the place map (Carr, 1993; Joris *et al.*, 1998; Konishi, 2003).

19.4.1 Delay Line – Coincidence Detection Circuits in Birds

The avian circuit conforms to the requirements of the Jeffress model, in that the axons from NM act as delay lines to create maps of ITD, and NL neurons act as coincidence detectors (see discussion below; Figure 2). Recent evidence from small mammals, however, suggests that the Jeffress model does not completely explain how ITDs are encoded in the mammalian auditory brainstem (McAlpine and Grothe, 2003). There are many shared features of ITD coding circuits in birds and mammals; principally, both encode the phase of the auditory stimulus, and both encode ITD, but there are significant differences between the two clades. We will therefore discuss birds, then mammals, and finally compare the coding strategies employed in each group.

In birds, the projections from NM to NL act as delay lines to create maps of ITDs, and NL neurons act as coincidence detectors (Figure 2a). NL neurons phase-lock to both monaural and binaural stimuli

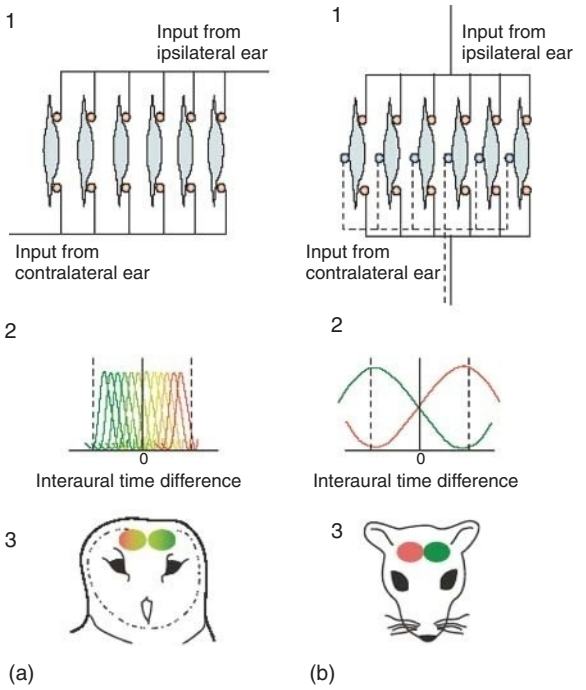


Figure 2 ITD coding strategies in birds and mammals. a, In birds, ITD detection depends upon a circuit composed of coincidence detectors and delay lines. (1) Each neuron in NL acts as a coincidence detector and fires maximally when inputs from the two sides arrive simultaneously. This occurs when the interaural phase differences are compensated for by an equal and opposite delay in the delay line inputs. (2) In the barn owl coincidence detector neurons are sharply tuned for ITDs relative to the width of the head (dotted lines). The lateral position of a sound source is read out as the position within the array that is maximally active. The different ITD tuning of single neurons is indicated by different colors. (3) Neurons in each brain hemisphere are tuned to different lateral positions in contralateral space. b, In mammals, the model of ITD-sensitive neurons does not depend on a map of ITDs created by delay line inputs. (1) In the gerbil, Grothe (2003) proposed that without inhibitory inputs, axonal conduction delays are distributed around zero ITD. The addition of glycinergic input from the contralateral ear (dotted lines) would shift the peaks of ITD functions toward longer ITDs. (2) The distribution of peak responses is positioned beyond the physiological range (dotted lines), centered on 45° interaural phase difference with respect to neural tuning for sound frequency. The sensitive slope of the broadly tuned functions is positioned within the physiological range. (3) The relative activation of the two brain hemispheres could provide a code of lateral position (McAlpine *et al.*, 2001). Reproduced from McAlpine, D. and Grothe, B. 2003. Sound localization and delay lines do mammals fit the model? *Trends Neurosci.* 26, 347 350, with permission from Elsevier.

but respond maximally when phase-locked spikes from each side arrive simultaneously, that is, when the difference in the conduction delays compensates for the ITD (Carr and Konishi, 1990; Overholt *et al.*, 1992; Pena *et al.*, 1996). Within the birds, the details of delay line circuit organization vary. In the chicken, NL is composed of a monolayer of

bipolar neurons that receive input from ipsi- and contralateral cochlear nucleus onto their dorsal and ventral dendrites, respectively (Rubel and Parks, 1975). These dendrites increase in length with decreasing best frequency. Evidence from brain slices suggests that only the projection from the contralateral cochlear nucleus acts as a delay line, while inputs from the ipsilateral cochlear nucleus arrive simultaneously at all neurons (Overholt *et al*., 1992). This pattern of inputs creates a single map of ITD in any tonotopic band in the mediolateral dimension of NL (Figure 2a; Overholt *et al*., 1992).

The barn owl is capable of great accuracy in detecting time differences, and its auditory system is hypertrophied in comparison to the chicken (Kubke *et al*., 2004). The nucleus laminaris is both much larger and reorganized when compared to the plesiomorphic condition exemplified by the chicken (Kubke *et al*., 2002, 2004). Magnocellular axons from both cochlear nuclei act as delay lines (Carr and Konishi, 1988). They convey the phase of the auditory stimulus to NL such that axons from the ipsilateral NM enter NL from the dorsal side, while axons from the contralateral NM enter from the ventral side. Recordings from these interdigitating ipsilateral and contralateral axons show regular changes in delay with depth in NL (Carr and Konishi, 1990). Thus, these afferents interdigitate to innervate dorsoventral arrays of neurons in NL in a sequential fashion, and produce multiple representations of ITD within the nucleus. Despite the differences in organization of NL in owls and chickens, ITDs are detected by neurons that act as coincidence detectors in both species (Joseph and Hyson, 1993; Kubke *et al*., 2002; Pena *et al*., 1996; Sullivan and Konishi, 1984).

A consistent feature of both avian and mammalian coincidence detectors is that they share physiological features with NM neurons and mammalian bushy cells (see Section 19.03.3). Coincidence detectors exhibit specific K^+ conductances that lead to a single or few well-timed spikes in response to a depolarizing stimulus *in vitro* (Kuba *et al*., 2002; Reyes *et al*., 1996; Smith, 1995). The low-threshold conductance channels should decrease the effective membrane time constant, that is, the average membrane time constant for a cell receiving and processing *in vivo* rates of EPSPs, which will be much shorter than the passive membrane time constant (Gerstner *et al*., 1996; Grau-Serrat *et al*., 2003; Softky, 1994). These fast conductances may be critical to coincidence detection, and current models suggest that fast membrane time constants are instrumental in keeping the firing rate near zero when the inputs are completely out of phase, and in allowing nonzero firing rate when the inputs are monaural.

19.4.2 ITD Detection Circuits in Mammals

The mammalian superior olive (MSO) contains neurons that receive excitatory input from the cochlear nucleus, and act as coincidence detectors to encode ITD (Goldberg and Brown, 1969; Yin and Chan, 1990). Despite this similarity with NL, the two structures may not be homologous, and their similarities may have emerged from the constraints of encoding ITD (Grothe, 2003). The reasons for assuming that the two nuclei are not homologous are both anatomical and physiological. First, the MSO is located in the ventral brainstem and forms part of the mammalian superior olivary complex, while the NL is dorsal and closely associated with NM. Thus, the embryological origins of the two structures may not be the same. Second, although MSO neurons act as coincidence detectors, it is not clear if their cochlear nucleus inputs act as delay lines to form maps of ITD. Anatomical data from the cat suggest that contralateral, but not ipsilateral inputs, could act as delay lines (Beckius *et al*., 1999), but there is as yet no unambiguous physiological evidence for a map formed by the incoming axons. Finally, the MSO receives fast, well-timed inhibitory inputs from the medial and lateral nucleus of the trapezoid body (Cant and Hyson, 1992; Grothe, 2003). These inhibitory inputs may enhance coincidence detection in several ways. Inhibition may produce a somatic shunt during coincidence detection to decrease the membrane time constant (Brughera *et al*., 1996). Inhibition may also modify the neural code for ITD. In the Mongolian gerbil, a small mammal with low-frequency hearing, precisely timed glycine-controlled inhibition in the MSO shifts the ITD curve so that the greatest change in firing rate falls within the physiologically relevant range of ITDs (Brand *et al*., 2002).

Inhibitory inputs are also found in the avian NL, but they are more diffuse, and appear to decrease excitability through a gain-control mechanism, rather than being phase-locked (Funabiki *et al*., 1998; Monsivais *et al*., 2000; Pena *et al*., 1996; Yang *et al*., 1999). Avian superior olivary neurons receive projections from the NA and NL and provide a GABAergic feedback projection to NM, NA, and NL (Lachica *et al*., 1994; Takahashi and Konishi, 1988b). This olivary input appears to provide tonic inhibition (Monsivais *et al*., 2000; Yang

et al., 1999), in contrast to the inhibitory projections in the mammalian MSO. It appears that the GABAergic input in birds maintains the coincidence detector in the optimal range of operation (Funabiki *et al*., 1998). *In vivo* recordings from the barn owl support this interpretation (Pena *et al*., 1996; Takahashi and Konishi, 2002).

The differences in how birds and mammals encode ITDs goes beyond differences in the neural circuit for ITD detection to the deeper issue of how ITDs are coded in the brain. Recent evidence from gerbils indicates that ITDs are not represented by maximal discharges of a few neurons, but rather by the relative activity in both MSOs. It has been proposed that this activity is regulated by inhibition, not delay-lines, and that there is no requirement for a map of azimuthal space in the MSO (Figure 2b; reviews in Grothe, 2003; McAlpine and Grothe, 2003). These observations, together with the lack of evidence for maps of ITD in the mammalian inferior colliculus, have been used to support the hypothesis that the neuronal representation of auditory space differs in birds and mammals, indicating again a parallel evolution of spatial hearing.

19.4.3 Models of Coincidence Detection and Dendritic Structure

In both birds and mammals, coincidence detectors are bitufted neurons with inputs from each ear segregated on separate sets of dendrites (Figure 3). Modeling studies suggest this dendritic separation improves coincidence detection (Agmon-Snir *et al*., 1998; Grau-Serrat *et al*., 2003). The dendritic separation may allow each dendrite to act as a current sink for inputs on the other dendrite, thus decreasing the amount of current to the soma when inputs arrive only on one side. This effect might be boosted by the presence of a low-threshold K^+ conductance similar to that found in NM and bushy neurons so that out-of-phase inputs are subtractively inhibited (Grau-Serrat *et al*., 2003). With only monaural input, the low-threshold K^+ conductance in the opposite dendrite is somewhat activated, producing a mild current sink. When, however, there are recent EPSPs in the opposite dendrite due to out-of-phase inputs, the low-threshold K^+ conductance is strongly activated and acts as a large current sink suppressing spike initiation. Thus, the model predicts the experimental finding (Carr and Konishi, 1990; Goldberg and Brown, 1969; Yin and Chan, 1990) that the monaural firing rate, while lower that the binaural in-phase rate, is higher than the binaural out-of-phase rate. The benefits conveyed by the neuronal structure of the coincidence detectors further supports the idea that the evolution of coincidence detectors in the bird NL and mammalian MSO may have occurred in parallel (Carr and Soares, 2002).

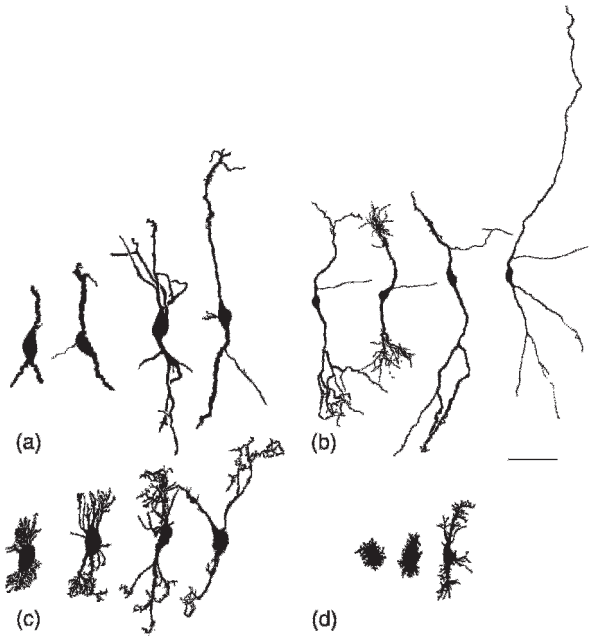


Figure 3 Coincidence detectors share bitufted morphology. a, Alligator NL neurons labeled with Golgi technique, from presumed high to low best frequency regions of NL (left to right; Soares, unpublished). b, Chicken NL neurons labeled with Golgi technique, from high to low best frequency regions of NL (Jhaveri and Morest, 1982). c, Guinea pig MSO neurons (Smith, 1995). d, Barn owl NL labeled with Golgi technique (Carr and Boudreau, 1993). Dendritic length increases from left to right except in the principal cells of the medial superior olive from the guinea pig, where a frequency gradient is not apparent (Smith, 1995). The bipolar architecture and the segregation of the inputs arriving from both ears are common to both mammalian and avian coincidence detectors. In the barn owl, coincidence detectors have lost this bipolar organization, except in low best frequency regions where their short dendrites radiate around the cell body. Reproduced from Carr, C. E. and Soares, D. 2002. Evolutionary convergence and shared computational principles in the auditory system. *Brain Behav. Evol.* 59, 294 311, with permission from Karger, Basel.

19.5 Summary and Conclusions

The cochlear nuclei of birds and mammals share similar features, including heterogeneous cell populations, and similar responses to sound. These shared characteristics may represent similar responses to selective pressures to encode the features of airborne sound. The principal reason for arguing that the similarities in the cochlear nuclei of birds and mammals may be due to similar

selective pressures, and not homology, is that the ancestors of birds and mammals separately developed true tympanic ears (Clack, 1997). A second reason is that close comparisons of bird and mammal cochlear nuclei reveal many differences. A third is that the observed similarity in the morphology and physiology of cochlear neurons is a plausible outcome of parallel evolution, because neurons in both birds and mammals experience similar constraints in detecting sound. Thus, although a common population of brainstem auditory neurons existed in the tetrapod ancestor, distinct evolutionary forces may have acted on these two groups allowing for the emergence of different ears and in turn, dissimilar organization in the brainstem.

Comparisons of temporal coding reveal shared computational principles. When compared with a simple integrate-and-fire unit, the auditory neurons that phase-lock, detect coincidences, and encode temporal patterns all exhibit a suite of physiological and morphological adaptations that suit them for their task. The core features of auditory coding are very similar in birds and mammals (and probably in other animals as well). Comparative studies of temporal coding can therefore add to the discussion of whether neuronal function follows form. A case can be made for this in time-coding neurons of the auditory brainstem of birds and mammals, and for phase-coding neurons in weakly electric fish (Kawasaki and Guo, 1998).

If there are computational advantages to particular neuronal architectures, similar forms should be expected. For example, we argue that the bitufted structure of coincidence detector neurons in birds and mammals is computationally advantageous. Therefore, morphological similarities might not support homology, but rather similar computational demands, and we can argue that the neurons of nucleus laminaris and MSO may have converged upon their similar form (Carr and Soares, 2002). In another example, it appears that large somatic terminals on NM or bushy cells are an ancestral feature of amniote auditory nerve. A shared pressure to encode higher-frequency sounds may have driven the parallel appearance of complex endbulbs in archosaurs and mammals.

Finally, phenotypically different neurons can produce similar computations. Neurons may differ in the expression and/or distribution of their ionic channels and still behave similarly. Thus, there may be numerous acceptable ways to carry out a particular computation. These may be revealed by comparative studies.

Acknowledgments

This work was supported by NIMH T32MH2004801 award to DS and by NIH DCD00436 to C.E.C.

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EVOLUTION OF MAMMALIAN BRAINS

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20 How Can Fossils Tell us About the Evolution of the Neocortex?

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Glossary

allometric	Applied to morphology, the measures of two different organs or of an organ and the whole body.
archaic orders	Orders of mammals that are entirely extinct.
encephalization	Enlargement of the whole brain relative to its expected size.
encephalization quotient (EQ)	Gross brain size relative to expected brain size; expected brain size is determined by the regression of brain size on body size in an appropriate set of species on which data are available, the empirical quotient is the residual from the regression on log log coordinates.
endocast	Cast molded by the cranial cavity in vertebrates. Also called endocranial cast.
foramen magnum	The hole at the entry of the spinal cord into the cranial cavity. It may be thought of as 'medulla' in an endocast, but is actually larger, containing blood vessels and sinuses in addition to medulla and anterior spinal cord.
fossil brains	Fossil endocasts interpreted as brains.
holarctic	Northern Hemisphere.
neocortex	A thin sheet of many millions of nerve cells in the telencephalon of the brain of all living mammals, often considered as involved in conscious thought. All sensory information (vision, hearing, smell, taste, etc.) is known to be represented in various localized regions in the neocortex, frequently in many 'projection areas'. Higher functions such as intention, motivation, and attention are correlated with neocortical activation. It is a uniquely mammalian structure, and in histological sections, in living species, it has six layers of nerve cells as distinct from other brain structures with fewer layers or without layering.
neocorticalization	Enlargement of neocortex relative to expected enlargement from brain body analysis. It can be measured as the ratio of neocortical surface area to total brain or endocast surface area.
neotropical	Southern Hemisphere (primarily South and Central America).
paleoneurology	The science that deals with the fossil evidence of the nervous system and behavior. Primary evidence is from endocasts, but also evidence of behavior inferred from skeletal adaptations for running or climbing, dental patterns, animal tracks, and nesting sites.
progressive orders	Orders of mammals in which living species have survived.
relative neocortical surface area	Ratio of neocortical surface area to total endocast surface area.

20.1 Introduction

The story of the brain's evolution is told by casts of the cranial cavities of extinct species. These endocasts document much of the evolution of the mammalian brain during the past 65 million years, the Cenozoic era. A single late Jurassic fossil (Simpson, 1927; Jerison, 1973) had extended the known evidence to about 150 Mya, and other

explorations (Hu *et al.*, 2005; Kielan-Jaworowska *et al.*, 2005; Novacek, 1996) fill gaps in our knowledge of the Cretaceous period (65–145 Mya). Mammals first appeared during the Triassic period of the Mesozoic, and it may one day be possible to trace the history of the mammalian brain almost to its beginnings, perhaps 225 Mya (see Reconstructing the Organization of the Forebrain of the First Mammals).

Encephalization is the increase in relative size of the brain as a whole over geological time. Its history was reviewed in depth in Jerison (1973) (cf. Falk, 1992; Falk and Gibson, 2001). Other recent evolutionary analysis emphasizes methodological innovations in cladistic analysis, with major revisions of mammalian phylogeny (McKenna and Bell, 1997; cf. Simpson, 1945). This article is consistent with those revisions.

Our central topic is neocorticalization, the increase of the relative amount of neocortex in mammals. ('Relative' in this case refers to the ratio of surface area to total surface area of the endocast.) Identifiable neocortex is a feature of the external morphology only of mammalian brains, but neural structures with similar functional significance have also evolved in birds and reptiles (Butler and Hodos, 2005; Karten, 1997; Reiner *et al.*, 2005). Avian and reptilian brain structures homologous with mammalian neocortex must first have appeared in the common amniote ancestor of these classes of vertebrates, but fossils are unlikely to be helpful in identifying these earlier ancestral connections. The question for this article is whether there was a change in neocorticalization within the mammals as they evolved during the past 225 million years (see The Origin of Neocortex: Lessons from Comparative Embryology). Like the avian Wulst, which is absent in endocasts of the earliest birds, evidence of neocortex may have been absent in endocasts of the earliest mammals. We don't yet know, but I review what we do know in this article.

We know that the neocortex, like the brain as a whole, became relatively larger in some but not all mammalian lineages (Jerison, 1990). Neocortex is present in all living mammals. Fossils tell their history, and it is reassuring that their evidence is consistent with inferences from the comparative neuroanatomy of living species (Butler and Hodos, 2005; Johnson, 1990; Shimizu, 2001). Opossums and hedgehogs (*Didelphis virginianus* and *Erinaceus europaeus*) can still be viewed as 'primitive', and cats and dogs and monkeys may be thought of as 'advanced', although one recognizes that this is an arbitrary dichotomy. (Spellings and orthography in this article follow the rules of taxonomic nomenclature. Genus and species are italicized, genus capitalized and species in lowercase.) From the fossils, we learn approximately when some identifiable changes in the brain occurred and how they differed in different lineages. They are the real proof of what is otherwise conjecture, that in most mammal species the brain evolved to relatively larger size and that this encephalization was usually accompanied by increased neocorticalization. Surprisingly, there appears to have been no comparable change in the olfactory bulbs other than their reduction in primates evident since the Miocene (about 20 Mya) and their complete disappearance in at least some cetaceans even longer ago.

All of the neural adaptations recognizable in the fossils are ancient, many occurring tens of millions of years ago. The most recent changes have been within the hominins, the human lineage, in which the most recent measurable increases in encephalization appeared about 250 000 years ago in the Neandertal and sapient species (*Homo neanderthalensis* and *Homo sapiens*). (References in the text to Neandertal follow the current German spelling and capitalization of nouns. The Neandertals were named for the Neander Valley in which the first specimen was found in 1856. German is famous as a language that combines words and elongates them rather than keeping them as short phrases; thus, the Neander Valley (capitalized in English as a place name) was the 'Neanderthal' before the German spelling reform of 1908, when it became 'Neandertal'. The rules of taxonomic nomenclature preserve the first published name of *Homo neanderthalensis*. The initial capital letter for the German was dropped in the species name in deference to the taxonomic usage of lower case for species. The genus '*Homo*' is as named by Linnaeus in 1758. Specimens shown here are as catalogued in AMNH (American Museum of Natural History, New York), BMNH (Natural History Museum of London, British Museum), FMNH (Field Museum of Natural History, Chicago), WISC (University of Wisconsin), and UT (University of Texas, Department of Paleontology).) The evolutionary evidence is at the generic and species level. I review a few within-species differences (Figures 3 and 4), but these are small compared to between-species effects.

20.2 Fossil Brains

Molded by the cranial cavity, endocasts such as those reviewed here have been called fossil 'brains' (Edinger, 1929; see Kohring and Kreft, 2003). The

brain, as soft tissue, does not fossilize, of course, but endocasts in birds and mammals resemble brains with dura intact, and they often show the superficial pattern of sulci and gyri in remarkable detail. Further analysis relies on relationships of external structures to the functional and microscopic anatomy of brains in living animals. In this chapter, brains and endocasts are treated as equivalent to one another. For most purposes, one can ignore the small differences between them in size and shape and use the same terminology for parts of an endocast as for comparable parts of the brain.

Neocortex can be distinguished from other structures visible on the surface of an endocast in mammals by using the rhinal fissure as the landmark. The evidence is exemplified by the brain of the living armadillo shown in Figure 1a. Rhinal fissure can be traced backward from the dorsal margin of the olfactory tract, and the fissure visible on the brain is also visible on endocasts. Figure 1b is a coronal section to show how the rhinal fissure serves as the boundary between neocortex and paleocortex, with paleocortex identified by the darkly stained layer of neurons in lamina II. Neocortex is taken as dorsal to the rhinal fissure on brains and endocasts.

Endocasts can be made from the cranial cavity of any skull. Those made from fossils are of special interest, because they are a physical record of the actual evolution of the brain. Figure 2, for example, presents snapshots of three-dimensional (3-D) scans of endocasts of two Eocene fossil mammals that lived about 40 Mya, a prosimian primate *Adapis parisiensis* and an even-toed ungulate (artiodactyl), *Anoplotherium commune*. Their endocasts may be compared with the brains of the living bush baby (*Galago senegalensis*) and the living llama (*Lama glama*), a camelid distantly related to *Anoplotherium*. An endocast of another Eocene fossil artiodacyl, *Bathygenys reevesi*, is shown in Figure 5 and that of an archaic Paleocene herbivore (*Phenacodus primaevus*, order Condylarthra, 58 Mya) is shown in Figure 9.

The olfactory tract and rhinal fissure are easily distinguishable on the lateral surface of the endocasts of *Anoplotherium* and *Bathygenys*; they are less clear but also identifiable in *Phenacodus*. We identify and measure neocortical surface area as dorsal to the fissure. The rhinal fissure in *Adapis* follows a course very much like that in the living bush baby, partly hidden by the temporal lobe. To be able to see the rhinal fissure in this endocast, it has to be rotated a bit to display more of the ventral surface. One objective of 3-D analysis reported here was to rotate virtual endocasts in primates to expose neocortex and include primate measurements in the analysis of mammalian data. Neocorticalization is the increase of neocortex relative to the rest of the brain, and it can be measured as a ratio of surface areas on an endocast.

This article is primarily on a quantitative analysis, but there is one interesting qualitative feature evident in Figure 2 that may be important. Comparing the Eocene fossils and their living relatives there is an obvious difference in the flexure of the brain, in the extent to which it is curved about the primary anterior–posterior axis, which is more marked in the living ungulates than the primates but evident in both. For example, in the fossils, olfactory bulbs, forebrain, hindbrain, and medulla are more or less in a line like railroad cars. The brains of their living relatives are bunched up and more globular. The difference probably results from the patterns of relative growth of the skull and skeleton compared to

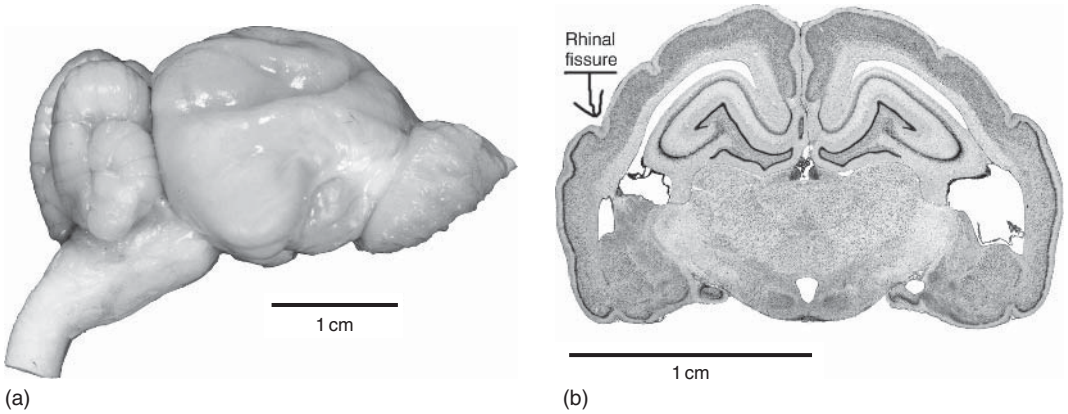

Figure 1 a, Brain of armadillo, *Dasypus novemcinctus*; rhinal fissure faintly visible dorsal to olfactory tract, then prominent further posteriorly. b, Coronal section of armadillo brain showing lamina II (dark stained layer) at border of rhinal fissure. Specimen WISC 60-465.

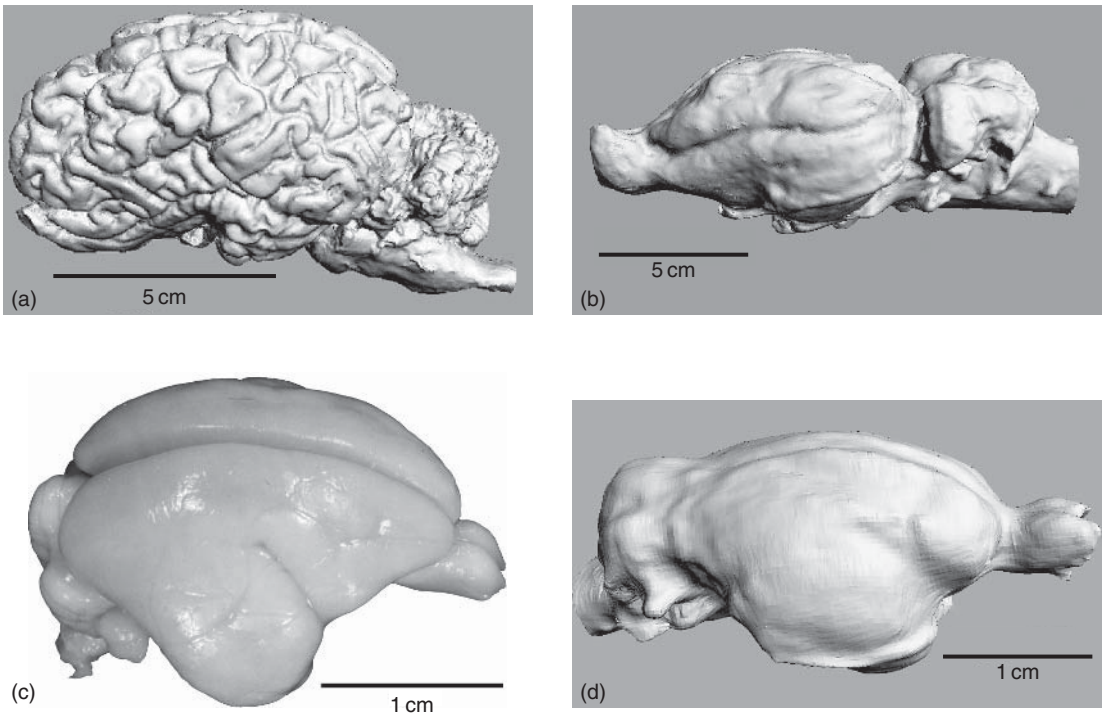

Figure 2 a, Brain and b, endocast in two artiodactyls, the living llama (*Lama glama*, WISC 65-139) and a 40-million-year-old camelid, A*noplotherium commune*, BMNH 3753. c, Brain of the living bush baby (*Galago senegalensis*, WISC 61-686) and d, endocast of a 40-million-year-old prosimian, *Adapis parisiensis*, FMNH 59259/BMNH 1340.

the growth of the brain, and is at least partly an epigenetic effect of squeezing a brain into the confines of the cranial cavity.

When an overall trend in skeletal growth resulted in enlargement of the cranial cavity, the brain can grow to fill and, to an extent, shape the cranial cavity. When trends toward encephalization became more prominent, the pressure was to maximize the amount of brain that could be packed into a given space. Later brains became more globular, primarily as an accommodation to maximize their volume relative to the space available for their growth. The change in shape as an evolutionary event would have been one of the changes that occurred at the Grande Coupure, the extinction of many species at the end of the Eocene and the beginning of the Oligocene about 33 Mya (Hooker *et al.*, 2004).

20.3 The Specimens and Their Measurement

The 78 ml plaster endocast of *Anoplotherium* in Figure 2 was prepared from the carefully cleaned cranial cavity of the fossil's skull (Palmer, 1913). This animal probably weighed about 80 kg, a weight comparable to that of the living llama in which the brain's volume is about 230 ml. *Bathygenys* (Figure 5) was a small artiodactyl that lived at the end of the Eocene, 35 Mya. It was about the size of the living chevrotain weighing about 5 kg. Its 12 ml natural endocast shown in Figure 5 is actually a piece of rock, but it unmistakably pictures the brain. One chevrotain (*Tragulus javanicus*) has been reported as weighing 4 kg with a 19 g brain (Nieuwenhuys *et al.*, 1998). The area of neocortex in both *Anoplotherium* and *Bathygenys* was 28% of the entire surface area of the endocast. In Oligocene species that lived about 30 Mya, such as the fossil horse Mesohippus, typical ratios are about 40% or more.

The volume of the endocast of *Phenacodus* (Figure 9) is 31 ml, and 16% of its surface area is neocortex. Its rhinal fissure is less marked than in the other endocasts illustrated here, but adequate for a measurement of the area of its neocortex. It was illustrated by Cope (1883). The endocast scanned for this analysis is a copy of the one made by Cope. Reconstructions of *Phenacodus* in life (Savage and Long, 1986) show it as living in a small herd of sheep-like five-toed animals. The weight of the fossil, estimated as 56 kg, is also appropriate for living sheep (*Ovis aries*) in which a brain weight of 130 g has been recorded.

Adapis, a prosimian primate that was a contemporary of *Anoplotherium*, weighed about 1.5 kg, and its endocast's volume was 8 ml. From skeletal

features, it has been reconstructed as a tree-dwelling lemuroid. The brain of the living galago, the bush baby, is shown for the comparison in Figure 2 because it is about the same size and shape as that of *Adapis*, but galago is a much smaller animal, weighing only about 250 g. A living lemur (*Lemur fulvus*) has been recorded with a body weight of 1.4 kg and had a 23 g brain.

The differences between the fossil and living brain sizes at comparable body sizes are examples of encephalization. Brains in most Eocene species averaged a quarter to half the size of living species in comparable niches and their relative size might be reported as 'encephalization quotients, EQs' – $0.25 < EQ < 0.50$ for Eocene fossils. Brain and body weight data on living species for comparisons are now available in many places. Some of the published data collections on living mammal species that I have seen have been in Count (1947), in Quiring (1950), and in Nieuwenhuys *et al.* (1998). I have published estimates on many fossil mammals (Jerison, 1973, 1990), and Holloway *et al.* (2004) has more data on primates in the human lineage.

EQs are not ratios of brain size to body size. They are ratios of measured brain or endocast size relative to expected size, and expected size is determined from the allometric relationship between brain and body size. That relationship is nonlinear and is usually described by the power function:

$$E = kP^{\alpha}, \qquad [1]$$

where E is brain size and P is body size in the same metric units (e.g., g or ml). There is some debate on correct values for the parameters k and α (see Jerison (2001)), but empirically the values $k = 0.06$ and $\alpha = 0.75$ are good approximate values as determined on large samples of living mammal species. When the equation is transformed logarithmically, it is

$$\log E = \alpha \log P + \log K. \qquad [1a]$$

Graphed on logarithmic coordinates, α is the slope and $\log k$ is the y-intercept of the best-fitting straight line. An encephalization quotient is the residual from that regression. For theoretical reasons (Jerison, 2001), I prefer $\alpha = 2/3$, in which case one must use $k = 0.12$ for computations. For a given set of parameters, it is an elementary exercise to compute an encephalization quotient.

To return to the specimens, the *Bathygenys* endocast in Figure 5 was made naturally. When this animal died, perhaps at a lakeside, its soft tissue decayed but its skull must have remained relatively undamaged. Sand and other debris could then pack the cranial cavity and could be covered and protected by layers of sediment. When the waters subsided, the skull and its contents eventually fossilized. Many millions of years later, the fossil was uncovered, presumably by erosion or earth movements. The fossilized skull must then have eroded, leaving only its hardened rock contents, the natural endocast. A lucky fossil hunter could find the specimen. Professor Jack Wilson of the University of Texas found the *Bathygenys* fossil, which he recognized as a natural endocast (Wilson, 1971), collected it for his paleontology department, and made it available for this report.

The plaster endocast of *A. commune* shown in Figure 2 is part of the history of anatomy and paleontology. A largely intact fossil skeleton of the whole animal was found in gypsum quarries in Montmartre, now part of Paris, and was named in 1804 by Baron Georges Cuvier. He noted that the fossil's canine teeth seemed short and ineffective as weapons – 'anoplos' is from the Greek for 'unarmed' and 'therium' for beast – hence, *Anoplotherium*. Serving under the Emperor Napoleon, Cuvier was director of the Muséum National d'Histoire Naturelle in Paris two centuries ago, and he undertook to demonstrate that fossils are evidence of the history of life.

At the time, fossils were sometimes considered to be mineral accretions that merely resembled living things. Cuvier accepted what we now recognize as the 'uniformitarian hypothesis', namely, that the present laws of nature have always been valid (Simpson, 1970). That he named the fossil according to his judgment of its teeth is a uniformitarian view that is natural for us. We share the judgment that they are not merely rocks that happened to look like teeth but were once teeth and had fossilized. The story is that in a public exhibition Cuvier 'dissected' his *Anoplotherium*, in which some of the fossilized vertebral column was exposed. The dissection was with hammer and chisel, and Cuvier pointed out that if what looked like the vertebral column had been the vertebral column of an animal that once lived, further exposure would reveal pelvic bones. It did. This was his way of proving that he had been working on the remains of an animal that was comparable anatomically to living animals.

Among the other endocasts and brains illustrated in this chapter, *Phenacodus* was collected and named by Edward Drinker Cope as mentioned earlier, and it was one of the bones of contention in the fossil feud of the late nineteenth century about discoveries in the American West (Wallace, 1999). The adapid endocast is from a skull presently at the Natural History Museum of London and was from the phosphorites of Quercy in southwest France, a Late Eocene site in which the fossils are about 40

million years old. Its endocast was first prepared a century ago under the direction of Elliot Smith (1903) and has had a prominent place in discussions of the evolution of the primate brain (LeGros Clark, 1962). This article's scan is from a later preparation for Professor Robert D. Martin, then at the Anthropology Department of University College, London (Martin, 1990). The brain of galago with which it is compared in Figure 2 is from the University of Wisconsin brain collection, a collection that I consulted for comparisons with almost all of the fossils analyzed for this chapter. At this writing, the Wisconsin collection can be viewed on the internet; see the 'Relevant Websites' section. Like the galago brain, the llama brain and the armadillo brain of Figure 1 are also in the Wisconsin collection. Some of that collection has been moved to Washington, D.C., and is now part of the National Museum of Health and Medicine of the Armed Forces Institute of Pathology.

Hundreds of fossil 'brains' have been collected throughout the world, either as natural endocasts or as latex or plaster casts made from fossil crania (Edinger, 1975), and they are available for study in the back rooms of many museums. The quality of an endocast as a model of the brain differs in different taxa. In fish, amphibians, and reptiles, the model is usually poor because when they mature their brains do not fill the cranial cavity. Semicircular canals and other auditory and vestibular structures are occasionally well preserved in many vertebrates (Rowe, 1996; Dominguez *et al.*, 2004). In mammals and birds, endocasts often provide accurate and detailed pictures of the external surface of the brain as in Figures 2, 5, and 9. Comparisons between brains and endocasts in living mammals indicate that only minor errors occur in treating endocasts as undissected brains.

Measurement of surface area in endocasts was essentially impossible until recently, when technologies were developed that enable us to scan and digitize irregular solids for computer analysis. The endocasts used for the 3-D analysis in this chapter were digitized with a laser scanner system and its associated software. After scanning, the surface areas were measured with that software to provide the data for Figures 7 and 8. At this writing, more details about the system are available on the internet; see 'Relevant Websites' section.

20.4 Brains and Endocasts

Why should we be concerned with simple-minded measurements of gross brain size? One obvious reason is that these are reliable measures that can be taken on fossils, and they enable us to quantify the evolution of the brain. Less appreciated is their utility for an understanding of the brain's work in living species, because gross brain size, either surface area or brain weight or volume, may estimate the total information-processing capacity of brains in living mammals. That relationship is inferred from Figure 3, which graphs surface area of living brains as a function of brain size. The number of neurons underneath a specific amount of surface appears to be constant in many species (Rockel *et al.*, 1980, but see also Haug, 1987 and Hofman, 1985, 1988). Since the total processing capacity of a neural system is related to the number of neurons in the system, total surface area must estimate the total number of information processing units in a brain. Analogously, the surface area of neocortex as a part of the brain measures the contribution of neocortex to the total amount of information processed by the brain. The surface–volume relationship as shown in Figure 3 is almost perfect with a product–moment correlation coefficient $r = 0.99$. Uniformitarianism suggests that this almost deterministic relationship was as true for fossil endocasts as it is for living brains, although there are questions raised at the end of this article among the 'caveats'.

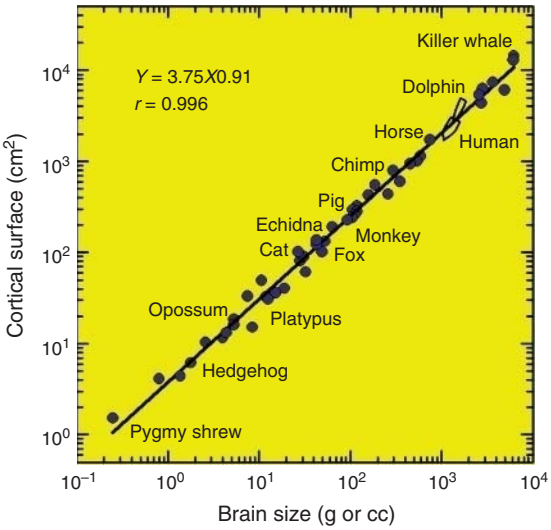

Figure 3 Total cortical surface area (including 'buried' cortex) as a function of brain size in 50 species of living mammals. Correlation: r 0.996; regression: Y $3.75X^{0.91}$. A few of the species are labeled to suggest the diversity of the sample. Human and dolphin data are presented as minimum convex polygons enclosing 23 points for humans and 13 points for dolphins to suggest within-species diversity. Data from Brodmann (1913), Elias and Schwartz (1971), Ridway (1981), and Ridway and Brownson (1984). From Jerison, H. J. 1991. Brain size and the evolution of mind. 59th James Arthur Lecture on the Evolution of the Human Brain. American Museum of Natural History.

Figure 3 also provides information about within-species variability compared to that between species. In two of the species, humans (*H. sapiens*) and dolphins (*Tursiops truncatus*), it was possible to show the full range of individual data by enclosing those data in convex polygons that incorporate the complete samples. It is evident that the polygons are only slightly larger than the individual points graphed for the other species, each of which is a single datum for the species.

How good is an endocast as a representation of a brain? The obvious answer is in the endocasts and brains illustrated in this article. The relationship has been quantified for gross size in the human species and is shown in Figure 4. Although partly obscured by the well-known variability in human brain size, there is a strong relationship between brain and endocast (cranial capacity) as indicated by the high correlation coefficients. Endocasts and brains are equivalent to one another for information on size, with a small difference (about 7%) due to the fluids and meninges that surround the brain. The regression lines are parallel to one another, showing that the difference between endocast and brain follows the same rule for women and for men. This article is not concerned with the sex differences in human brain size, but that difference is also shown to complete the graphic summary of the data as published by Davis and Wright (1977).

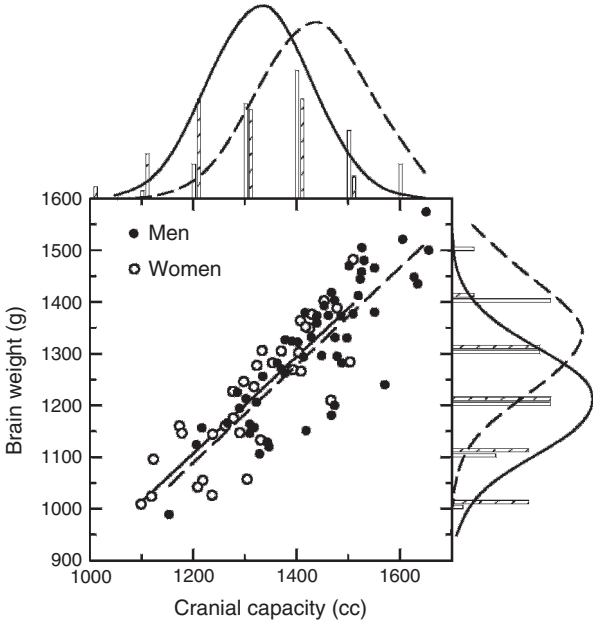

Figure 4 Relationship between brain size and cranial capacity (endocast volume) in 54 human male and 33 female cadavers. Solid lines and normal curves for males; dashed lines and normal curves for females. Mean brain size: male 1308 g; female 1221 g. Mean cranial capacity (endocast volume): male 1431 ml; female 1322 ml. Correlation coefficients, r 0.84 for men; r 0.85 for women. Regression equations: male, Y 0.94X 44; female, Y 0.94X 16. Marginal distributions shown by fitted normal curves. Data from Davis, P. J. M. and Wright, E. A. 1977. A new method for measuring cranial cavity volume and its application to the assessment of cerebral atrophy at autopsy. *Neuropathol. Appl. Neurobiol.* 3, 341 358.

20.5 Two-Dimensional Analysis of Neocorticalization

The first quantitative analysis of neocorticalization presented measurements of the areas of neocortex and olfactory bulbs in 2-D lateral projections in a sample of 35 fossil and 24 living species of carnivores and ungulates (Jerison, 1990). It was based on profiles of the endocasts in which rhinal fissures were visible, and the measure was the area of neocortex dorsal to the fissure. The 2-D analysis was also performed on the areas of the olfactory bulbs. Data for the analysis are illustrated in Figure 5a on the endocast of *Bathygenys reevesi*, discussed earlier; Figure 5b sketches the areas that were measured.

The 2-D results are graphed in Figure 6 and show how the neocortical and olfactory bulb quotients in this sample changed with geological age during the Cenozoic era. The quotients are ratios of measured brain areas relative to their expected areas, with the latter determined by the regression of brain areas on the height of the foramen magnum (medulla). The measure on the foramen magnum followed a suggestion by Radinsky (1967) to use the foramen measure to control for body size differences in different species. The quantitative analysis was limited to neocortex and olfactory bulbs. Paleocortex and hindbrain were not analyzed because the curvature of the brain hides much of the paleocortex and because hindbrain regions such as the cerebellum are partly hidden under overlying neocortex. (Regression analysis such as this is often referred to as allometric analysis, the analysis of the measures of two organ sizes relative to one another.)

The results of the 2-D analysis as discussed in the original report (Jerison, 1990) were, first, that neocorticalization occurred, which is shown by the significantly positive slope of the regression of the neocortical quotient on geological age. Second, the olfactory bulbs did not change in relative size in these species during the Cenozoic. Taken together, these two results validated the method in that it could discriminate between the change and absence of change. A third result was that the 'archaic' fossil species, that is, species from orders of mammals that are now entirely extinct, were

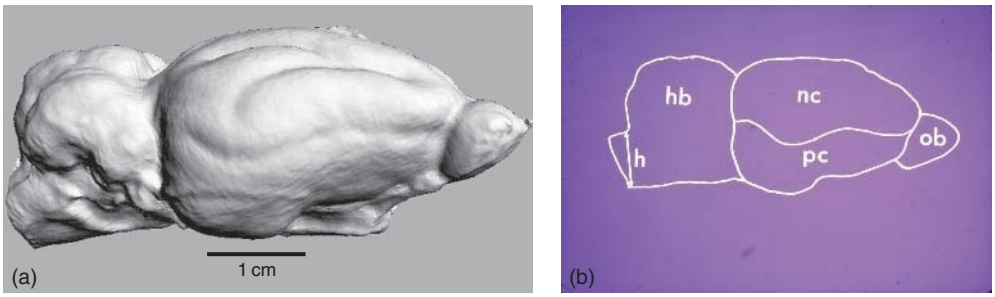

Figure 5 a, Natural endocast of *Bathygenys reevesi* (UT 40209-431). b, Profile of endocast, showing areas measured for Figure 6. nc, neocortex; ob, olfactory bulbs; h, height of foramen magnum. Not measured: pc, visible paleocortex; hb, visible hindbrain, including cerebellum.

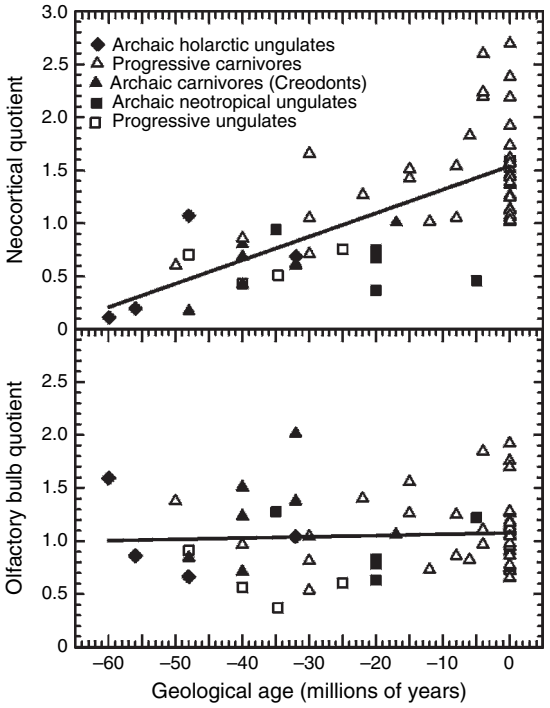

Figure 6 The 2-D analysis showing increased relative neocortical surface area (top) and stasis in olfactory bulbs (bottom) during the past 60 million years; see Figure 5. Quotients are residuals from regression of neocortex area and olfactory bulb area on foramen magnum height. They are interpreted as ratios of measured areas relative to expected areas on the basis of body size in this sample. 'Progressive' neocortical change noted here (positive slope of regression line; r 0.75) demonstrates neocorticalization over time. Each point is a species. Archaic carnivores are from the order Creodonta. Other groups are discussed in the text. Redrawn from Jerison, H. J. 1990. Fossil evidence on the evolution of the neocortex. In: Cerebral Cortex (*eds.* E. G. Jones and A. Peters), vol. 8A, pp. 285 309. Plenum.

significantly below average in neocorticalization, falling below the regression line determined for the entire sample. This suggests positive selection for neocorticalization, that it improved fitness in an evolutionary sense.

The 2-D data on brain size are flawed because they are limited to profiles of the brain and do not measure the actual areas of the curved surfaces of the endocasts. They are also limited to species in which rhinal fissure is visible on the lateral surface, and this excluded primates from the analysis. When the 2-D analysis was published, it was not possible to perform an equivalent 3-D analysis with the technology available at that time. Such a technology has since been developed, and the analysis of 3-D images of endocasts is published here for the first time.

20.6 Three-Dimensional Analysis: Neocortex

The analysis of the newly acquired 3-D data on a larger sample of fossil and living mammals, which includes primates (Figure 7), confirms that there was progressive neocorticalization in mammals during the Cenozoic. The positive slope of the regression (Figure 7) is similar to that found in the 2-D analysis. The sample of 106 mammals included 84 fossil species and 22 living species. There were seven fossil primates: two hominins and five prosimians, including the *A. parisiensis* shown in Figure 2. The hominins were two Plio-Pleistocene australopithecines, *Australopithecus africanus* (the Taung specimen discovered by Raymond Dart in 1923) and *Australopithecus robustus* (SK 1585) from South Africa (see Tobias, 1971; Holloway *et al.*, 2004). There are partial endocasts for Oligocene and Miocene anthropoids (*Aegyptopithecus*, *Libypithecus*, and *Proconsul*; see Radinsky, 1979), which were sufficient to indicate that frontal and temporal lobes were in a primate-like configuration (Jerison, 2006), but they were too incomplete otherwise for this quantification. The 22 living mammal species included eight anthropoids (simians) and two humans. Primates have evidently always been above average in neocorticalization,

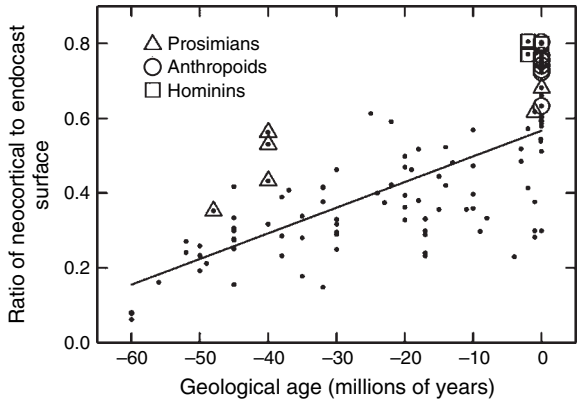

Figure 7 Relative size of neocortex measured in the 3-D analysis of the endocasts of 106 species of mammals during the past 60 million years. Points for prosimians are marked by triangles, hominins (including australopithecines) by squares, and anthropoids by circles. (All 106 species are shown as points, and identifying symbols surround the points.) Regression of neocortical ratio on geological age: $Y = 0.007X + 0.57$; $r = 0.72$. In living mammals, 57% of the endocast surface area is devoted to neocortex; the increase was about 7% per 10 million years. In living monkeys and living and fossil hominins, the ratio averages about 75%.

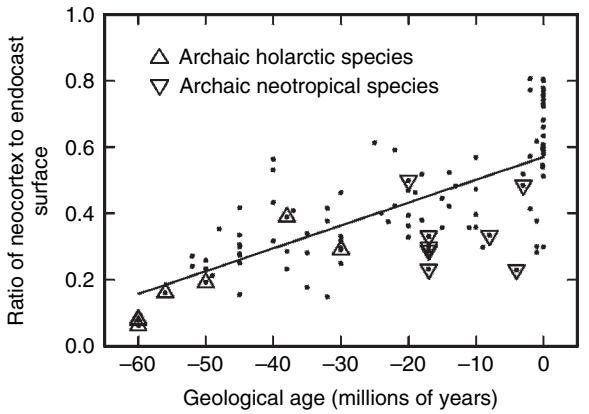

Figure 8 The same mammal species as in Figure 7, marked to distinguish 'archaic' from 'progressive' species, that is, species that are members of presently extinct orders or suborders of mammals from those that are members of surviving groups. Upright triangles for holarctic species, inverted triangles for neotropical species. Regression line is for the entire sample, and is the same as in Figure 7. Archaic species on average fall below the regression line.

that is, their data lay above the regression line determined for the entire sample as shown in Figure 7. Living and fossil hominins are typical primates on these measures. The highest ratio of neocortex to endocast surface area was a langur's (*Cercocebus albigena*) at 80.4% followed by one living human at 80.0%. A second living human endocast measured 77.7% and was topped by two other monkeys.

As in the 2-D analysis, it was possible to compare species from archaic orders with those from 'progressive' orders, and the data showing the difference are presented in Figure 8. The species in which endocasts were illustrated earlier in Figures 2 and 5 were progressive in that there are even-toed ungulates (order Artiodactyla) and primates (order Primates) living today.

Several orders of Miocene and Pliocene South American mammals, originally discovered as fossils by Charles Darwin in the 1830s on the voyage of the *Beagle*, are 'archaic', having no surviving species. Their data are included in Figure 8 and marked with inverted triangles. Erect triangles mark holarctic species from Europe and North America, also from extinct orders. Thirteen of the 15 archaic species fell below the regression line. The probability that this was a random departure from 'average' is less than 0.05 (chi-square test). The 3-D analysis thus supports the conclusion that neocorticalization contributes to fitness, that is, there was positive selection for neocorticalization.

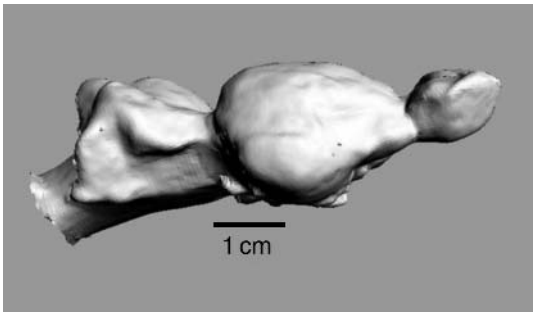

Figure 9 Endocast of *Phenacodus primaevus* (FMNH 59042/AMNH 4369), a late Paleocene archaic holarctic species (58 Mya).

Endocasts of archaic species are not superficially unusual. That of *P. primaevus* discussed earlier is shown in Figure 9. It might be distinguished from the other fossil endocasts because of slight differences in appearance, but it is also different quantitatively. At the animal's estimated body weight (56 kg), its expected endocast volume is 176 ml according to my preferred parameters of eqn [1]. The measured volume of the endocast at 31 ml results in an encephalization quotient of 0.18. Its ratio of neocortical area to total endocast area is 0.16, one of the lowest in the sample, and it is an example of the grade of encephalization and neocorticalization in most Paleocene mammals.

20.7 Three-Dimensional Analysis: Olfactory Bulbs

The 3-D quantitative analysis of neocorticalization reported here supplements but does not entirely replace the 2-D analysis. It omits the olfactory bulbs, which could not be measured reliably on too many of the fossil endocasts. There were obvious artifacts in many of them in the representation of olfactory bulbs. In preparing plaster endocasts from a skull, the region of the olfactory bulbs is cleaned out, and it is easy to make mistakes. The cribiform plate and the region of the turbinals has sometimes been excavated, resulting in artificially enlarged bulbs. In others, the olfactory bulbs may be incompletely excavated in preparing latex endocasts. Many natural endocasts, unlike the *Bathygenys* endocast illustrated in Figure 5, are also obviously distorted in the region of the olfactory bulbs. There were enough uncertainties in the sample of endocasts that were scanned for this article to make it inappropriate to present 3-D data on the olfactory bulbs without further study. Olfactory bulbs in the 2-D analysis were all sketched by neurobiologists familiar with normal living brains, who used that information in their reconstructions (see Jerison, 1990). The sketches were all published prior to the later quantitative analysis, and the areas in the 2-D analysis were measured independently of the sketching. The result that showed no change in the relative size of the olfactory bulbs was unexpected and unanticipated. Clearly unbiased, the conclusion of the 2-D analysis can be accepted at least provisionally, namely that the relative size of the olfactory bulbs remained more or less unchanged during the Cenozoic.

The evidence of the reduction of olfactory bulbs in primates and cetaceans is from comparative anatomy. The fossils suggest that their reduction in primates occurred after the Oligocene, when *Aegyptopithecus* lived; Radinsky's (1979) sketches indicate olfactory bulbs in *Aegyptopithecus* that were comparable to those in fossil prosimians (cf. *Adapis* in Figure 2) and relatively larger than in later anthropoid species. In Miocene and Pliocene anthropoids, the olfactory bulbs appear as reduced as in living species, and australopithecine olfactory bulbs are reduced comparably to those of living chimpanzees and humans. Fossil data on cetaceans were reviewed in Jerison (1973) and indicate either reduced or completely absent olfactory bulbs.

20.8 Caveats and Conclusions

Neocorticalization occurred in many lineages, and there appeared to be some increase in all mammal species after the Paleocene epoch. The overall increase is evident in the positive slope of the regression lines of the neocortical ratio on geological age. The increase was most dramatic in primates, where it is evident in the earliest record of their brains in the Eocene epoch, but even in 'primitive' living marsupials such as the koala (*Phascolarctos cinereus*), neocorticalization to the extent of 30% of the endocast surface is in advance of the Paleocene grade of the archaic *Phenacodus*.

Another conclusion is about the diversity of neocorticalization. The range between 30% in the living marsupial koala and 80% in living humans and langurs suggests the variety of niches for which neocorticalization could be selected. When I published the 2-D data 15 years ago, I thought that the correlation of 0.7 between geological age and neocorticalization and the scattered points in its graph (Figure 6) might be due to the inadequacies of 2-D measurements. The better method for determining and measuring surface area, and the larger sample for the measures in Figures 7 and 8, indicate that the variability is real and reflects the true diversity of adapations for neocorticalization in mammals.

The third conclusion which also verified the 2-D analysis indicated that neocorticalization contributed to fitness. The evidence is in the fate of archaic species, which were on the average less neocorticalized than progressive species. This kind of conclusion may seem obvious and hardly worth special mention, but it is difficult to find reasonable evidence for the fitness of quantifiable traits that evolved to different extents in different taxa. The unusual history of the olfactory bulbs in mammals is as instructive as that of the neocortex. Based on this trait living anthropoid primates (including *H. sapiens*) are a 'degenerate' order, and one can interpret the reduction in their olfactory bulbs as evidence of the relative unimportance of olfactory information in their lives. Stasis in the evolution of the olfactory bulbs is presumably the norm, and if primates had been included in the 2-D analysis their degeneracy might have been clearer. But humans write the histories, and in our accounts of the history of the brain, large olfactory bulbs have erroneously been taken as a sign of primitiveness rather than normality. Olfactory bulbs, large or small, are adaptive to specialized niches.

The final caveat is to be wary of conclusions based on endocasts rather than brains and to be wary of conclusions based on externals rather than on the fundamental structure and function of the brain. On the other hand, conclusions based only on the fundamentals have to follow a cladistic analysis of data on living species, comparing apparently

homologous traits and taking into account their differentiation in different living species. Johnson (1990) has reviewed cladistic analyses of neural evolution, and there have been several other important publications on phylogeny, which were cited in the opening paragraphs of the article. The conclusions, based on endocasts and limited to externals, provide a time dimension for the brain's evolution and broadly date the events.

I have emphasized the place of endocasts as providing direct evidence on the evolution of the brain and that the relevance of the evidence comes partly from relationships between superficial data such as brain size and more fundamental measures of the brain's structure and function. Figure 3, showing the relationship between the gross size of the brain and the extent of its cortical surface area, is a good example of the approach, illustrating a likely relationship between the gross measure of the brain itself and neural information processing capacity. In that graph, the dependent variable was total cortical surface, including surfaces buried in the fissures. For a closer look, consider Figure 10, which graphs the measured surface area of endocasts rather than brains as a function of their volume. Although the relationship is equally strong for brain measures and endocast measures in which cortex buried in the fissures is not measured, the difference between the slopes on logarithmic coordinates (the allometric exponent) is instructive.

In the endocasts (Figure 10), the slope is about two-thirds, which is the expected relationship among similar solids of different size. For example, in graphs of the surface–volume relationship in spheres of different size, the slope is exactly 2/3, as it is in cubes or any other solid object of any shape if shape is conserved as size changes. In an equation like eqn [1], $\alpha = 2/3$ for a given solid, and the differences among solids are in the parameter k. Regardless of their sizes, for all spheres, $k = 4.84$; for all cubes, $k = 6$. Figure 10 tells us that our endocasts were more alike in shape than we might have guessed, at least with respect to this aspect of their geometry. Figure 3, on the other hand, tells us that we had been able to work with the brains of these fossils rather than their endocasts we should have expected convolutedness to increase as volume increased, that is, convolutedness would be greater in larger brains. The change in convolutedness is reflected by the exponent $0.91 > 2/3$. That information is lost in working with endocasts. The high correlation coefficients save the day for a uniformitarian view. They indicate that the surface areas of portions of the neocortex buried in the sulci and fissures are also related in an orderly way to brain or endocast size. It is, therefore, likely that like actual brain surface area, the surface area of endocasts also estimates the information processing capacity.

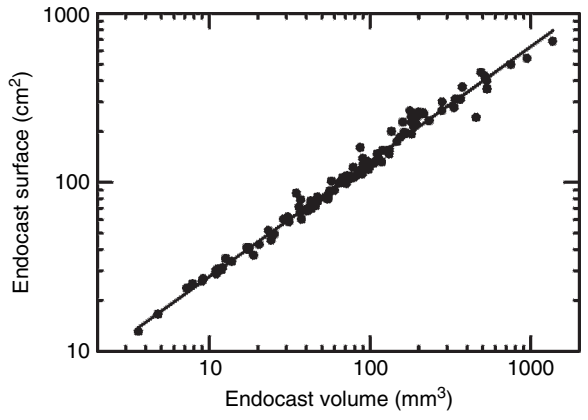

Figure 10 Surface volume relations in endocasts as measured in the sample of 106 species used in the 3-D analysis. Product moment correlation, r 0.992; regression, Y $5.8X^{0.68}$.

Acknowledgment

The research reported here was begun on a Fellowship at the Hanse-Wissenschaftskolleg (Institute for Advanced Study) in Delmenhorst, Germany, and I am grateful to that institute for the support.

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http://www.neurophys.wisc.edu Department of Physiology, University of Wisconsin at Madison.

21 The Origin of Neocortex: Lessons from Comparative Embryology

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Glossary

cell migration The cortex comprises two types of neurons: glutamatergic pyramidal projection neurons and GABAergic interneurons. The two cortical cell populations are generated at different sites within the forebrain. The pyramidal neurons are generated in the local cortical germinal zone and migrate radially (perpendicular to the pial surface) to reach their final destination in the cortex. In contrast, most of the interneurons are born in the distant ganglionic eminence (neuroepithelium of the embryonic pallidum) and migrate tangentially (parallel to the pial surface) to reach the cortex.

germinal zone In the embryonic brain, epithelium lining the ventricles (the ventricular zone) is a neurogenic source. In mammals, there is an additional adjacent layer of mitotic activity termed the subventricular zone. These compartments differ in size, organization, and in their modes of division. The proliferative cells in the ventricular zone undergo interkinetic migration, and have radially aligned nuclei. The subventricular zone contains cells with randomly oriented nuclei.

infragranular and supragranular The mammalian neocortex (or isocortex) consists of six layers. Layer 4 is also called the granular cell layer. Layers 5 and 6 are referred to as infragranular and layers 2 and 3 are also called supragranular layers. Different cortical areas show considerable variations in the proportions of these components.

layers of the cortex These are cells spanning the entire thickness of the wall of the embryonic forebrain extending perpendicular to the pial surface. These cells were previously assumed to act merely as scaffolds for newly born neurons to migrate along. They are now considered to be the source of most neurogenesis in the developing cortex.

radial glia At the ventricular zone, radial glia divide to yield a new glial cell and a daughter cell which migrates away from the ventricular surface. This is termed asymmetrical division. The subventricular zone also participates in neurogenesis. Here the intermediate progenitors mostly undergo division to produce two identical daughter neurons, which migrate to the cortex. This is termed symmetrical division.

symmetrical and asymmetrical divisions

21.1 Constant Features of the Mammalian Isocortex

The mammalian six-layered cortex (neocortex or isocortex) is a great achievement of cortical

development and evolution (see How Can Fossils Tell us About the Evolution of the Neocortex?). Cortical neurons are arranged into distinct layers according to their input and output in a very specific and conserved manner (Lorente de No, 1949; Toyama *et al.*, 1974; Peters and Jones, 1985). In spite of the enormous variations in cortical surface area, and in the sulci and gyri (Krubitzer, 1995; Krubitzer and Kahn, 2003), the basic cortical circuitry is similar. The laminar allocation of cells connecting to the thalamus, spinal cord, or intracortical areas is remarkably conserved among all mammals studied. Rockel *et al.* (1974) counted the number of neuronal cell bodies in a narrow radial strip (30 μm wide) through the depth of the neocortex in several different functional areas (motor, somatic sensory, area 17, frontal, parietal, and temporal) in different mammalian species (mouse, rat, cat, monkey, and human) and found that the numbers were surprisingly constant. The same absolute number (congruent to 110) even characterized all areas of all species, with only one exception (see below).

This conserved cortical cell number dogma was established before the availability of various neuronal markers. It would be important to revisit this idea with modern neuroanatomical tools. The principal neuronal types of the cerebral cortex are the excitatory pyramidal cells, which project to distant targets, and the locally projecting inhibitory nonpyramidal interneurons (Peters and Jones, 1985). Pyramidal neurons are generated in the cortical neuroepithelium and migrate radially to reach the cortex following an inside-first outside-last gradient (Rakic, 1995, 2005). Interneurons produced from the neuroepithelium of the embryonic pallidum also contribute to the formation of the cerebral cortex (Parnavelas, 2000; Corbin *et al.*, 2001). These cells migrate tangentially through the striatocortical junction to reach the cortex (Marín and Rubenstein, 2003). In rodent, only a few nonpyramidal cells are generated in the cortical ventricular zone (VZ). It is known that around 70–85% of cortical neurons are excitatory glutamatergic pyramidal neurons, while the rest are GABAergic interneurons. These basic proportions also seem to be constant in all mammals (DeFelipe, 2002).

In spite of the constant number and neuronal cell types in the cortex, the mammalian cortices exhibit remarkable variations in their thickness, relative proportions of their layers, and patterning of cells and fibers (Ramón y Cajal, 1911; Figure 1). The differences within the same brain across various cortical areas are equally fascinating (Brodmann, 1909). The transition between primary and secondary visual cortical areas in primate is perhaps the most striking change in cortical organization (Rakic, 1988), as the binocular part of area 17 of primates (macaque and human) has approximately 2.5 times more neurons (Rockel *et al.*, 1974, 1980). The structure of layer 4 is so different in primate primary visual cortex that it can be seen as the line of Gennari with the naked eye (Figure 1e). However, this transition should be considered as an exception rather than the rule, since this is the only currently known aerial boundary where the numbers of cortical cells in a unit column changed. The cytoarchitectonic differences between cortical areas reflect the subtle changes in different components of the cortical circuits needed to perform the computational function of that particular area. For example, motor cortical areas have a more prominent output layer, layer 5, while the granular layer is nearly absent. On the other hand, in primary sensory areas there is more emphasis on granular and supragranular layers (layers 4 and 2–3) at the expense of the infragranular layers (Brodmann, 1909).

21.2 Dorsal Cortex Contains Fewer Neurons in Nonmammalian Vertebrates

The major divisions of the brain are comparable and are remarkably well conserved across sauropsids (birds and reptiles) and mammals (Puelles *et al.*, 2000). The exception to this is the organization of telencephalic derivatives (Striedter, 1997, 2005). There are several hypotheses about the evolutionary rearrangement of different sectors of the forebrain to produce the neocortex in mammals (Northcutt and Kaas, 1995; Molnár and Butler, 2002a, 2002b; Butler and Molnár, 2002). In spite of the emerging anatomical and gene expression data (Fernandez *et al.*, 1998), identifying structures at the striatocortical junction of the forebrain of various vertebrates and elucidating homologies remains difficult (Bruce and Neary, 1995; Butler and Hodos, 2005; Molnár *et al.*, 2006). In contrast to the six-layered mammalian cortex, reptiles possess a three-layered cortex which is similar to layers 5 and 6 of mammals (Goffinet, 1983; Goffinet *et al.*, 1986; Reiner,

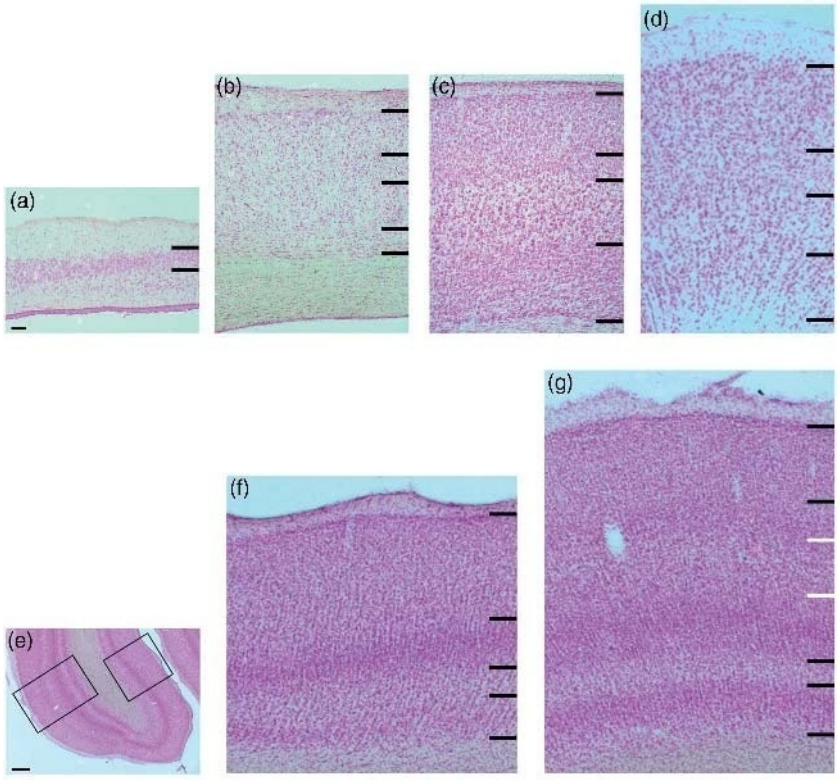

Figure 1 Nissl-stained coronal sections of four different amniote brains that demonstrate the similarities and differences in the cortical lamination in: a, Crocodile (Australian); b, hedgehog; c, mouse; d, cat; e g, rhesus monkey. e, Low-power image was taken from the border of 17 18. Boxes in (e) depict the location of the images taken from areas 17(g) and 18(f). Bars mark the layering of the different cortices. White bars in (g) mark the partitioning of layer 4 into sublayers (a) (c). Scale bar: 100 μm (a d, f, g); 500 μm (e).

1991; Figure 1a). The isocortical homologue of birds is a pseudolayered structure, which is considerably different in organization to that of mammalian neocortex (Medina and Reiner, 2000). The most notable difference is that the mammalian cortex contains dramatically more neurons. Therefore, a question arises: where did the extra cortical cells come from in the mammalian brain?

21.3 What are the Major Changes in Cortical Neurogenesis in Mammals?

Much debate exists about the progression from the postulated primitive ancestor to the modern-day mammalian and reptilian cortices (Northcutt and Kaas, 1995). There are two theories on the (total) increase of mammalian cortical neurons. Both theories suggest that there are accessory sites of neurogenesis for the mammalian cortex. First, the equivalent cell migration hypothesis suggests that a considerable population of mammalian neurons are generated outside the neocortex and migrate into the cortex during development. This theory predicts relocation of corresponding cell groups in ancestral species at the reptilian mammalian transformation (Karten, 1997). Second, the dorsal cortical germinal zone elaboration hypothesis suggests that the generation of extra cortical neurons for the expanding sheet of cortical neuroepithelium and elaboration of the granular and supragranular cortical layers in mammals required an accessory site of proliferation within the cortical subventricular zone (SVZ) and the appearance of an intermediate progenitor population (Martínez-Cerdeno *et al.*, 2005; Molnár *et al.*, 2006).

Although one cannot directly study ancestral brains, study of comparative cortical development of extant species can still reveal the developmental mechanisms that change most considerably in mammals and other vertebrates, elucidating the steps of evolutionary progression. Unfortunately, current neurodevelopmental studies are limited to very few vertebrate species, making generalizations and comparisons difficult. Our own comparative

developmental analysis is based on six species: (1) turtle, (2) chick, (3) mouse, (4) rat, (5) macaque, and (6) human.

21.4 Are Cortical Neurons Produced Outside the Mammalian Cortex Supportive for the Equivalent Cell Migration Hypothesis?

It has been argued that equivalent circuits are present in the visual and auditory pathways in avian and mammalian telencephali (Karten, 1968, 1997). Although the basic components of these circuits (thalamic recipient cells, interneurons, and efferent projection neurons) are present in both avian and mammalian cortical circuits, their arrangement is very different. While these components are arranged into cortical layers in mammals (layers 4, 2–3, and 5–6 respectively), they are mostly situated subcortically in birds (Karten, 1991). In lizard, turtle, and bird, this subcortical structure is a large mass of cells protruding into the lateral ventricle above the striatum, called the dorsal ventricular ridge (DVR). The DVR hosts most of the neurons required for information processing in the equivalent circuits. As shown by a study in iguana (*Iguana iguana*), highly organized multiple representations involved in visual processing were observed in the DVR (Manger *et al.*, 2002). In comparison to the DVR, the three-layered dorsal cortex in the iguana appears rudimentary compared to the neocortex of mammals (Figure 1).

The recent discovery that the subpallium, a region outside the cortical neuroepithelium, contributes tangentially migrating neurons to the mammalian cerebral cortex generated a good deal of excitement (de Carlos *et al.*, 1996; Anderson *et al.*, 1997; Tamamaki *et al.*, 1997). In rodents, most of these neurons are derived from the medial ganglionic eminence (Lavdas *et al.*, 1999; Sussel *et al.*, 1999; Wichterle *et al.*, 1999; Xu *et al.*, 2004). This region of the forebrain did not correspond to the sector which was suspected to be homologous to the DVR. Moreover, these neurons are exclusively GABAergic interneurons and not excitatory pyramidal cells, which one would expect for the equivalent projection circuits.

21.5 Generation and Mode of Migration of the GABAergic Interneurons

The proportion of GABAergic neurons in avian pallial regions and their rather uniform distribution closely resemble the patterns seen in other vertebrates, including mammals (Veenman and Reiner, 1994; Jarvis *et al.*, 2005). In both cases, generation and differentiation of GABA neurons in the ventral forebrain regions specifically require *Dlx* family transcription factor expression (Stuhmer *et al.*, 2002). Likewise shared are *Emx* genes expressed in the cortical domain, which primarily generates excitatory glutamatergic neurons (Anderson *et al.*, 2002; Gorski *et al.*, 2002). The molecular mechanisms and the genetic pathways are conserved in phyla as distant as amphibians, reptiles, birds, and mammals. Orthologues of *Dlx* genes have been cloned in these vertebrates (Fernandez *et al.*, 1998; Puelles *et al.*, 2000), and these genes define homologous ventral forebrain domains. The DVR, which contains the neuronal elements of the equivalent circuit, according to Karten's (1997) hypothesis, lies between the *Dlx* and *Emx* expression domains (Fernandez *et al.*, 1998; Puelles *et al.*, 2000; Jarvis *et al.*, 2005) (Figure 2).

Though the dorsal cortex of sauropsids lacks several cell types found in the mammalian isocortex, both structures are comprised of two basic components required to build a functional cortex: the excitatory glutamatergic projection neurons and the inhibitory GABAergic interneurons (Goffinet *et al.*, 1986; Blanton *et al.*, 1987; Reiner, 1991, 1993).

During embryonic development, GABAergic neurons originate in the ventral subpallium and progressively colonize the dorsal pallium following distinct routes (Nadarajah and Parnavelas, 2002; López-Bendito *et al.*, 2004; Métin *et al.*, 2006). These streams are generally oriented tangentially to the brain surface throughout their path, but at the pallium/subpallium boundary, or within the VZ before reaching the cortical plate, where some cells reorient radially (Nadarajah *et al.*, 2002).

Comparative analysis of tangentially migrating neurons in birds revealed that, just as in mammals, most GABAergic interneurons originate in the ventral telencephalon (Cobos *et al.*, 2001). In slice cultures prepared from embryonic chick brains, GABAergic cells follow similar tangential routes in both subpallium and pallium, and show similar branched leading processes (Tuorto *et al.*, 2003). The similarities in the migration route and morphology to mammalian tangentially migrating interneurons suggest common mechanisms of development (Bellion *et al.*, 2005). Accordingly, the developmental sequence of GABAergic interneurons in the turtle cortex is reminiscent of tangential migration from ventral territories (Blanton and Kriegstein, 1991). In bird and turtle, the relative contribution of pallidum (or medial ganglionic eminence of the telencephalon) and paleostriatum (or lateral ganglionic eminence of the telencephalon) to the GABAergic population is debated (Cobos *et al.*, 2001; Tuorto

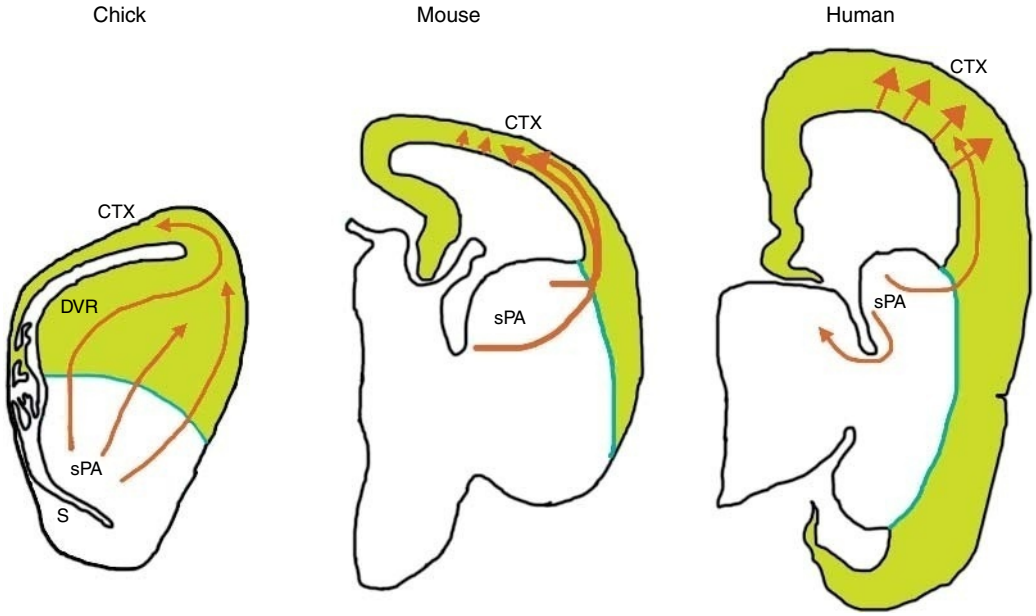

Figure 2 Common mechanism of subpallial origin and tangential migration of GABAergic neurons in bird, rodent, and human. Schematic outlines represent the cross sections through chick, mouse, and human forebrains. Orange arrows depict the migratory patterns of GABAergic neurons from subpallium (sPA). S, septum; CTX, dorsal cortex. See text for details. The left panel was inspired by Cobos *et al.* (2001) and the right panels by Tan (2002). Adapted from Molnár, Z., Métin, C., Stoykova, A., *et al.* 2006. Comparative aspects of cerebral cortical development. *Eur. J. Neurosci.* 23, 921 934, with permission from Blackwell Publishing.

et al., 2003). In rodent and primate, there also seem to be great differences in the proportion of GABAergic neurons generated locally in the pallium and the striatum/pallium (lateral and medial ganglionic eminences). In human, gene expression evidence suggests that a substantial fraction (65%) of cortical interneurons are generated by the pallium (Letinic and Rakic, 2001), whereas in rodents this estimate is only 5% (Letinic *et al.*, 2002; Tan, 2002).

In spite of all these current uncertainties, the neuronal production observed outside the cortex in mammals is not supportive of the equivalent cell migration hypothesis. Several expectations were not fulfilled by the tangentially migrating neurons. (1) The migrating neurons are purely GABAergic and do not contain any excitatory pyramidal neurons. (2) The origin of the migrating cells in mammals does not coincide with the domain which is considered homologous to the DVR. (3) Tangential migration is not unique to mammalian brains. In avian and probably reptilian brains, GABAergic interneurons also arise from a *Dlx* domain and migrate tangentially to the dorsal cortex. Therefore, it is more likely that changes in the local dorsal cortical neurogenetic program, together with some major rearrangements at the striatocortical junction (Molnár and Butler, 2002a), provided the foundation for remodeling the mammalian cerebral cortex (Molnár *et al.*, 2006).

21.6 Basic Pattern of Cortical Neurogenesis

The predecessor of the mammalian cortical plate is the preplate (or primordial plexiform zone), which contains a heterogeneous population of the earliest-born neurons of the cortex, and is considered to be the reptilian component of the mammalian neocortex (Marin-Padilla, 1978). The first neurons of the rodent and human cortex probably originate from subpallium and not from cortex (Bystron *et al.*, 2005). This is in line with the notion that Cajal–Retzius cells migrate in from various sources (Bielle *et al.*, 2005). Neurons subsequently generated from the cortical plate split the preplate into an outer plexiform layer and an inner subplate (Marin-Padilla, 1978; Smart and McSherry, 1982; Smart and Smart, 1982; Luskin and Shatz, 1985). Newly produced neurons migrate out of the germinal zone from the VZ towards the pial surface according to a strict timetable. In mammals, the cortical plate is destined later to become the six-layered structure of the mature cortex. The cortex is formed in an inside-first outside-last neurogenic gradient (Angevine and Sidman, 1961) where younger cohorts migrate beyond previously generated neurons to settle at the upper border of the cortical plate. Consequently, the oldest neurons of the cortex occupy the deep layers, whereas the upper layers are made of late-born neurons.

In all veterbrates, embryonic neurogenesis provides the majority of neurons that compose the adult brain. In the embryonic brain, epithelium lining the ventricles (the VZ) has long been known to be a neurogenic source (Sauer, 1936; Sauer and Walker, 1959; Sidman *et al.*, 1959). In mammals, an additional, adjacent layer of mitotic activity was also observed (termed the SVZ). It was believed that gliogenesis commences during late corticogenesis and continues perinatally in the SVZ (Privat, 1975), and hence neurogenesis and gliogenesis were thought to occur in the VZ and SVZ respectively (Sturrock and Smart, 1980; Bayer and Altman, 1991). These compartments differ in size, organization, and in their modes of division. The proliferative cells in the VZ undergo interkinetic migration, and have radially aligned nuclei. The SVZ contains cells with randomly oriented nuclei (Smart, 1973).

Radial glia were previously assumed to act merely as scaffolds for newly born neurons to migrate along, and until recently, their importance was unappreciated. They are now considered to be the source of most neurogenesis in the developing cortex (Malatesta *et al.*, 2000; Noctor *et al.*, 2002). At the VZ, radial glia divide asymmetrically to yield a new glial cell and a daughter cell which migrates away from the ventricular surface. Elegant time-lapse photography of individual neurons migrating in brain slices demonstrated that the SVZ also participates in neurogenesis and the VZ is not the sole neurogenic compartment (Noctor *et al.*, 2004). Noctor and his colleagues also showed that the daughter cells generated from asymmetrical VZ divisions head directly to their locations in the future cortex, while others choose to arrest in the SVZ, where they are termed intermediate progenitors. Here the intermediate progenitors mostly undergo symmetric division to produce two identical daughter neurons, which migrate to the cortex (Kriegstein and Noctor, 2004; Noctor *et al.*, 2004) (Figure 3). This work was further confirmed *in vivo* using direct labeling of neurogenic progenitors lying outside the VZ (Wu *et al.*, 2005), which demonstrated that cells marked in the SVZ subsequently gave rise to upper-layer pyramidal neurons.

Analysis of gene expression in the germinal zone revealed that the VZ and SVZ progenitor cells are controlled by different genes. Embryonic expression of *Svet1* (Tarabykin *et al.*, 2001), *Cux1*, and *Cux2* (Nieto *et al.*, 2004) are only seen in the SVZ but expression of these genes is absent in the VZ. Postnatally, these genes are confined to the supragranular and granular layers of the cortex. On the other hand, the transcription factor Otx-1 and Er81 labels cells in the VZ exclusively during embryonic stages, and later is confined to layer 5 and 6 or layer 5, respectively (Frantz *et al.*, 1994; Tarabykin *et al.*, 2001; Yoneshima *et al.*, 2006). Thus, the SVZ gives rise to neurons that go on to populate the upper cortical layers (2–4), whereas the VZ produces the earlier-born neurons which give rise to the deeper layers (5 and 6) of the cortex. Differential fate is a product of transcription factor action on divergent dividing and migratory properties.

In addition to the proliferation in the VZ and SVZ, it was recently found that there are further scattered divisions within the intermediate zone, cortical plate, and marginal zone in rodent and human. These abventricular divisions compose less than 10% of all divisions at any stage of any cortical area during development (Carney *et al.*, 2004; Carney, 2005). Nevertheless, it shows that scattered progenitor cells are also capable of division outside the two main proliferation compartments (Figure 4).

In summary, we favor the dorsal cortical germinal zone elaboration hypothesis on two accounts. By examining the cortical development in macaque, where the variety of supragranular layer cells is much more diverse, the germinal zone differentiates

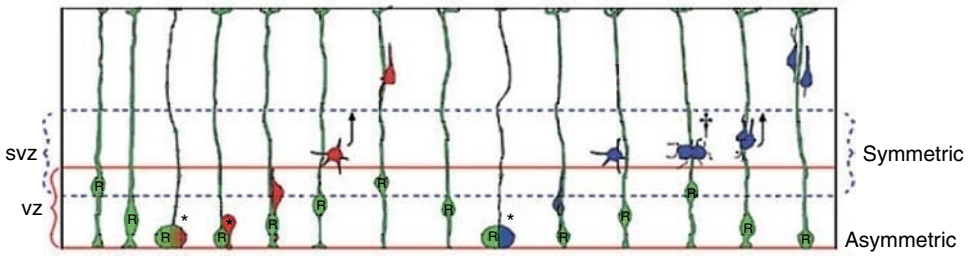

Figure 3 Two distinct programs of division and migration are observed in the germinal zone. Radial glia (R) self-renew and directly give rise to neurons (red) through asymmetric division in the VZ (*). Other neurons are generated from intermediate progenitor cells (blue) in the subventricular zone (SVZ) with terminal symmetric divisions (dagger). Reprinted by permission from Macmillan Publishers Ltd: *Nat. Neurosci.* (Noctor, S. C., Martinez-Cerdeno, V., Ivic, L., and Kriegstein, A. R. 2004. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. *Nat. Neurosci.* 7, 136 144), copyright (2004).

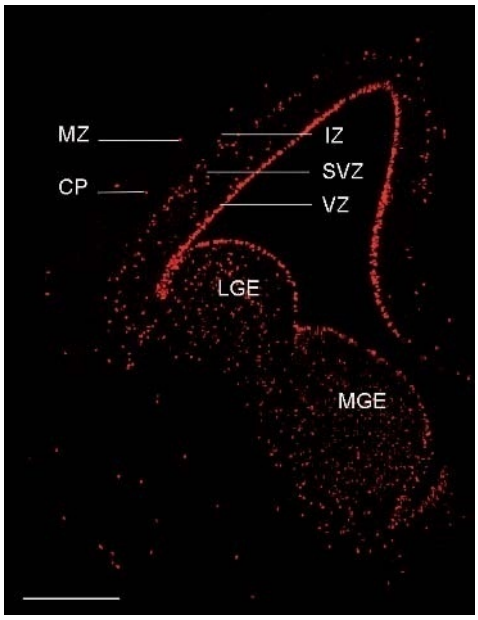

Figure 4 Coronal section through the right hemisphere of an E14 rat brain stained with phosphohistone H3 antibody to reveal the sites of cell divisions. Most of the divisions occur in the VZ lining the cortical neuroepithelium. There is a second major row of divisions in the SVZ, but there are further scattered divisions in the marginal zone (MZ), cortical plate (CP), and intermediate zone (IZ). There are large number of divisions in the medial and lateral ganglionic eminences (LGE, MGE). Scale bar: 100 μm. Unpublished figure from Carney *et al.* (2004) and Carney (2005).

further, with unique features not seen in rodents. On the other hand, the dorsal cortical neuroepithelium of nonmammalian vertebrates (turtle and chick) does not contain subventricular zone.

21.7 Neurogenesis in Primate Cortical Neuroepithelium

The developing primate cortex contains a unique compartment of the SVZ termed the outer SVZ (OSVZ) (Smart *et al.*, 2002). This site of proliferation has been shown to produce the majority of the supragranular layers (Lukaszewicz *et al.*, 2005). In rat and mouse, the supragranular layers are significantly smaller compared to macaque; thus, correspondingly, the SVZ is also much smaller (Smart *et al.*, 2002).

The work of Kennedy, Dehay, Smart, and colleagues produced some very interesting comparisons between mouse and macaque in cortical development (Smart *et al.*, 2002; Lukaszewicz *et al.*, 2005; Figure 5a). In mouse, the VZ is the major source of proliferation up until E15, after which it begins to regress in size. The SVZ appears at E13 but lags behind in proliferative terms, and begins to regress after E15 (Smart, 1973; Smart and McSherry, 1982). The relatively low germination activity in the SVZ is mirrored in the smaller proportion of the upper layers in the mouse neocortex. The SVZ in rodents accounts for no more than 35% of cortical proliferative population at E15 (Takahashi *et al.*, 1995). Accordingly, the supragranular layers occupy no more than a third of the thickness of the mature neocortex (Smart *et al.*, 2002). In contrast, the monkey has a very different process of proliferation. At a gross histological level, the size of the proliferative compartment (i.e., VZ + SVZ) rapidly increases in size from E65 onwards (Figure 5b). At this point, on the basis of histological appearance, the SVZ appears to have two distinct components: an inner SVZ (ISVZ) and a larger, OSVZ. Between E65 and E72, the OSVZ rapidly increases in size and becomes the major proliferative area of the SVZ, with the ISVZ contributing little. The dense, radially oriented precursors of the OSVZ constitute a unique feature, and birth-dating experiments showed that it generates the supragranular layers of the neocortex (Lukaszewicz *et al.*, 2005). The predominance of OSVZ in macaque could be due to the increased importance of the corticocortical connections and therefore the supragranular layers, where most corticocortical connections are formed. After E72, the OSVZ begins to decrease in size, accompanied by a corresponding increase in cerebral wall thickness, suggesting that the postmitotic cells are migrating to their future home in the cortex. By E78 the proliferative compartment has been fully split by the complete appearance of the inner fiber layer, with the ISVZ now attaching to the VZ but not the OSVZ (Figure 5) (Smart *et al.*, 2002).

It is possible that localized transcription factor expression of the SVZ and the VZ is responsible for the creation of certain neuronal subtypes. It is also possible that further compartmentalization of SVZ in primates is a correlate of higher neuronal diversity of supragranular layers (DeFelipe *et al.*, 2002). The contribution of SVZ in neuronal production seems to grow in evolution as the complexity of the cortex increases. The emergence of an additional proliferation zone and its diversification during cortical evolution might have been triggered by the necessity to produce more neuronal subtypes in different morphological compartments.

21.8 Cortical Neurogenesis in Nonmammalian Cortex

Our group became interested in examining the dorsal cortex of nonmammalian (turtle and chick) brains because these vertebrates do not possess a

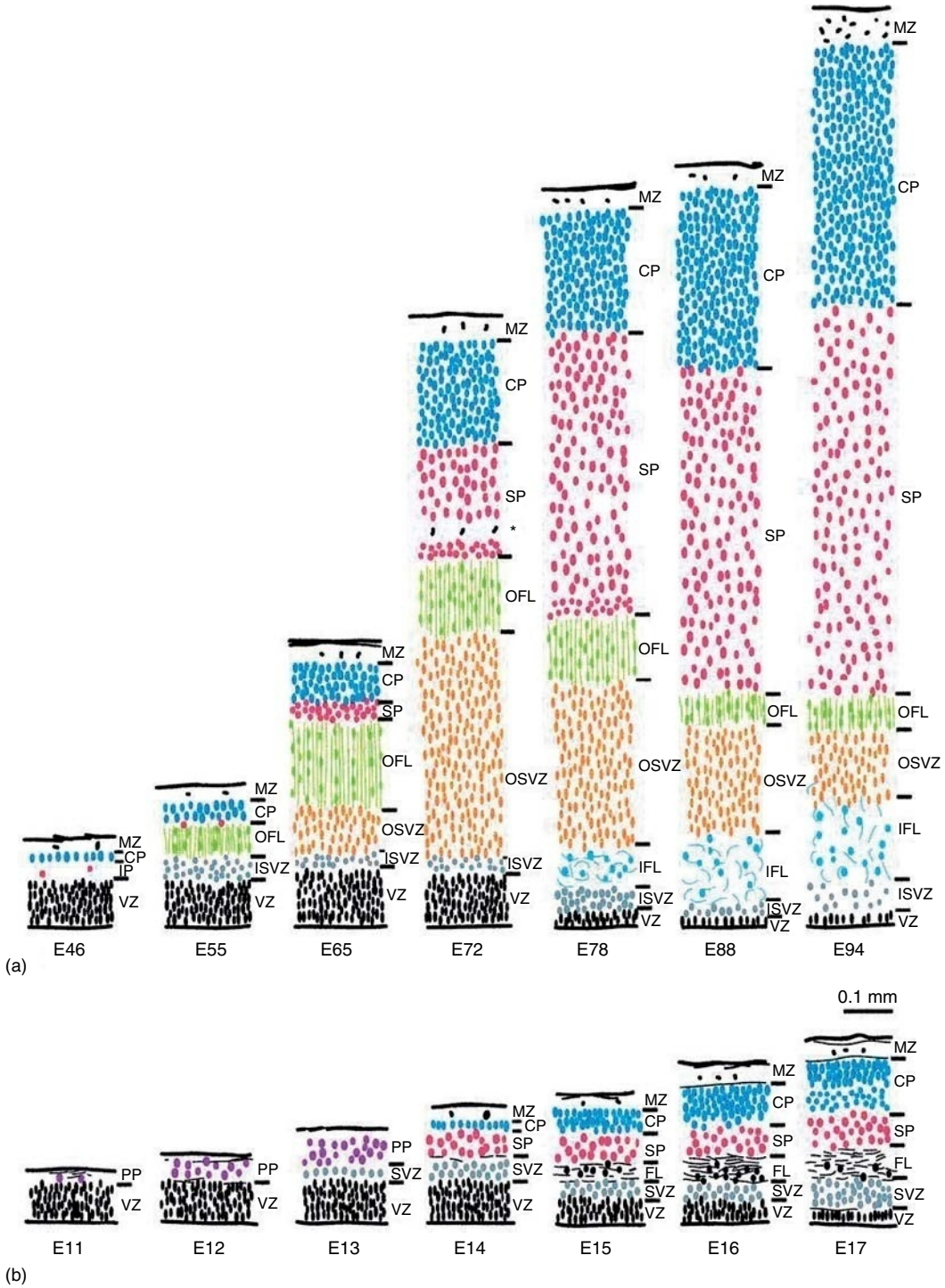

Figure 5 Comparison of histological sequences in the developing mouse and monkey telencephalic wall. These drawings are of transects through putative area 17 in monkey (a) and mouse (b) at comparable developmental stages. The depth of each layer is drawn to a common scale. The internal detail of each layer is not to scale but depicts the orientation, shape, and relative packing density of nuclei in each layer. The vertically aligned pairs have been chosen with reference to birth-dating experiments to illustrate corticogenesis at equivalent developmental stages. A curiously conspicuous clear layer marked by an asterisk (*), located in the deep subplate (SP), is transiently present at E72. At later stages it appears to merge into the SP. CP, cortical plate; IFL, inner fiber layer; ISVZ, inner subventricular zone; MZ, marginal zone; OFL, outer fiber layer; OSVZ, outer subventricular zone; SP, subplate proper; VZ, ventricular zone. Reproduced from Smart, I.H., Dehay, C., Giroud, P., Berland, M., and Kennedy, H. 2002. Unique morphological features of the proliferative zones and postmitotic compartments of the neural epithelium giving rise to striate and extrastriate cortex in the monkey. *Cereb. Cortex* 12, 37 53, Oxford University Press.

six-layered cortex, and the complexity and variety of neuronal types within them is more limited than in mammals. If indeed the generation of the upper layers of cortex found in mammals required an accessory site of proliferation, such as the SVZ, in addition to the VZ (Martínez-Cerdeno *et al.,* 2005; Molnár *et al.*, 2006), then this zone should be rudimentary or nonexistent in the reptilian and avian dorsal cortical neuroepithelium during embryonic development.

We examined the site of mitotic divisions in embryonic turtle and chick brains, paying special attention to the investigation of the presence or absence of abventricularly generated cells and the SVZ. Using phosphohistone H3 (a G2 and M-phase marker), we found that the distribution of mitotic cells in embryonic turtle and chick is fundamentally different from each other, and from rodent and primate (Lukaszewicz *et al.*, 2005). In turtle and chick cortex, there is a single major zone of proliferation with additional scattered abventricular divisions within the dorsal cortex (Figure 6). However, in certain regions of the chick brain (mesopallium, nidopallium, and striatum), two distinct proliferative zones are visible, whereas the turtle has only one (ventricular) organized zone of mitotic activity throughout the brain (Figures 6c and 6d). In turtle a significant proportion of division occurs abventricularly (highest in the striatum and septum), but these are scattered across the entire depth of the forebrain structures and never align into a distinct zone. VZ mitosis peaks in earlier stages (S18.5 and S20) before shifting to an increasingly abventricular site of proliferation in later stages (S23 and S25). In turtle, the major zone of activity is the VZ. Using Nissl-stained preparations, Martínez-Cerdeno *et al.* (2005, 2006) also suggested that the majority of the divisions are in the VZ, but noted the presence of a rudimentary SVZ. According to our own observations on the distribution of phosphohistone H3 immunoreactivity, there appears to be no organized zone outside the VZ anywhere in the embryonic turtle brain. Thus, one must conclude that the SVZ is absent in turtle (Figure 6a).

In the chick the situation is more complicated. An SVZ-like structure has been identified (Striedter and Keefer, 2000), but this sector appears restricted to the dorsolateral portion of the basal telencephalon. This finding remains controversial, especially as the SVZ is only found in this region. In the homologue of the dorsal cortex of the chick brain, the hyperpallium has a similar distribution of phosphohistone H3-labeled cells to turtle (i.e., VZ only; Figure 6b). Outside the cortex there appears to be a clear and distinct secondary zone of proliferation in the mesopallium and nidopallium (and, to a certain extent, in the striatum) above the VZ. This is clearly

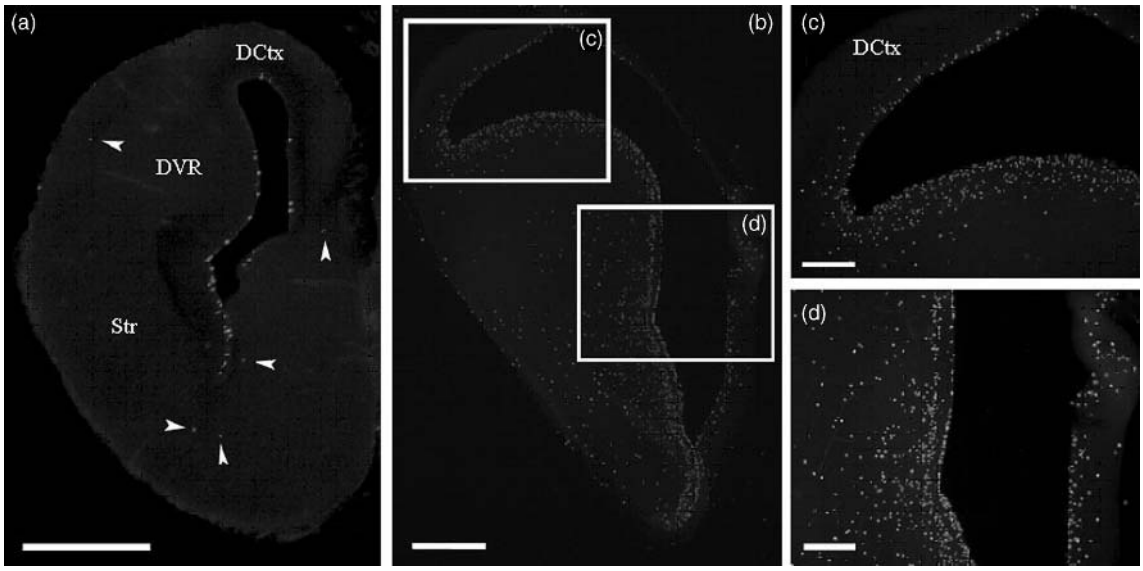

Figure 6 The lack of SVZ in turtle and chick dorsal cortex has been demonstrated with antiphosphohistone H3 immunohistochemistry. H3 immunofluoresence demonstrates mitotic activity in S18.5 turtle (a) and E8 chick (b) in the VZ and occasionally scattered abventricular proliferations (arrowheads in (a)). In turtle there is no organized SVZ in any part of the neuroepithelium. In chick, higher magnification (c, d) demonstrates an additional layer of proliferation superficial to the VZ in mesopallium and nidopallium in addition to more numerous scattered abventricular proliferation profiles. DCtx, dorsal cereberal neocortex; Str, Striatum. Scale bars: 500 μm (a, b); 200 μm (c,d).

demarcated by the presence of a band which separates the two zones, lacking in phosphohistone H3-labeled cells. Interestingly, this arrangement is not detected at any stages in the hyperpallium. This suggests that the SVZ is not a purely mammalian phenomenon in the forebrain, but it is unique for mammalian dorsal cortex together with the six-layered isocortex, to which SVZ provides neurons for more superficial layers (Noctor *et al.*, 2004; Wu *et al.*, 2005). As SVZ was absent in the hyperpallium, it seems likely that the presence of a precortical SVZ is an exclusive hallmark of mammals (Molnár *et al.*, 2006). This is one of the forces driving their cerebral complexity over other taxonomic classes. In support of this, recent data also suggest the SVZ is absent from amphibians (Wullimann *et al.*, 2005).

21.9 Evolving Cortical Progenitor Populations in VZ and SVZ

The absence of SVZ in turtle and chick hyperpallium indicates that the VZ directly provides the cells that subsequently form the dorsal cortex of the postnatal brain. Indeed, by examining the neurotransmitter organization and connections of turtle cortex, Reiner (1993) suggested that only the infragranular layers produced by the ventricular progenitors are present. It would be interesting to study further whether the fate of the reptilian or avian ventricular progenitors could be modified by providing appropriate gene expression patterns in the SVZ. Perhaps the turtle and chick dorsal cortical progenitor cells do have some innate ability to arrest in an SVZ-like region for additional symmetrical division. Moreover, several transcription factors show strong regional expression and could be used to map out VZ (*Pax6*) and SVZ (*NeuroD* and *Tbr2* co-expression) (Hevner *et al.*, 2006). The role of *Svet1* could be explored further by electroporating DNA into the VZ of turtle and chick isocortical homologues. The cell cycle parameters in different sectors of the turtle and chick germinal zone should be further studied. Measurements made using proliferating cell nuclear antigen (PCNA) immunohistochemistry and ^{3}H-thymidine pulse labeling revealed that cell cycle differs in duration in the germinal zones of monkey (Lukaszewicz *et al.*, 2005). The cell cycle is longer in the monkey OSVZ than in VZ (or comparable mouse), which may allow for the precise generation of a greater diversity of neurons that compose the supragranular layers (Figure 7) (Smart *et al.*, 2002; Molnár *et al.*, 2006).

Further study of the different cortical progenitor populations and their evolutionary origin could have general clinical implications. Various developmental diseases have a background of disrupted cortical formation (Francis *et al.*, 2006) and examining these cases could help expound the principles of cortical neurogenesis. The mechanisms involved in the formation of sulci and gyri of the brain are not fully understood (Van Essen, 1997). The lissencephalic cortex of mouse has been demonstrated to be a result of a less active SVZ. Drawing from this observation, lissencephaly in human may also result from inadequate SVZ proliferation. However, generalizations should currently be avoided until cortical neurogenesis is examined in gyrencephalic rodents and lissencephalic primates. According to the dogma on the cell numbers within a unit column of mammalian cerebral cortex, all mammalian cortices in all areas possess the same number of cells, with the exception of the primate primary visual cortex (Rockel *et al.*, 1974, 1980). If so, then the compartmentalization of the germinal zone should correlate more with the size of the cortical sheet and with the proportions and cell diversity of the supragranular cell layers. The cortical SVZ appears to be crucial for generating the increased cortical size and complexity (particularly the diversity of the upper layers) seen through the progression of mammalian evolution. While the SVZ is not unique to mammals, the development of a cortical SVZ appears to have been a crucial step in cortical evolution.

21.10 Summary and Conclusions

Comparative studies contribute to the debate on the possible evolutionary progression from the developmental mechanisms present in the postulated primitive ancestor to the modern-day mammalian and other vertebrate cortices (Northcutt and Kaas, 1995). We examined two theories that address the increased number of mammalian cortical neurons. Both suggest that there are accessory sites of neurogenesis for the mammalian cortex. The analysis of embryonic development does not support the equivalent cell migration hypothesis, although there is indeed a considerable population of mammalian neurons generated outside the cortex that migrate into the cortex during development; these neurons are exclusively GABAergic. More importantly, this process is not unique to mammals. We believe an increasing volume of work supports the dorsal cortical germinal zone elaboration hypothesis. In light of recent comparative investigations on embryonic cortical neurogenesis in frog, turtle, chick, rat, mouse, and macaque, it is more likely

Figure 7 There is a strong correlation between the increase in supragranular layer complexity and the increase in subventricular zone between Turtle and chick (a); mouse (b); and monkey (c). The left panels for mouse and monkey are from Figure 5 from an E15 mouse and an E72 monkey. The right panels represent the layering in the adult. VZ and infragranular layers (6 and 5) are labeled red; SVZ and supragranular layers (2 and 3) are colored yellow. Note that the increase in the complexity of supragranular layers is accompanied by an increase in the SVZ during development. For clarity, SVZ includes ISVZ and OSVZ in the monkey panel. Adapted from Molnár, Z., Métin, C., Stoykova, A. *et al.* 2006. Comparative aspects of cerebral cortical development. *Eur. J. Neurosci.* 23, 921 934, with permission from Blackwell Publishing.

that the generation of extra cortical neurons for the larger cortical sheet and increasingly elaborate granular and supragranular cortical layers in mammals required the adoption of an accessory site of proliferation within the cortical SVZ, as well as the appearance of an intermediate progenitor population (Smart *et al.*, 2002; Kriegstein and Noctor, 2004; Noctor *et al.*, 2004; Martínez-Cerdeno *et al.*, 2005; Molnár *et al.*, 2006).

Acknowledgments

The original work of ZM's laboratory was supported by grants from Medical Research Council (GO300200), the Wellcome Trust (063974/B/01/Z), Human Frontier Science Program (RGP0107/2001), European Community (QLRT-1999–30158), and St John's College, Oxford.

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22 Reconstructing the Organization of the Forebrain of the First Mammals

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Glossary

area	A major subdivision of neocortex. Each area performs a specific set of functions. Areas were called the "organs of the brain" by Brodmann (1909).
column or module	Subdivisions of areas that mediate a function or functions that are repeated many times within other modules of the same type within the area. Areas may have two or more types of intermixed modules.
cortical magnification	The greater representation in cortical areas of important parts of sensory surfaces in proportion to receptor density.
motor cortex	Subdivisions of cortex that are specia lized to elicit and control body movements. Movements can be evoked by electrically stimulating motor cortex.
representation	Areas are said to represent a sensory surface, such as the retina, skin, or cochlea, when stimulation in different parts of the sensory surface activates neurons in different parts of the area in a matching or isomorphic pattern. Muscles and movements are also represented in areas of motor cortex. Some areas may have more abstract, higher order representations.

22.1 Introduction

The evolution of the large human brain intrigued early investigators, such as Smith (1906) and Clark (1959), but their efforts to describe this evolution were greatly constrained by the limited information on brain organization and the few techniques to evaluate brain function available at that time. What they did have were extensive collections of brains, preserved in jars of fixative, and thin sections of brains stained for the cell bodies of neurons or for the myelin that wraps the axons of these neurons. Thus, they could carefully observe ways in which the brains of extant mammals varied greatly in size, the locations of fissures that indent the cortex and even in the architectonic appearance of cortex and other parts of the brain. Such early investigators recognized that the neocortex was a part of the brain that varied the most in size and that some portions of neocortex could be recognized as similar

enough that they were likely to be homologous across species. They concluded that early mammals had small brains with little neocortex, and mammals leading to humans had brains that were progressively larger and more complexly organized, with proportionally more neocortex. While these deductions were based on the appearances of the brains of a large array of species, they primarily depended upon detailed considerations of the brains of a few key mammals that were thought to represent stages or levels in the course of the evolution of human brains (for review see Kaas, 2002; Preuss, 2000).

Today, we are in a position to greatly expand on the efforts of such early investigators. Most importantly, we know so much more about the organization of the brains of a wide range of present-day mammals. The traditional Nissl, myelin, and Golgi stains have been supplemented by an ever-increasing array of histochemical and immunochemical protocols for revealing the architecture of brains and their structurally distinguishable parts. Electrophysiological approaches, especially microelectrode recording and stimulation procedures, have greatly expanded our understanding of brain organization, and this knowledge has been fortified and advanced by newer methods such as the optical imaging of patterns of evoked neural activity. We also know something about the sizes and shapes of the brains of long extinct mammals from the ever-increasing fossil record based on the endocasts of the brain cases of the preserved skulls (Jerison, 1973; Kielan-Jaworowska *et al*., 2004). Although this record tells us little about the functional organization of the brains of extinct mammals, it does yield information about brain sizes and fissure patterns.

Conceptual advances have been important as well (see Hodos and Campbell, 1969; Preuss, 1995a; Striedter, 1998). Early investigators assumed that various extant species could be used to represent stages or levels of mammalian evolution. The problem with this assumption is that any extant mammal is likely to contain a mixture of ancestral (plesiomorphic) and newer, apomorphic (specializations derived from an earlier state) traits or features. Of course, some mammals appear to have mainly primitive brain features, while others have many obviously advanced features. Thus, methods were needed to distinguish primitive from advanced traits. Otherwise, an advanced trait in a generally primitive brain could be mistaken for a primitive trait and vice versa. The current approach to this problem is to use cladistic analysis (see Eldredge and Cracraft, 1980; Wiley, 1981). In brief, some brain traits are recognized as present or absent in members of a clade of mammals (any group of mammals that have all descended from a common ancestor). The distribution of the trait across the phylogenetic tree of related mammals, the cladogram, tells you whether it is more likely (more parsimonious to assume) that the trait was present in a common ancestor, and was retained in many or all of the descendents, or subsequently evolved in one or more lines. Given this approach, there is a logical way to distinguish primitive from advanced (derived) traits, other than from their appearance or association with other traits. A common mistake of early and even current investigators was to consider simple or undifferentiated traits as primitive. For example, a few current investigators still support the theory of Sanides (1970) that poorly differentiated regions of neocortex are older and highly differentiated regions are newer. This may generally be the case, but it can be demonstrated not to be the case in some instances. For example, primary visual cortex (V1 or area 17) in the hedgehog has poorly differentiated cell types and cell layers are not very distinct (Kaas *et al*., 1970), while primary visual cortex in tarsiers is perhaps more distinctly laminated than in any other mammal (Collins *et al*., 2005). Yet, V1 is an equally old area in both mammals, having been present in the reptilian ancestors of mammals (see below). The histological structures of V1 had simply differentiated more in the line leading to tarsiers than in the line leading to hedgehogs (see The Evolution of Visual Cortex and Visual Systems).

To be fair, early investigators such as Eliott Smith and Le Gros Clark had some appreciation of the advantages of broad comparisons across members of a clade. They recognized that the easily identified corpus callosum, the bundle of axons that interconnects the two cerebral hemispheres, was a new feature in placental (Eutherian) mammal brains because all members of the placental clade have a corpus callosum, no members of the monotreme or marsupial clades have a corpus callosum, and no reptiles or other vertebrates have a corpus callosum. But only easily identified traits could be examined across a wide range of mammals at the time of their investigations. Recognizing many brain characters is still difficult, and thus premises about brain evolution still depend on too few observations (see Kaas, 2002). Fortunately, we have procedures to correct mistakes, and powerful ways of determining brain organization. A great aid to current cladistic studies of brain evolution is the progress that has occurred in understanding the details of the phyletic radiation of mammals. This understanding is based on both the fossil record, and the results of recent

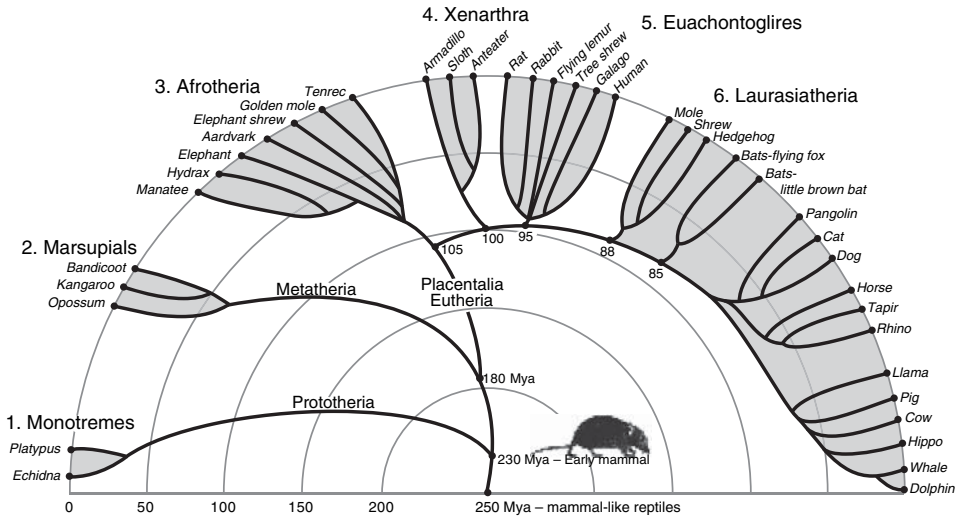

Figure 1 A broad overview of current understanding of the phyletic radiation of mammals. While uncertainties remain, current evidence supports the division of extant mammals into six major clades or superorders (1 6). The molecular evidence, together with the fossil evidence, allows estimates of the times of divergence for these major branches of the radiation and their sub-branches. Thus, early mammals emerged from mammal-like reptiles around 230 Mya. Most early mammals were shrew-like in appearance, and they changed little until the extinction of dinosaurs some 65 Mya. An early mammal is depicted at the center of the radiation, the starting point for mammals. Based on Murphy, W. J., Pevzner, P. A., O'Brien, J. O. 2004. Mammalian phylogenomics comes of age. *Trends Genet.* 20, 631 639.

molecular studies of phylogenetic relationships. As an example, we know from the discovery of fossil whales (Cetacea) with retained hind limbs (Gingerich *et al*., 2001), that whales evolved from a branch of even-toed ungulates (Artiodactyls), and molecular evidence supports the same conclusion (Shimamura *et al*., 1997). A version of a modern phylogenetic tree of the mammalian radiation, showing only the main branches, is shown in Figure 1. This depiction usefully guides the following discussion of brain evolution in mammals, which focuses on neocortex as a flexible structure that has been modified in many ways.

22.2 The Basic Structure of Neocortex and the Transition from Dorsal Cortex of Reptiles

In order to understand the different ways that neocortex has changed in the evolution of various mammals, it is useful to briefly review the basic organization of neocortex, and contrast it with its homologue, the dorsal cortex of reptiles (Figure 2; Northcutt and Kaas, 1995). The cortex is the outer sheet of tissue of the forebrain. In reptiles, three major regions are generally distinguished (Figure 3). A dorsomedial region forming the medial walls of the cerebral hemisphere has long been identified as a homologue (the same structure) of the mammalian hippocampal formation (e.g., Smith,

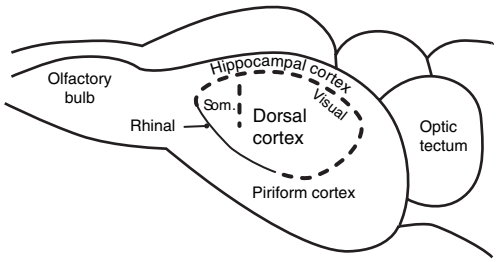

Figure 2 A dorsolateral view of the brain of a turtle showing subdivisions of the forebrain. The large olfactory bulb provides input to the lateral cortex (cf. Figure 3) that is the homologue of piriform cortex of mammals. A small dimple, referred to as the rhinal sulcus in mammals, separates dorsal cortex from lateral cortex, the homologue of piriform cortex. Dorsal cortex is the homologue of neocortex of mammals. Dorsal cortex has visual inputs from the lateral geniculate nucleus, as in mammals, and there is evidence for somatosensory (som.) inputs as well. The medial hippocampal cortex is the homologue of the mammalian hippocampus. The optic tectum is the homologue of the mammalian superior colliculus. Based on Hall, W. C., Ebner, F. F. 1970. Thalamotelencephalic projections in the turtle (*Pseudemys scripta*). *J. Comp. Neurol.* 140, 101 122.

1910; see Striedter, 1997 for review). In reptiles, this tissue does not have the coiled form of the mammalian hippocampus, but its location and appearance, with a single dense row of cell bodies sandwiched between layers of fibers, help identify it as the hippocampus. A dorsal sector, the dorsal cortex of reptiles, is the homologue of mammalian neocortex. Nevertheless, dorsal cortex is only

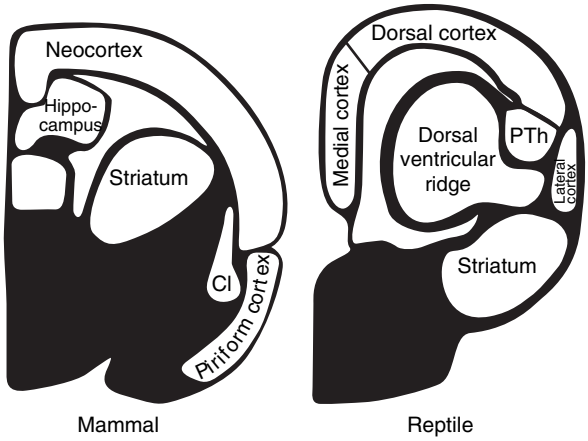

Figure 3 Schematic cross sections through the forebrain of a reptile and a small-brained mammal. Note that the dorsal cortex of reptiles, although smaller, corresponds in position to neocortex of mammals. PTh, pallial thickening; lateral (piriform) cortex; Cl, claustrum. Based on Striedter, G. F. 1997. The telencephalon of tetrapods in evolution. *Brain Behav. Evol.* 49, 179 213.

slightly modified in appearance from the medial hippocampal cortex. Dorsal cortex has a thin deep layer of fusiform neurons with horizontal dendrites, and a more superficial layer of more pyramid-like neurons with dendrites extending vertically into an overlying fiber layer (e.g., Ulinski, 1986). Together these two cell layers are rather thin, only a few cells thick, and unimpressive. Afferents from the thalamus enter from the lateral margin of dorsal cortex and course in the outer fiber layer to contact the dendrites of the cells below (Hall and Ebner, 1970). The ventrolateral part of dorsal cortex, termed the pallial thickening, is now thought to be the reptilian homologue of the mammalian claustrum (a thin sheet of cells internal to layer 6 of ventrolateral neocortex with interconnections with neocortex (see Dinopoulos *et al.*, 1992 for review). The lateral cortex of reptiles is considered the homologue of piriform (olfactory) cortex of mammals. The point of this brief description is to stress that mammalian neocortex is not really a new structure in mammals. Hence, many comparative neuroscientists prefer to call it isocortex for its uniform appearance in mammals. However, a comparison of dorsal cortex with neocortex illustrates that neocortex has new features and in that sense can be called new.

In contrast to dorsal cortex of reptiles, neocortex in all mammals is a rather thick structure, packed with neurons, that is traditionally described as having six layers of different functions (Figure 4). Across these layers, a species variable numbers of fewer than 100 to over 200 neurons are aligned in a vertical row. As neurons in this row (sometimes called a minicolumn) are densely interconnected, they constitute a functional unit that integrates the functional roles of neurons across all the layers. The functionally dominant inputs for such minicolumns consist of only a few closely related inputs from a location in the thalamus or other parts of cortex, which terminate largely on the small stellate or granular neurons of layer 4. Large pyramidal neurons of layer 5 provide outputs over long distances, mainly to subcortical structures such as the superior colliculus and spinal cord, but also to the other hemisphere and to other parts of cortex in the same hemisphere. Smaller pyramidal neurons in layer 3 provide most of the connections between different sectors of cortex while pyramidal and spindle shaped neurons in layer 6 provide 'feedback' projections to whatever thalamic nucleus or cortical area provides the driving input to layer 4 neurons, and projections to the claustrum (see Dinopoulos *et al.*, 1992). Other inputs to any sector of cortex include modulating feedback inputs to superficial (1, 2, and 3) and deep (5 and 6) layers from other areas of cortex, modulating inputs from the thalamus, and modulating inputs from various neurotransmitter specific nuclei (dopamine, serotonin, acetylcholine). Many of these modulating inputs terminate on the distal ends of dendrites of pyramidal cells. Neurons in different rows or minicolumns of neurons interact via horizontal connections stemming largely from layer 3 pyramidal cells. Layer 2 has small pyramidal cells. All layers contain stellate-shaped inhibitory neurons (~20% of the total) of several types that inhibit nearby neurons when they are activated by inputs from neurons in other structures or by nearby neurons. The great computational power of neocortex comes, in part, from this basic arrangement of neurons with specialized roles within and across layers, and the activation of a complete row of neurons by a few powerful inputs. All this while this activity is subject to modification by nearby inhibitory neurons, horizontal intrinsic connections, feedback connections from other areas of cortex and the claustrum, and various neurotransmitter modulating inputs from the brainstem and thalamus. All these connections allow the outputs of such a row of neurons to be modified by their activating patterns. Many of the modifications are instantaneous and short-lived, but highly active inputs can induce long-term changes in responsiveness as well (plasticity and perceptual learning). The result is great flexibility. In addition to this flexibility based on sensory experience and activity patterns unique to each individual, natural selection can genetically modify all the features of

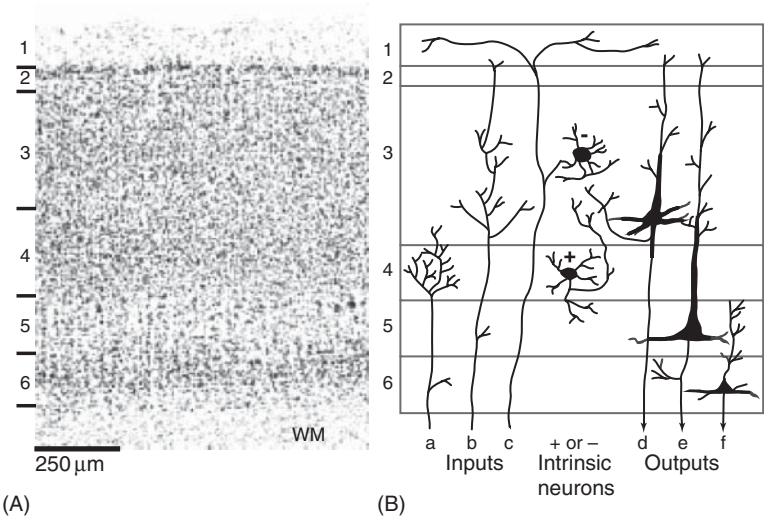

Figure 4 The laminar organization of neocortex in mammals. A, A thin slice of neocortex through primary visual cortex (V1) of a prosimian primate (galago) that has been stained to reveal the cell bodies (dark dots) of neurons, but not axons or dendrites. The cell bodies vary in shape and packing density across the thickness of cortex in a laminar pattern. Traditionally, six layers have been recognized, along with varying numbers of sublayers. Different cortical areas within a species vary in the appearance of the layers, from more to less distinct, and the same (homologous) area across species varies in appearance, but often they retain enough similarities to be identified. This cortex is just over 1 mm thick, with the cortical surface at the top of the photomicrograph and the white matter (WM) or fibers (axons) that connect cortex with other areas and structures at the bottom. B, A schematic of the cortical layers showing some of the types of neurons and connections. The major activating inputs are axons (a) from other areas of cortex or from the thalamus that terminate on stellate or granule cells in layer 4 (and inner layer 3). These 'spiny' stellate cells, thought to be modified pyramidal cells, have short dendrites within the layer, while sending axon branches to activate neurons in layers 3 and 5. Typically, the axons (a) activating neurons in layer 4 also have a branch that provides some activation of neurons in layer 6. Other inputs (b) include feedback, activating axons from other areas of cortex that terminate in layers 1 3 and layer 5. Other modulating inputs (c) from the thalamus and brainstem terminate in layer 1, and in layers 2 and 3. Most of the neurons in layers 2, 3, 5, and 6 are pyramidal neurons with short basal dendrites in the same layer, and a long apical dendrite that usually reaches layer 1. All layers have a portion of inhibitory stellate neurons ( ) with short nonspiny dendrites and short axons. Axon inputs, spiny stellate cells (+) and pyramidal cells activate other such cells and the inhibitory neurons, which synapse on excitatory neurons to dampen their activity. Layer 3 pyramidal cells project to other areas of cortex and to subcortical targets. Layer 5 pyramidal cells project mainly to subcortical targets. Layer 6 cells provide feedback connections to the thalamus or other areas of cortex, as well as to the claustrum.

such rows of cortical neurons over generations, to alter and adjust functions. For example, the functions of any row of neurons can be changed by evolving different activating inputs, or different targets for the outputs.

There are two other basic features of neocortex that have contributed to it being such an important part of the brain in all mammals, and the dominant part of the brain in most mammals. The sheet of cortex that is made up of vertical rows of cells that form layers is subdivided across its surface into functionally distinct and specialized regions called areas that are often divided into smaller functionally distinct regions called columns or modules (the term, module will be used here). The concept of a cortical area goes back to early investigators, such as Brodmann (1909), who defined cortical areas as the 'organs of the brain', and identified areas by structural differences in the layers and neurons between sectors of cortex. The early evidence for functionally specialized areas came from observing impairments in abilities of humans and other mammals after damage to specific regions of neocortex. Areas can be small or large, depending on functional role and overall size of neocortex, but the general concept is that they constitute a region of cortex with sharp boundaries where neurons are related in function as a consequence of having specific types of inputs and specific targets for outputs (Kaas, 1982). The prototypical example of an area is V1, also known as area 17 or striate cortex, which receives most of the output of the dorsal lateral geniculate nucleus of the visual thalamus, and provides activating visual inputs to other areas of cortex. Each row of cells in V1 deals with inputs corresponding to a specific location in visual space, and adjoining rows deal with adjoining locations in space so that the V1 of each cerebral hemisphere forms a map or representation of the contralateral visual hemifield. Other areas receive inputs from other sensory modalities, or from other cortical areas, and have outputs to other types of structures. This allows areas to have great flexibility in function, while relying on similar minicolumns as the basic computational unit. Most

importantly, the relatively simple computations of cortical minicolumns, can result in very complex outcomes, just by the process of reiteration. The outputs of any cortical area provide inputs to other cortical areas where the computational functions are repeated. This means that the more cortical areas there are, the more steps in processing are possible, resulting in more sophisticated computations. Mammals with large brains and large sheets of neocortex generally have more cortical areas and more variable and sophisticated behavior. Cortical evolution in mammals is largely characterized by modifications that change the functions of cortical areas, and by the addition of cortical areas, thereby adding steps to the information processing sequences.

A further feature of cortex that adds to its flexibility is the cortical column or module (Kaas, 1982). Many, perhaps all, cortical areas are not uniform in function. Relative to its surface, a cortical area may be divided into a tile-like or band-like pattern of alternating blocks of neurons of related, but slightly different functions. Sensory receptors, and the computations based on inputs from these receptors, create different functional classes of neurons at various levels of processing in the nervous system. The projections of these classes to other structures can be combined for various types of integration, or segregated for further processing. Modules, which include neurons across cortical layers, are one way of maintaining functional segregation. As an unusual (perhaps unique) example of modular segregation, the ancestors of the highly specialized monotreme, the duck-billed platypus, evolved electroreceptors in the skin of its nose (bill), which is also highly sensitive to touch. What to do with this new type of input? The platypus uses the somatosensory system for analyzing the electroreceptor input by dividing the structures for processing tactile inputs into modules for tactile receptor inputs and modules for electroreceptor inputs. Thus, the part of primary somatosensory cortex of the platypus that represents the bill is subdivided into alternating modules of neurons processing information form either electroreceptors or tactile receptors (Krubitzer *et al.*, 1995). The reorganized and subdivided somatosensory system can now be used to detect the electrical activity of the muscles of prey as the platypus feeds in the water. Mammals have evolved different types of modules within cortical areas in the process of acquiring new and expanded functions. This ability of cortex to be modified by forming modules is another reason why cortex has been so important in brain evolution.

As another sign of the ability of cortex to evolve in different ways, separate classes of input to a cortical area may be segregated in sublayers rather than in modules. For example, in primates, two classes of visual input, stemming from two classes of retinal ganglion cells (M and P), form segregated pathways to primary visual cortex, where they terminate either in the upper or lower half of layer 4, forming two functionally and morphologically distinct sublayers (Casagrande and Kaas, 1994). As another example, a different type of segregation occurs in the visual system of tree shrews, where the classes of retinal ganglion cells that respond to light onset (ON cells) or light offset (OFF cells) form segregated pathways that terminate in upper or lower sublayers of layer 4 of primary visual cortex (Norton *et al.*, 1985). Thus, different types of sublayers of the same layer can form in the same cortical area in different lines of mammalian evolution. Given this and other modes of modification, no wonder that neocortex has played such a critical role in mammalian evolution.

Before ending this section, we need to consider another variable feature of neocortex, the sensory representation. In some sense, cortical sensory areas constitute maps or representations of peripheral arrays of sensory receptors: the mechanoreceptors of the skin, the photoreceptors of the retina, and the row of sound sensitive hair cells of the inner ear. Mammals evolve different processing systems for these receptors by distributing them differently in the receptor sheet, and by providing some of them with proportionally more neurons in representations. Thus, behaviorally significant parts of receptor surfaces evolve greater numbers and densities of receptors, and by maintaining a set number of cortical neurons for each receptor, these parts of the receptor sheet activate larger parts of the cortical representations of the receptor sheets than do receptor surfaces with fewer receptors. Traditionally, this has been called the 'cortical magnification' of important sensory surfaces. Thus, the tongue and fingers are 'magnified' in the representation of body receptors in somatosensory cortex of humans, while the whiskers of the face are magnified in S1 of rats and mice. The auditory hair cells of echolocating bats that respond to the echo frequency have a large cortical magnification in primary auditory cortex, and many mammals have a magnified representation of the receptors of the central retina, used for detailed vision. The cortical processing machinery is reassigned in evolution as the distribution of receptors in the receptor sheet is changed to allow various behavioral specializations.

A related modification of the representations in cortex has been called 'afferent magnification' (Catania and Kaas, 1997) when some afferents, those with enhanced behavioral significance, gain more cortical space and cortical neurons than do others. Thus, when the receptors of the fovea of the retina, via their ganglion cells, project to the thalamus, they activate a larger cortical territory in V1 than predicted from the number of afferents (Azzopardi and Cowey, 1993). As another example, afferents from the behaviorally important 11th ray of the nose of the star-nosed mole activate more of primary somatosensory cortex than predicted from the number of afferents (Catania and Kaas, 1997). Thus, sensory systems can evolve to devote more cortical space and neurons for some inputs than for others in the same system. Both cortical magnification of receptor-dense sensory surfaces and afferent magnification of behaviorally important receptors and afferents have led to many well-recognized modifications of cortical representations (see Johnson, 1990).

In summary, we have outlined some of the major ways in which neocortex can be modified in the course of evolution to accommodate the behavioral specializations of various mammals. Examples indicate that these modifications do occur, but present understandings of brain organization in most mammals are too limited to allow an extensive survey of the brains of different species and a listing of derived features that have emerged as adaptations. Instead, this section of the review serves to remind us that neocortex is a uniquely organized but highly variable part of the mammalian brain, and suggests that this is the case because neocortex emerged in early mammals as an extremely flexible part of the brain where functions could be modified and extended in so many useful ways. Now we go on to a discussion of how neocortex in early mammals was probably organized, and then to some of the modifications that have occurred in some of the lines of descent. In doing this, we will briefly consider the implications of increasing the size of neocortex and cortical areas, something that has occurred repeatedly in cortical evolution. Even more briefly, we will note the implications of decreasing the size of the cortex from that of ancestors.

22.3 The Fossil Record: How Much Neocortex Did Early Mammals Have?

Mammals evolved from cynodonts, mammal-like reptiles, at least 230 Mya (Figure 1). The transition involved a loss of body mass, as early mammals were quite small, mostly shrew to mouse size, but occasionally cat size to coyote size (Hu *et al*., 2005; Kielan-Jaworowska *et al*., 2004). This small size dominated for over 150 million years, during which time the three major surviving lines of mammalian evolution emerged. The egg-laying Prototherian monotremes diverged very early from Therian mammals some 230 Mya, and the Metatherian marsupials diverged more recently from the Eutherian (placental) mammals some 180 Mya. Dental structures suggest that most early mammals were carnivorous and their small size indicates that they likely ate mostly insects. They may have foraged during the night, as most small mammals do today. Along with their small size, early mammals had small brains and little neocortex. Mammals maintained this body type until 65 Mya, when the massive dinosaur extinction took place, after which they rapidly diverged to occupy a diverse range of ecological niches via modifications in body size (see Falkowski *et al*., 2005) and form, including modifications in the brain. Today, there are over 4600 species of mammals. Here we briefly consider some of the fossil remains of some of the early mammals in order to develop some concept of what their brains were like. While there have been a number of publications describing these fossils, they have been collectively described in an extensive review (Kielan-Jaworowska *et al*., 2004).

Brain size varies with body size, as large mammals generally have larger brains than small mammals. Thus, comparisons of brain size across taxa to see if the brains have gotten bigger or smaller depend on comparisons of brain size of a given mammal to the predicted brain size for that mammal's body mass based on averages of mammals of given body masses (Jerison, 1973). For their body sizes, mammal-like cynodont reptiles had brains that were smaller than current mammals, and early stem mammals also had brains smaller than that predicted from body size (brain sizes were based on the size of the brain cavity in the skull). The reconstructed brain from a 85-million-year-old placental mammal is shown in Figure 5. The brain of this early mammal was obviously small. A hint of a rhinal fissure marks the transition from piriform (olfactory) cortex to neocortex. The high position of rhinal fissure indicates that neocortex was quite small. In addition, neocortex did not extend caudally to cover the midbrain, as it does in most extant mammals. The proportionally large olfactory bulb and piriform cortex indicate that much of the forebrain was olfactory in function, while the small neocortex apparently had a limited role in behavior.

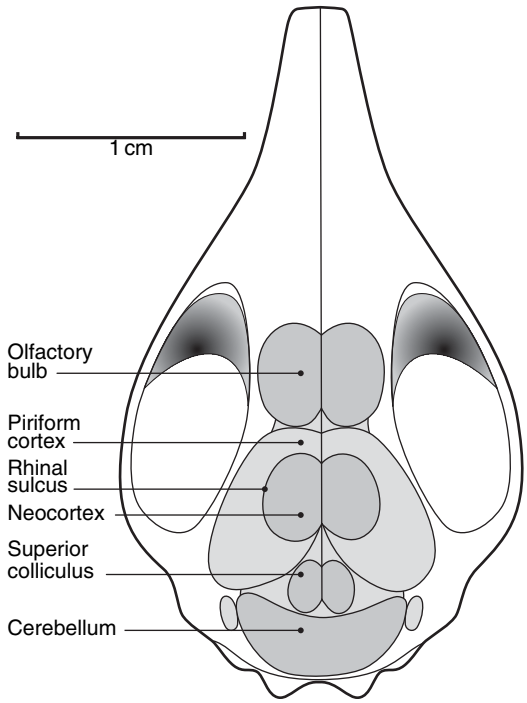

Figure 5 A reconstruction of the skull and brain (from the endocast) of an early (Late Cretaceous) Eutherian mammal (85 Mya). Primitive features of the brain include proportionally little neocortex compared to the olfactory bulbs and piriform cortex. The hemispheres are widely separated posteriorly, and they fail to cover the superior colliculus of the midbrain. Modified from Kielan-Jaworowska, Z., Cifelli, R. L., Luo, Z.-X. 2004. Mammals from the Age of Dinosaurs. Columbia University Press.

In summary, the fossil record provides some information about brain size in early mammals. Their brains were generally smaller for their size than the averages for current-day mammals, and they were not much different from the sizes of the mammal-like reptiles from which they emerged. Much of their forebrains were devoted to olfactory (piriform) cortex and the olfactory bulb. The suggestion of a rhinal fissure high on the lateral surface of some brain endocasts provides evidence that neocortex was proportionally very small.

22.4 How was the Neocortex of Early Mammals Subdivided into Functionally Distinct Areas?

As mentioned earlier, modern mammals are not necessarily completely modern. That is, some parts or features of their brains are likely to have been retained from ancient ancestors, while others have been greatly modified or created in more recent ancestors. It is said that evolution proceeds by tinkering, rather than by redesign (Jacob, 1977). Allman (1999) likened brain evolution to the process he noted when he visited a giant power plant of a major city. As greater and greater demands were placed over the years on the power plant, modifications and modernizations were needed to keep up with the demands. Yet, the power plant could never be shut down for a complete redesign. Thus, new control systems were added to old systems and a mixture of ancient to new features were integrated into one functional system. If we can identify the retained parts of brains in the brains of extant mammals, then we can reconstruct the major features of the brains of ancestors. The problem is how to do this. It was tempting for early investigators to infer that simple and undifferentiated brain features are the old ones retained from an ancient ancestor, while structurally complex and internally subdivided features are relatively new. This inference may generally work, as the assumption behind it seems logical, but it can lead to big mistakes, as the same (homologous) cortical area may range from highly differentiated to poorly differentiated in different extant mammals. Only the changes in the differentiation of the area, and not the area itself, differ in age. To avoid such mistakes, rules for reconstructing ancestral character traits have emerged (e.g., Brooks and McLennan, 1991) that assign widely shared traits among members of a clade (any group of mammals that have descended from a common ancestor) to a common ancestor. As members of any such group would have diverged from another at different times in the past and from different shared ancestors, each previous time of divergence is called a node in a cladogram, and characters that diverged from a node are compared, as well as characters inferred for a node from those of other previous nodes. By proceeding backwards in time, characters can be inferred for the last common ancestor of the clade. The optimization of the reconstruction, based on maximum parsimony criterion, can be a bit more complex, using downpass, uppass, and final optimizations (Cunningham *et al.*, 1998). In any case, the questionable assumption of simple-means-primitive is avoided.

Here we use the cladistic approach in a less formal way, largely because many brain features are difficult to identify without extensive investigation. The costs and time in investigation mean that few members of any clade have been well investigated. The difficulties in using a cladistic approach in studies of brain evolution, and the use of a truncated approach have been outlined elsewhere (Kaas, 2002). Here, we simply use the comparisons available to infer some of the major organizational features of neocortex of the first mammals.

A basic assumption of our truncated cladistic approach is that information about the organization of brains of extant mammals is not all equally useful. We know from the fossil record that early mammals had small brains with little neocortex. A number of current mammals, such as opossums, hedgehogs, tenrecs, and to a lesser extent, even rats and mice, have small brains with little neocortex, while several others, such as ourselves, have large brains with proportionally huge amounts of neocortex. Obviously, the large brains must have changed a lot, while the small brains may have changed relatively little. Thus, it should be easier to find the common features in small brains, if only because the needle is in a small haystack. Of course, more extensive cladistic comparisons are important, and they remain the ultimate test of any proposition. For instance, the small brains of echolocating bats have highly specialized (derived) areas of auditory cortex, and studying auditory cortex in these bats alone would lead to a highly misleading idea of how auditory cortex was organized in early mammals. Nevertheless, a productive approach seems to be to study small-brained mammals in as many of the major branches of the mammalian radiation as is practical, make inferences about the brains of early mammals from this data set, and then see how consistent these inferences are with what is more widely known about mammalian brains, including human brains.

We start our analysis by considering the organization of neocortex in the brains of members of four of the six major branches or superorders of the mammalian radiation (Figure 1). The earliest branch (230 Mya) with surviving members, the monotremes, is barely surviving as they are represented today by only two families, one genera of platypus and two of echidna. As these survivors are highly specialized in body form and brain organization as adaptations to unusual niches, we will return to them later. The marsupials or Metatherians, a line some 180 million years old, have been more successful, representing today about 7% of extant species in 16–18 families. They vary in brain size and shape, but most of the American opossums and Australian possums have small brains, with little neocortex, and the brains of some of these, especially the North American opossums, have been well studied. The highly varied superorder, Afrotheria, over 100 million years old, includes the very impressive and very large-brained elephants, but also the small-brained tenrecs, now almost completely restricted to the island of Madagascar. Tenrecs look like the reconstructions of stem mammals as if they stepped out of the distant past (Figure 6). Tenrecs were once

Figure 6 A photograph of the tenrec, *Echinops telfairi*, which is found in southwestern Madagascar. This small member of the Superorder, Afrotheria, retained many primitive characteristics in body form and appearance, so that it was previously classified with the insectivores of the Laurasiatherian superorder. Reconstructions of the appearance of early mammals look a lot like current day tenrecs. Reproduced from, figure 1, Krubitzer, L. A., Kunzle, H., Kaas, J. H. 1997. Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J. Comp. Neurol.* 379, 399 414, with permission from Wiley-Liss, Inc.

placed in the Insectivore order with hedgehogs and shrews, but molecular evidence indicates that they are only distantly related, and their many shared features are retentions of ancient mammalian features. Thus, their brains are small with very little neocortex. Another superorder that is nearly as old, Xenarthra, includes sloths, anteaters, and armadillos. These mammals do not have much neocortex, but little is known about how their neocortex is organized. However, somatosensory (S1) motor (M1), visual (V1 plus more temporal visual cortex) and auditory areas have been identified (e.g., Royce *et al.*, 1975). The highly varied members of the Laurasiatherian superorder include the large-brained whales and dolphins, as well as small-brained moles, shrews, and hedgehogs. As quite a bit is known about their brains, we will use them to represent the superorder. The other remaining superorder, Euarchontoglires, includes humans and other primates, and thus, they are of special interest. It also includes tree shrews and flying lemurs as our closest nonprimate relatives, and lagomorphs and rodents. As rats have rather small brains that have been intensively studied, they represent the superorder as we deduce common features across the superorders.

22.4.1 Primary Sensory Areas

The brain of the tenrec (Figures 7a and 7b) closely resembles that of early stem mammals (Figure 5) in

external appearance. In a dorsolateral view of the intact brain, a small cap of neocortex, marked by the shallow indentation of the rhinal 'sulcus', rests over the much larger olfactory brain, the piriform cortex, olfactory tubercle, and olfactory bulb. As with early mammals, olfactory processing dominated the forebrain. This is even more evident in the flattened brain. In tenrecs and other mammals,

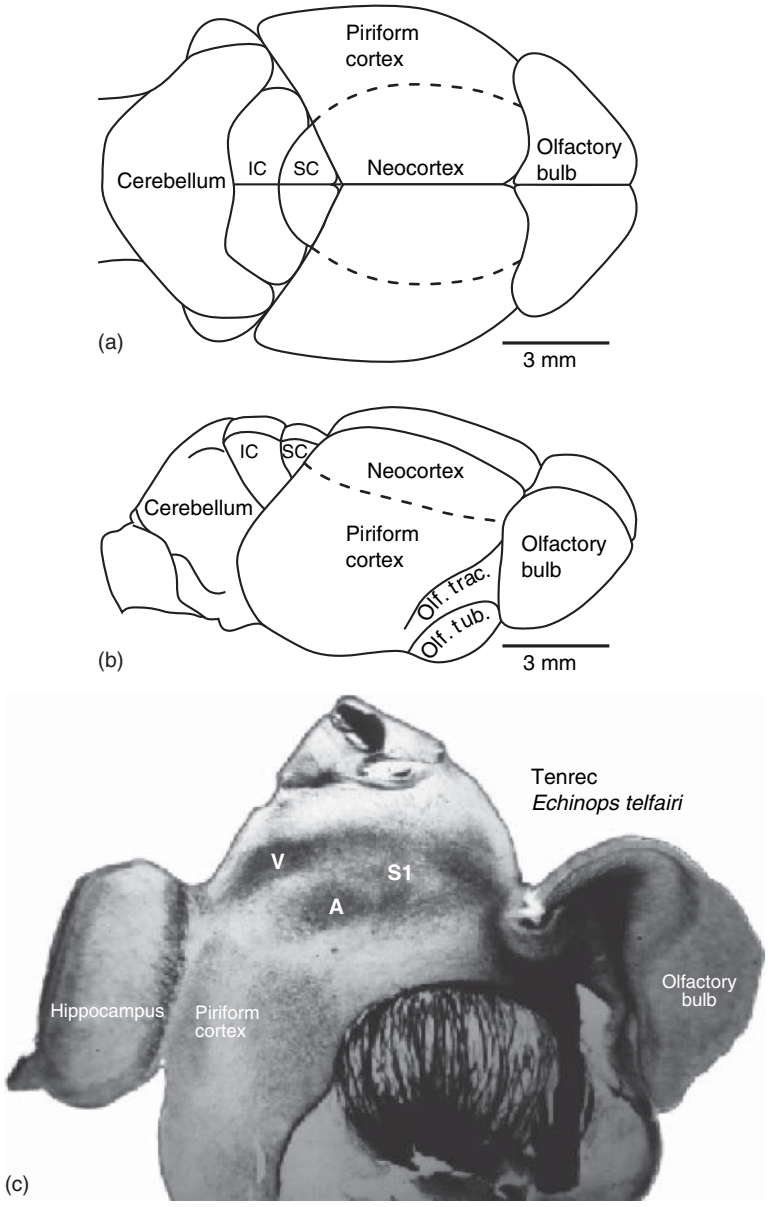

Figure 7 The brain of a Madagascan tenrec (*Echinops telfairi).* a, A dorsal view of the brain showing the small amount of neocortex relative to the large olfactory bulbs and piriform (olfactory) cortex. The small neocortex fails to cover the midbrain, as in the brains of early mammals (compare with Figure 5). b, A lateral view of the brain, showing the greater extent of piriform compared to neocortex. The line dividing neocortex from piriform cortex represents the rhinal sulcus, which is no more than a dimple in tenrecs. The olfactory tract (olf. trac.) and tubercle (olf. tub.) are apparent. c, A brain section stained for myelin that has been cut parallel to the surface after the cortex, hippocampus, and olfactory bulb have been separated from the rest of the brain and flattened. This preparation allows all of neocortex to be viewed as a single sheet, containing piriform cortex and the hippocampus. As in other mammals, the primary visual (V), auditory (A), and somatosensory (S1) areas stain darkly for myelin and are easily identified. Other areas of neocortex of tenrecs are shown in Figure 8b. At the top of the figure, neocortex of the medial wall of the cerebral hemisphere has been unfolded, and the fornix, a bundle of axons, is very dark, while the corpus callosum, a bundle of axons connecting the hemispheres, is less so. Next to the corpus callosum, the cingulate (limbic) cortex is lightly stained. The myelin dense region rostral to S1 may be primary motor cortex, M1. IC, inferior colliculus; SC, superior colliculus. Adapted from, figures 2 and 8, Krubitzer, L. A., Kunzle, H., Kaas, J. H. 1997. Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J. Comp. Neurol.* 379, 399 414, with permission from Wiley-Liss, Inc.

it is possible to separate the cortical sheet from the underlying basal ganglia and thalamus, flatten the whole sheet, cut it into thin sections parallel to the cortical surface, and stain these sections to reveal architectonically distinct subdivisions. For the small tenrec brain, it is relatively easy to also include the septal region below the corpus callosum on the medial wall of each hemisphere, the unfolded hippocampus, and the olfactory cortex and olfactory bulb (Figure 7c). In such sections, it is apparent that olfactory cortex is three or more times larger than neocortex, and that both the hippocampus and the olfactory bulb are nearly as large as neocortex. In a section stained for myelin, four regions stain darkly. One is primary visual cortex, V1 or area 17, another is primary somatosensory cortex, S1, or in primates, area 3b (Kaas, 1983). A third region is primary auditory cortex, and the fourth in frontal cortex may demarcate primary motor cortex, M1.

We find three of these areas of tenrecs, V1, S1, and A1, in opossums, hedgehogs, and rats (Figure 8), representing three other superorders. Indeed, these fields appear to exist in all examined mammals, with the possible exception for V1 of mammals with no functional object vision. Yet, even in the subterranean 'blind' mole rat, a small 'visual' area may be architectonically apparent (Cooper *et al*., 1993), although involved in nonvisual functions. These three sensory areas may be not only early areas present in the first mammals, but also areas that emerge early in the development of cortex, possibly to organize the overall arrangement of other later developing areas in all mammals (Krubitzer and Kaas, 2005). Thus, V1, S1, and A1 may have been retained in extant mammals as necessary components of the developmental plan for neocortex. These three areas of cortex can be identified by a number of features, including direct

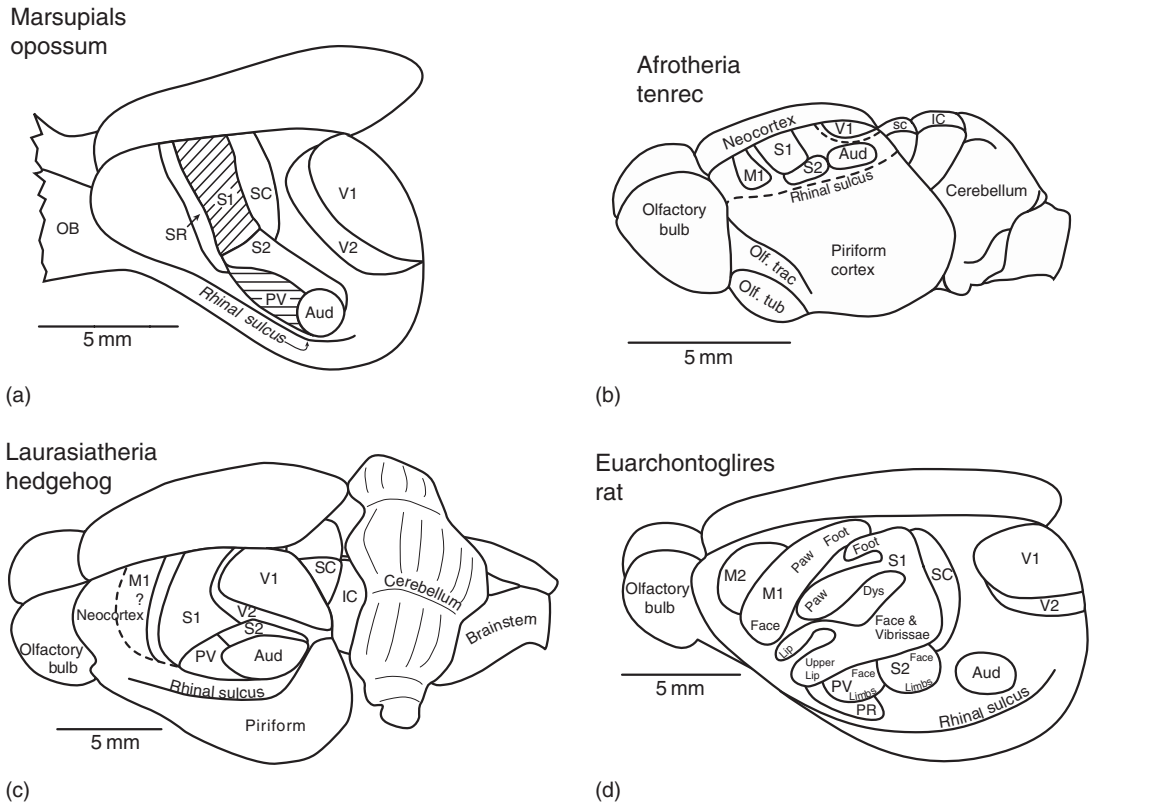

Figure 8 Dorsolateral views of the brains representing small-brained members of four of the major superclades of mammals. In each of these four brains, only a few areas of neocortex have been identified, and most of these are present in all four brains. All have primary and secondary visual (V1 and V2) and somatosensory (S1 and S2) areas, as well as a primary auditory area (Aud). The parietal ventral (PV) somatosensory area has been identified in three of these brains, but was not detected in tenrecs. Somatosensory fringe areas rostral (SR) and caudal (CR) to S1 may exist in all these mammals. A primary motor area, M1 has been identified in many placental mammals, but not in opossums. The presence of some areas in members of all four clades suggests that these areas were present in a common ancestor. a, The brain of a North American opossum (see Beck *et al*., 1996, for details). b, The brain of a tenrec (see Krubitzer *et al*., 1997, for details). c, The brain of a hedgehog (see Catania *et al*., 2000). d, The brain of a rat (see Remple *et al*., 2003). In (c) and (b), the brainstem is attached and the superior colliculus (SC) and the inferior colliculus (IC) both are apparent, as neocortex is so small that it fails to cover them. Adapted from Kaas, J. H. 2004. Evolution of Somatosensory and Motor Cortex in Primates. *Anat. Rec. Part A* 281A, 1148 1156, with permission from Wiley-Liss, Inc.

inputs from specific 'relay' nuclei of the thalamus, orderly representations of the corresponding receptor sheet in a characteristic manner, and a 'sensory' type of histological structure. At least one of these fields emerged in cortex well before the advent of mammals, as a dorsal lateral geniculate nucleus with inputs from the retina has been identified as projecting to a 'V1' of dorsal cortex of reptiles (Hall and Ebner, 1970). Auditory and somatosensory relay nuclei also exist in the thalamus of reptiles, but they appear to have largely subcortical (basal ganglia) targets. However, a small somatosensory region of dorsal cortex has been described in turtles (Figure 2). Cortical targets for the auditory thalamus apparently emerged with or directly before the first mammals.

The existence of a fourth area of neocortex of tenrecs, the primary motor area (M1), in the stem mammals remains unsettled. M1 has been identified in all placental (Eutherian) mammals that have been appropriately studied, so it is nearly certain that the common ancestor of all placental mammals had an M1. However, the evidence for M1 in marsupials and monotremes is uneven and a bit confusing. In placental mammals, M1 is located rostral to S1, with a narrow strip of somatosensory cortex separating the two (see below). In contrast, there is no convincing evidence of an M1 in marsupials (see Beck *et al*., 1996). M1 is usually identified by electrically stimulating cortex, as this will evoke movements that vary in type depending upon which part of M1 is stimulated. Hindlimb movements are evoked medially in the M1 strip; forelimb movements in the middle, and face, vibrissae, and tongue movements are evoked laterally in the M1 strip. In opossums (e.g., Beck *et al*., 1996; Frost *et al*., 2000), no movements could be evoked from the expected region of M1, although some movements were evoked by electrically stimulating S1, as in placental mammals. In addition, M1 can also be identified by architectonic characteristics that reflect the major function of M1 in motor control. Specifically, M1 does not have a distinct layer 4 of cortex, which is well developed in sensory areas, and it has at least a somewhat larger layer 5 containing large pyramidal neurons, as these are the motor output neurons to the brainstem and spinal cord. In opossums and other examined marsupials, there is no architectonic evidence for M1. Finally, patterns of connections can help identify M1. In Eutherian mammals, M1 receives somatosensory inputs from several somatosensory areas, as such sensory inputs are necessary to guide motor control. In opossums, such areas as S2 do not project to the expected location of M1. More significantly, M1 pyramidal cells project to motor neuron pools in the brainstem and spinal cord. In opossums, none of the cortex in the expected location of M1 projects to the spinal cord (Nudo and Masterton, 1990). While a small number of neurons in S1 and the second somatosensory area, S2, do project to the spinal cord, they terminate in the dorsal sensory part of the spinal cord, as do corticospinal projections from S1 in placental mammals, rather than in the motor neuron groups in the ventral spinal cord, as M1 projections do in placental mammals. Thus, it appears that opossums do not have a motor cortex, and whatever weak motor control cortex has on guiding motor behavior, it comes from somatosensory cortex. This lack of motor cortex is thought to account for the relative lack of skilled forelimb movements in opossums compared to rats (Ivanco *et al*., 1996).

A remaining puzzle is that opossums do appear to have a motor thalamus, a ventrolateral complex, VL, as defined by inputs from the deep cerebellar nuclei (Walsh and Ebner, 1973). VL projects to motor cortex in placental mammals, but its target in opossums appears to be S1 (Killackey and Ebner, 1973). One theory for the evolution of M1 is that M1 differentiated out of S1 (Frost *et al*., 2000; Lende, 1963), but there is no clear evidence for this, and M1 could have emerged in some other way. Another intriguing possibility that further study could rule out or verify, is that some of the marsupials with larger brains and better motor skills have independently evolved a motor cortex. In support of this speculation, the projections from the motor thalamus (VL) in some marsupials, appear to include some cortex rostral to S1 (e.g., Haight and Neylon, 1981).

Given that there is no evidence for a motor area in dorsal cortex of reptiles, and apparently no motor cortex in marsupials, together with the assumption that once evolved, a motor cortex would be too useful to abandon, a reasonable inference is that motor cortex evolved in the stem placental mammals after they diverged from marsupials. If so, monotremes should not have any motor cortex. However, this is presently uncertain. There is some evidence in both echidna and platypus that movements can be evoked in a region of cortex rostral to S1, and this cortex has architectonic features suggestive of M1 (see Krubitzer *et al*., 1995). It would be useful but probably difficult (because of limited opportunities for study) to obtain definitive evidence for M1 in monotremes. If M1 is present, we need to consider the possibility that M1 emerged with early mammals and was somehow lost in at least some marsupials. For now, the scenario that M1 evolved later with placental mammals seems more likely. If so, it is important to remember that neocortex still participated in motor control in

non-placental mammals. The somatosensory areas are more sensory than motor, but they do have motor functions.

22.4.2 Other Cortical Areas

What other subdivisions of neocortex were likely present in stem mammals? In all appropriately studied mammals, a second somatosensory area, S2, has been described as adjoining S1 on its lateral border (see Beck *et al*., 1996). S2 gets somatosensory inputs from S1, receives additional somatosensory inputs from the somatosensory thalamus, and projects to nearby sectors of somatosensory cortex and, in placentals, to motor cortex. S2 appears to be a component of neocortex in all mammals. An adjoining oval of cortex, the parietal ventral area, has been identified as present in a range of mammals, including many placentals (Krubitzer *et al*., 1986), marsupial opossums (Beck *et al*., 1996), and monotremes (Krubitzer *et al*., 1995). However, parietal ventral (PV) somatosensory area has not always been found, even after a detailed exploration of the appropriate region of cortex (Frost *et al*., 2000). PV closely resembles S2 in connections and architectonic features, so that it can be difficult to distinguish from S2. However, PV does have a separate representation of the contralateral body receptor sheet that mirrors that of S2, and evidence of two mirror-image representations provides clear evidence for PV. The evidence for PV in some species of the three major branches of mammalian evolution suggests that PV evolved in the early stem mammals, and has either been difficult to detect, or has been lost in some small-brained mammals (this is discussed in the following).

In mammals that have been tested, S1 projects to a narrow strip of cortex just rostral and just caudal to S1, as well as to S2 and PV (see Beck *et al*., 1996). These two strips of cortex appear by connections to be somatosensory in function, although they may be multisensory as well (Wallace *et al*., 2004). In either case, early mammals likely had four or five somatosensory areas, as well as perirhinal cortex with S2 inputs that would serve as a relay to the hippocampus.

In regard to visual cortex, most mammals appear to have several visual areas. With a few known exceptions, all carefully studied mammals have demonstrated a second visual area, V2 (Kaas and Krubitzer, 1991; Rosa and Krubitzer, 1999). V2 constitutes a visual area that borders V1 laterally (or rostrally in primates) along the representation of the vertical line of decussation of the retina (the vertical meridian through the center of gaze) of V1, and forms a smaller, mirror image of the representation of the contralateral visual hemifield that is in V1. Some investigators have interpreted the region of V2 of rats as consisting of a row of smaller visual areas (e.g., Montero, 1999), but this interpretation appears to stem from confusing modules within V2 as separate visual areas (Kaas *et al*. 1989). In many mammals, V2 is not homogeneous in function, but contains a row of patches or bands along its length that alternatively have somewhat different connections and functions. In a few other mammals, V2 appears to be absent. Thus, there is no evidence for V2 in the 'blind' mole rat (Cooper *et al*., 1993). In mammals with little visual function, V2 and other visual areas were likely lost in evolution. In addition, the least shrew (*Cryptotus parva*) has no V2 (Catania *et al*., 1999). Instead, V1 directly adjoins S1, leaving no space for V2. However, the 4–5 g adult least shrew is one of the smallest of mammals, probably near the limit in small size for mammals, and it may have lost V2 in evolution to allow other areas, such as V1 and S1, to remain large enough to preserve functions in its small brain. As cortical areas became smaller, they do so by having fewer neurons, and below some number of neurons, the functions of areas cannot be maintained. As the extremely wide distribution of V2 across mammalian taxa indicates that V2 evolved very early with or before the first mammals, the evidence for the absence of V2 in some mammals argues that cortical areas can be lost as well as gained.

By determining the connections of V1 and V2, other cortical areas with visual input can be identified. In a range of placental and marsupial mammals, V1 demonstrates connections with cortex just medial to V1 and just lateral to V2 (e.g., Martinich *et al*., 2000). Both the medial and lateral regions may contain one or more visual areas, and presumptive fields in these regions have been identified with different names by various investigators. A small region medial to V1 has been called prostriata in primates and the splenial visual region in other mammals (Rosa *et al*., 1997). Prostriata likely existed as a visual area in early mammals, as did one or more small visual areas lateral to V2. As a conservative estimate, early mammals had V1, V2, a prostriata, and a small visual area in temporal cortex for a minimum of four areas, but possibly five or six.

All mammals examined have a region of auditory cortex that is typically located in temporal cortex caudal to S1 and ventral to V2. Much of this auditory region has been characterized as primary auditory cortex, A1, on the basis of several criteria, including architectonic characteristics of sensory cortex,

auditory inputs from the principle (ventral) nucleus of the medial geniculate complex, and often, physiological evidence, not only for neurons responsive to auditory stimuli, but for a systematic representation of tone frequencies across the taxa (a tonotopic representation). A problem here in comparing species is that many taxa have more than one area that has some of the characteristics of primary auditory cortex. It is likely that across species different areas have sometimes been identified as the primary auditory area, A1, a field first identified in cats (see Kaas, 2005). A survey of studies on auditory cortex suggests that an area that represents tones from high to low frequencies in a rostrocaudal dimension across the field, as in cats, is present in a number of other studied placental mammals, and in opossums. This A1 may have been present in early mammals. But, the evidence for another primary-like anterior field, with a reversed order of tonotopic organization is also common, as well as for a bordering fringe of two or more secondary fields. From this evidence, it seems likely that early mammals had at least one primary auditory field, A1, and perhaps two, with a bordering belt of two or more secondary fields, for a total of three to five auditory areas. At least one of these fields may also have responded to somatosensory stimuli.

As a part of a system that is devoted to evaluating the quality of food, early mammals are expected to have one or more taste areas in neocortex. Comparative data are not extensive enough to allow this conclusion, but rodents (e.g., Sugita and Shiba, 2005) and monkeys (see Kaas *et al*., 2006) appear to involve both the tongue representation of primary somatosensory cortex, S1, and a laterally adjacent 'insular' region of cortex in processing gustatory information. This information in turn is relayed to a portion of orbital frontal cortex for an evaluation of the hedonistic properties of the ingested food. While there are great uncertainties, it seems reasonable to postulate that part of S1 as well as neurons lateral to S1 in dysgranular 'insular' cortex were involved in taste in early mammals, and the reward value of food objects was processed further in a orbital frontal area. In primates, the insula is an island of cortex in the depth of the lateral sulcus. The term is used here for the equivalent region of cortex in mammals without a lateral sulcus.

As with taste, there have been few comparative studies of the involvement of neocortex in nociception (pain) and temperature perception. Evidence from primates, cats, rabbits, and rodents implicates S1 and thus other somatosensory areas in at least the sensory-discriminative component of pain, while a region of anterior cingulate cortex on the medial wall of the cerebral hemisphere appears to be important in the affective-motivational component of pain (e.g., Johansen *et al*., 2001; Treede *et al*., 1999). A portion of 'insular' cortex lateral to S1 and S2 may be specialized for the affective component of pain. The same or a similarly located region of insular cortex may be important in processing thermal stimuli (e.g., Davis *et al*., 2004). What is not known is the extent to which pain and temperature depended on subcortical rather than cortical processing in early mammals, but the possibility or even likelihood of specialized cortical areas in insular cortex and/or cingulate cortex remains.

In regard to cingulate cortex of early mammals, current theory holds that this limbic cortex has perhaps four subdivisions, at least in some mammals (Vogt, 2005). Anterior cingulate cortex is involved in the important and basic functions of fear and avoidance behaviors, middle and posterior cingulate areas are sensory in some sense having to do with body and spatial orientation and somatosensory and visual functions. The retrosplenial region, adjacent to the splenium of the corpus callosum, is linked to the hippocampus and is thought to be involved in memory processing. Indeed, all of cingulate cortex appears to be involved in memory via associations with the hippocampus involving thalamic connections and cortical connections. Architectonic evidence for subdivisions of cingulate cortex have been described in a number of mammalian taxa, and nuclei of the anterior thalamus which project to these divisions have been recognized in the common laboratory mammals (Jones, 1985). Overall, more comparative study is needed, but the evidence supports the conclusion that the medial wall of the cerebral hemisphere of early mammals contained three to four, and possibly more, functionally distinct areas.

As noted above, cortex lateral to S1 includes S2 and other areas generally referred to as insular cortex. The insular region adjoins perirhinal cortex, the cortex along the rhinal sulcus. Perirhinal cortex appears to receive afferents from secondary somatosensory and visual areas, and have a role in fear-potentiated startle, via projections to the amygdala (Rosen *et al*., 1992), and memory via the hippocampus (Lin *et al*., 2000). A similar involvement of a perirhinal area in startle and memory may have characterized the neocortex of early mammals.

Finally, the significance of an orbital frontal area or region of cortex in taste has been mentioned, and this and other subdivisions of frontal cortex have been considered to be fundamental components of mammal brains (Preuss, 1995b; Uylings *et al*., 2003), although there are uncertainties about how to identify areas, and how to recognize them across species. As a

result, a broad comparative appreciation of the subdivisions of frontal cortex is lacking. Nevertheless, it seems reasonable to postulate that early mammals had two to four subdivisions of frontal cortex, including one or more divisions of orbital frontal cortex.

In summary, the fossil record indicates that early mammals had small brains with little neocortex. Comparative evidence from extant mammals suggests that this cortex was already subdivided into a considerable number of functionally distinct areas, including four or more visual areas, four or more somatosensory areas, two to three auditory areas, possibly a taste area separate from S1, two to four areas of frontal cortex, one or more perirhinal areas, and three or four cingulate areas. This produces an estimate of 17–21 cortical fields, and this could be somewhat of an underestimate. However, it seems unlikely that early mammals had more than 30 fields or many less than 17. As early mammals had little neocortex, perhaps 150–200 mm^2 per hemisphere, cortical areas would have been very small, possibly averaging about 10 mm^2 in surface area, with some areas being larger (e.g., S1) and others considerably smaller (e.g., S2). As areas were quite small, they may not have been subdivided into different classes of modules. Alternatively, a few areas such as V2 may have already been modular. However, more comparative evidence is needed to address these speculations. Finally, cortical areas differed in architecture (patterns of cell arrangements and other structural features), but the differences were not marked. The different functions of cortical areas depended more on connections, the inputs and outputs, than on specializations of areas in cellular and laminar structure.

22.5 What Happened to Neocortex in the Radiation of Mammals?

The short answer to the question above is 'different things' – but sometimes very little. As discussed above, the brains of some mammals appear not to have changed very much over the course of 230 million years. Opossums, hedgehogs, shrews, tenrecs, armadillos, and even rats and mice have retained small brains with relatively little neocortex. This cortex remains relatively undifferentiated in structure and cell types. Neocortex remains divided into a few areas, most or all of which were present in the first mammals. The placental mammals, hedgehogs, shrews, tenrecs, rats and mice, have added a cortical motor area, M1, rats and mice have differentiated a 'barrel field' of barrel-shaped modules representing individual whiskers of the face in primary somatosensory cortex, the smallest of shrews lost V2, and so on. But these are rather minor changes. Clearly, environmental niches can be found where brainpower is less important than reproductive capacity and other factors, and ancestral brains did not need to be changed very much.

22.5.1 Impressive Modifications of Small Brains

Even in small-brained mammals, several rather remarkable modifications of neocortex have occurred. To mention only a few, the brains of echolocating bats have specialized, without an expansion of cortex, to facilitate the tasks of flying and echolocating. The echolocating bats have an altered auditory system that over-represents the echo frequency and have specialized several auditory areas of cortex that perform computations based on echoes to locate and identify flying prey and avoid objects (Suga, 1995). The somatosensory system of bats has been modified (Calford *et al.*, 1985) for flying by representing the specialized sensory receptors, the Haarscheiben or touch domes with a protruding hair, on the wing and other parts of the body so that flight can be guided. Without these receptors, bats tumble and fall (J. M. Zook, personal communication). Some rodents and squirrels have emphasized vision by enlarging the visual midbrain structure, the superior colliculus, to 10 times its size in other rodents, and differentiating it structurally into more prominent layers and cell types (Kaas and Collins, 2001), while also adding, expanding, and differentiating subdivisions of visual cortex (Kaas *et al.*, 1989). The star-nosed mole has devoted much of its cortex to three large representations of the mechanosensory receptor structures, called Eimer's organs, that are tightly packed on the fleshy appendages of its nose (Catania and Kaas, 1997). This specialization allows the mole to detect and consume small prey at a rate that exceeds that of any other mammal (Catania and Remple, 2005). The duck-billed platypus blindly searches for prey with its eyes and ears shut in murky water by using sensitive tactile and electroreceptors on its rubbery bill (Krubitzer, 1998). To successfully occupy this niche, the platypus depends on a greatly modified neocortex that is dominated by several large representations of the receptors of its bill. The platypus devotes little cortical territory to visual or auditory cortex (Krubitzer *et al.*, 1995). Such specializations in even small-brained mammals indicate the great flexibility of neocortex as a computational structure. The vertical rows of neurons, modules composed of rows of neurons, and areas composed of modules in neocortex

can be reassigned in evolution to various tasks as needs arise. Similar modifications are seen in mammals that have also enlarged their brains, and modified them in other ways. For example, one can marvel at the great skill that raccoons have in blindly locating food items in water. Using their hands, they rapidly locate the source of tiny ripples and currents that are created by moving objects. This ability is made possible by extremely expanded representations in several areas of neocortex of the receptors of the hand (Welker and Seidenstein, 1959). Finally, it is hard not to be impressed with cebus monkeys, as we are members of a clade of primates without tails. Cebus monkeys use receptors on the glabrous pad of the tip of their tail to actively explore their environment and use their tail to retrieve objects of interest. This ability, of course, depends on devoting large portions of somatosensory areas of cortex to the tactile receptors of the tail (Felleman *et al*., 1983).

22.6 The Implications of Changes in Brain Size

Brains also change in other ways. One way that was obvious to early investigators such as Smith (1906) and Clark (1959) was that brains vary in size from very small to very large. Part of this variation, for uncertain reasons, is related to body size, so that brains tend to increase in size by an average factor of ~0.75 with increases in body size (Allman, 1999; Jerison, 1973). Such increases in brain size result typically in disproportionately large expansions of neocortex (Finlay *et al*., 2001), but such increases in neocortical size often do not seem to correlate with the acquisition of notably new abilities. For example, the behavior of lions, with much larger brains, do not seem to be remarkably different from those of domestic cats. Thus, we suspect that closely related mammals that are large or small might have brains with similar organizations, although the brains differ in size (this assumption needs careful evaluation). For example, the arrangement of sensory areas of neocortex seem to be roughly the same in the smaller brains of guinea pigs than the larger brains of capybaras (Campos and Welker, 1976), both related South American rodents. Yet, this cannot be completely true, as brains have a basic scaling problem. Large brains cannot simply be large versions of small brains, because the computational unit of the brain, the neuron, does not scale to large sizes with the brain, as the functions of neurons depend on their size (Bekkers and Stevens, 1970). Obviously, transmission times increase as the dendrites and axons of neurons get longer, unless they are modified by making them thicker, and in other ways. Neurons with longer, thicker axons result in brains that devote more of their volume to axons than the computational parts of axon terminations, dendrites, and cell bodies. As the cell bodies and dendrites of neurons do not vary much in size, larger brains are larger, in the main part, because they have more neurons and even more supporting glial cells. Having more neurons means that each neuron, while maintaining roughly the same number of contacts with other neurons, contacts a smaller proportion of the total number of neurons. This changes the organization of the processing network. To evolve large brains, these scaling problems can be reduced or solved in several ways, but mainly via increases in modular organization and local processing that decreases the need for long connections (Kaas, 2000b). To achieve this solution, large brains with much neocortex should have more cortical areas, and more functional subdivisions of areas into different types of modules, than small brains with little neocortex. There is much evidence to support this premise when species are considered across taxa, but not when one considers closely related members of a taxonomic group of different brain sizes. These later mammals may add neurons and disproportionately axon volume to neocortex with increases in brain size, while maintaining the basic cortical organization of the group. Without organizational adjustments, larger brains may just maintain brain functions, without appreciable gains in functions.

Another problem emerges if one considers the consequences of increasing the size of neocortex without adding modules or areas. If bigger brains are simply expanded versions of smaller brains, then the cortical areas must be bigger. Specific areas do vary greatly in size. V1, for example, is 700 times larger in surface area in a human than a mouse, and the cortex in humans is over twice as thick as in mice. This means that V1 in humans has many more neurons. The functions of cortical areas must, in part, depend on their sizes. Of course, one limit on any reduction in the size of a cortical area is that it must have enough neurons to perform its function. Cooper *et al*. (1993) have previously shown that the small sliver of primary visual cortex that is found in the 'blind' mole rat is too small to allow even a crude image. Thus, this cortex has become nonfunctional, at least for the purpose of object vision. Somewhat larger visual areas, with more neurons, may mediate object vision, but the pixels would be large and the image would not have much detail. This is largely due to the scope of the dendritic trees of neurons as they gather information over some limited portion of cortical area. As areas further increase in size, the

scope of the dendritic window gets proportionally smaller, and the area becomes more specialized for detailed vision at the cost of global vision. However, neurons are also influenced by thalamic inputs that terminate outside their dendritic arbors, via the horizontal intrinsic connections of neurons activated by those inputs. In V1 of a mouse, these short (~1 mm) intrinsic connections can tie all parts of V1 together so that the output of V1 can reflect global processing and directly mediate useful vision, but as V1 gets bigger and bigger, the outputs of individual neurons reflect less and less of what is happening in the total visual scene. Thus, the outputs of a large V1, as in macaque monkeys or humans, provide important details about a visual image, but not enough global information about the visual scene to guide most visual behavior. Other smaller visual areas would seem to need to make sense from the outputs of V1. A macaque monkey with V1 intact, but other visual areas missing, should be virtually blind (this has been difficult to test; see Nakamura and Mishkin, 1986). This line of reasoning suggests that in mammals with large amounts of cortex only a few cortical areas should be large. This general supposition seems well supported. Indeed, even V1 in large-brained mammals is not as large as it would be if it maintained a constant proportion of neocortex. Thus, V1 occupies proportionally more of neocortex in the smaller macaque brains than in the larger human brains (Kaas, 2000a).

The limited variability of neuron size and dendritic arbor size relative to the greater variability of brain size has the added implication that it is easier to change the functions of small cortical areas by adjusting dendritic arbor size than those of large cortical areas (Kaas, 2000b). In brief, increasing or decreasing the scope of the arbors in small areas has more impact on the sizes of receptive fields, and on the nature of processing from regional to global. Thus, pyramidal neurons in large areas may have smaller dendritic arbors than neurons in smaller areas (Elston *et al.*, 1996), as larger arbors would not enlarge receptive field sizes enough to alter functions in a significant way.

22.6.1 Larger Brains Often Have More Areas

We have discussed the possibility that in closely related species of different body size, the larger species with larger brains may not differ very much in terms of number of areas. However, when comparisons are made more extensively across mammalian taxa, brains do vary in number of areas, and larger brains tend to have more areas. While early investigators, such as Brodmann (1909), came to this same conclusion by studying cortical architecture in many different mammals, the evidence was not very strong as areas were subjectively defined and identified by subtle differences in histological appearance. More recently, it has been possible to define areas with more certainty by using the multiple criteria of architectonics, connectional, physiological, and gene expression differences. Unfortunately, such studies require a huge experimental effort, and results are more credible if verified by several research groups. It is fair to conclude that all of the cortical areas have not been defined with a high degree of certainty in any mammal, and for most taxa, very little is reliably known about how cortex is subdivided (see Kaas, 2005). Yet, it is clear from the results based on a few well-studied species that the number of areas is quite variable across species. For example, it is easy to see from only the shapes of the brains that squirrels and tree shrews have devoted proportionally more of their cortex to vision, as the visual occipital and temporal regions of neocortex are expanded over frontal motor, somatosensory, and prefrontal parts (Figure 9). Moreover, we know from previous studies that hedgehogs have few visual areas, perhaps four (Figure 8). While the full number of visual areas in either squirrels or tree shrews has not been determined, the number is certainly more than four. For squirrels, seven visual areas have been proposed, while nine have been described in tree shrews (Kaas, 2002). As these two mammals of about the same size are not closely related (Figure 1), more visual areas and proportionally more visual cortex

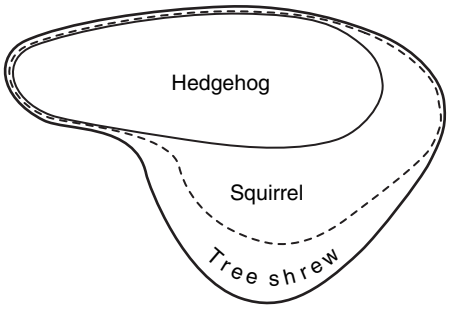

Figure 9 Differences in the shapes of brains suggest changes in function. Here, lateral views of the neocortex of a hedgehog, squirrel, and tree shrew are outlined. The brains have been scaled so that frontal cortex on the left is the same size to emphasize the differences in shape of temporal (ventral) and occipital (posterior) cortex. The highly visual squirrel and tree shrew have greatly expanded temporal and occipital regions of neocortex, and these regions are largely visual in functions. This trait is more pronounced in tree shrews than squirrels. These observations on extant mammals provide a rationale for deducing functional specialization of the brains of extinct mammals from the shapes revealed by skull endocasts.

evolved independently in both lines of descent. Domestic cats have been extensively studied, and they appear to have at least ten visual areas (see Grant and Shipp, 1991, for review). While there is uncertainty about how to divide visual cortex in macaque monkeys, recent proposals number over 30 visual areas (Felleman and Van Essen, 1991). The total number of visual areas in human brains is unknown, but clearly the number is large, as some estimates of total number of cortical areas place the number in the range of 150, possibly 10 times the number present in the first mammals.

Having more areas allows more sophisticated processing via the reiteration process, but also by increasing the number of parallel streams of processing (Kaas, 1989). Another important consequence is that the total amount of wiring (connections) in the cortex is decreased due to greater emphasis on regional processing (Mitchison, 1991). Finally, in large brains, the two hemispheres become less symmetrical in organization, so that areas in one hemisphere no longer have mirror-image counterparts in the other hemisphere. This effectively increases the number of areas while reducing the need for connections between the hemispheres via long axons that cross in the corpus callosum to connect matched pairs of areas (Ringo *et al*., 1994).

While we presently know far too little about how cortical areas are subdivided into classes of modules, we know that this does occur in some brain areas (see Purvis *et al*., 1992; Catania, 2002), and that the same area may be subdivided in different ways in different species. Monkeys have a V2 that is divided into repeating blocks of three types of band-like modules with different connections, architecture, and neural functions (Roe, 2003). In some mammals, including tree shrews and opossums, V2 is subdivided in another manner into modules with and without interhemispheric connections (Cusick and Kaas, 1986). Such modularity adds to the ways neocortex can vary across taxa, and provides a mechanism for grouping types of neurons that need to work together, thereby reducing the connection problem.

22.7 Summary and Conclusions

22.7.1 Neocortex Varies in Size and Complexity

An overview of the sizes and parts of brains of extant (living) mammals indicates that neocortex varies most in size and complexity relative to the rest of the brain. This indicates that neocortex is an important part of the brain for further study if one is interested in brain evolution. Such studies reveal that neocortex varies in many ways across taxonomic groups. Variations include those in morphological types of neurons, morphological specializations of the six layers that characterize cortex, types of modules that subdivide cortical areas into smaller functional units, proportions of cortical areas that are devoted to specific inputs, absolute size of areas, the size of areas relative to neocortex, intrinsic connections of areas, inputs and output targets of areas, number of areas, types and proportions of modulating inputs, the extent and distribution of interhemispheric connections, and so on. The great variability of these features suggests why neocortex has held center stage in studies of brain evolution in mammals. Neocortex varies in so many ways, allowing so many different adaptations to the environment.

22.7.2 Shared Features of Neocortex Organization Across Species Suggest Why Neocortex Is So Modifiable and So Important

The fundamental unit of computation is the vertical array or column of 100–200 neurons that are tightly interconnected, and driven by only a few specific inputs, while modulated and influenced by many other inputs. The grouping of neurons by functional role into layers, modules, and areas simplifies the process of modulating neuron responses by the responses of the most relevant other neurons via direct and indirect connections, as these neurons are nearby. The computations within each column transform the inputs to one or more different types of output, which can be sent to other cortical columns so that the computation process can be repeated with the addition of other inputs. Multiple cortical areas allow cortex to function in serial steps that transform simple computations into complex outcomes, and produce parallel streams that allow information to be used in many different ways.

22.7.3 The Fossil Record Indicates that Early Mammals Had Small Brains with Little Neocortex

The olfactory bulb and olfactory (piriform) cortex were relatively large, indicating olfaction was an important source of information about the external world. The small amount of neocortex suggested it played a modest role in regulating behavior. Some mammals with small brains and little neocortex have persisted up to present times, indicating that behavioral niches remain for mammals with limited brainpower. Perhaps because early mammals may have had brains close to the lower limit in size for mammals, few subsequent lines of evolution lead to

smaller brains. Instead, the fossil record indicates that increases in brain size, especially that of neocortex, occurred independently many times over, while some mammals with small brains continued to survive.

22.7.4 The Probable Organization of the Neocortex of Early Mammals Can Be Reconstructed, Using an Analysis That Identifies Brain Characters That Are Broadly Distribution Across Mammalian Taxa as Those Likely to Have Been Retained from a Common Ancestor

In a process known as a cladistic analysis, the emergence of novel brain features can be assigned to more or less distinct branching points in a phylogenetic tree (cladogram) for any group of mammals descendant from a specific common ancestor (recent or distant) based on parsimony. As identifying brain characters can be labor intensive and depend on costly experimental procedures, the process of reconstructing the organization of the neocortex of early mammals can be simplified by initially focusing on mammals with brains that resemble those of early mammals in size and proportions. The brains of hedgehogs, shrews, and other insectivores, together with those of tenrecs and opossums are strong candidates, but the brains of other mammals, such as rats, have only slightly increased the proportional size of neocortex, and thus they provide additional comparisons of clear value. Conclusions based on this limited sample can then be validated as consistent or challenged as inconsistent with observations from mammals with more derived brains, in terms of neocortex size and shape, so that all the major branches of the mammalian radiation are considered.

22.7.5 Early Mammals Had Poorly Differentiated Neocortex and Few Areas

A comparative analysis indicates that the neocortex of the first mammals was rather poorly differentiated into layers and different neuron types, although six layers of different types of connections and functions were present, as well as pyramidal cells, stellate calls, and two or more types of local circuit inhibitory neurons. Neocortex was divided into ~20 cortical areas, a small number in comparison to the 50–150 proposed for some extant mammals. More specifically, early mammals had a primary somatosensory area and 3–4 other somatosensory fields, primary and secondary visual fields and perhaps two other visual areas, a primary and one or more additional auditory fields, as well as areas of limbic, orbitofrontal, and endorhinal cortex. Motor functions depended on somatosensory areas until the advent of placental mammals, which were characterized by a primary motor area and possibly a secondary motor area. Thus, the neocortex of early mammals was dominated by areas devoted to analyzing sensory information. In several subsequent lines of descent, more sensory areas were added, increasing the complexity of the analysis of sensory information, and motor areas were sometimes added, increasing the sophistication of behavioral responses. Additional multisensory areas sometimes emerged that allowed computational outcomes to be more easily influenced by several sources of information.

22.7.6 Theories of the Subsequent Evolution of Neocortex in Mammals Can Be Guided by a Theoretical Consideration of the Implications of Increasing Brain Size

Larger brains with larger expanses of neocortex would not function efficiently without structural modifications. As neurons do not scale up very well with increases of brain size, large brains have more neurons. This generally means that neurons in large brains have connections with a smaller proportion of the total number of neurons than neurons in small brains. Thus, large and small brains function differently. In addition, larger brains require longer connections and thus more time for transmission unless axons are increased in thickness. In part, these connection problems can be addressed in evolution by devoting proportionally more of the larger brain to connections, resulting in decreases in neuron cell body densities in cortex, and having at least some longer, thicker axons. Connection problems can partially be addressed by evolving brains that are more modularly organized, as they became larger with an emphasis on local processing via short axons. Thus, mammals with larger brains and expansive neocortex are expected to have more cortical areas, more areas divided into more modules, more connections overall, and some connections over thick, long axons, but relatively fewer of the longer interhemispheric and subcortical connections. The functions of areas also depend on their size. As areas become larger, their intrinsic horizontal connections may become longer, but not in pace with the expansion of the cortical surface. Thus, neurons become less influenced by the activities of the increasingly distant other neurons in the area. This means that large areas transmit information to other parts of the brain only about a small subset of inputs to the areas. They provide detailed, but focused information. Neurons in smaller areas are influenced by neurons that are more widely distributed across the area, and the computation of local circuits of such neurons provides a more global (but

less detailed) picture of what is going on. Global views of sensory inputs are obviously more useful for directing behavior, but details can be valuable. Thus, a few large areas, and many small to moderately sized areas, would seem to provide the most useful system.

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23 Captured in the Net of Space and Time: Understanding Cortical Field Evolution

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Glossary

analogous	Having the same function.
Baldwin effect	The ability of an animal to respond optimally to a given environment.
cortical domain	The portion of cortex devoted to a given sensory system.
cortical field	The fundamental organizational feature of the cortex.
cortical field magnification	The amount of cortex within a cortical field devoted to processing inputs from a behaviorally relevant body part is enlarged.
evolvability	The ability of an organism to generate heritable, selectable phenotypic variation.
genetic assimilation	How an environmentally induced phenotypic characteristic becomes genetically coded in a population.
homologous	A characteristic inherited from a common ancestor.
homoplaseous	An independently evolved characteristic that looks the same across species.
module	Smaller units of organization within a defined cortical field.
pleiotropy	A single gene controls numerous activities during development resulting in various phenotypic effects in the adult organism.

23.1 Introduction

Examination of a number of different mammalian brains demonstrates that brain organization, particularly the neocortex, varies dramatically across species. This variation in neocortical organization is accompanied by a considerable degree of behavioral diversity. Specifically, differences in cortical sheet size, organization, number of cortical fields, and connections are associated with differences in sensory, perceptual, cognitive, and motor abilities. How these differences in neocortical organization in mammals arise in evolution and how these alterations generate variable behavioral repertoires are difficult questions to investigate directly because the evolutionary process is highly dynamic, and alterations to the brain occur over hundreds of thousands to millions of years. Despite the fact that evolution cannot be studied 'head on', we can circumvent the problems associated with studying evolution in two ways. First, we can examine the products of evolution, namely extant mammals, and compare their brain organization, to make inferences about the evolutionary process. Alternatively, we can study the developmental processes that generate different aspects of brain organization, since the evolution of the neocortex is the evolution of the developmental mechanisms that give rise to adult phenotypes. We can then postulate how developmental mechanisms may have been altered to produce different phenotypes (see The Origin of Neocortex: Lessons from Comparative Embryology).

The use of the comparative approach has led to number of important insights regarding brain evolution. Likewise, studies of development, particularly recent molecular studies, have provided much needed information on the genes that are involved

in various aspects of cortical development and organization. However, utilizing the comparative or the developmental approach in isolation in an attempt to uncover principles of brain evolution is problematic. In terms of the comparative approach, examining any extant mammal allows us to observe only a static moment in the evolutionary process. In essence, we have captured, in our net of space and time, a number of individual phenotypes, or individual snapshots, in a process that is constantly in a state of flux. We take these snapshots out of our net, use a number of different tools to dissect and examine them, and then put them together to make an evolutionary moving picture. The problem is that each extant mammalian brain that we observe is a frozen frame or moment in its own moving picture; it has its own evolutionary history and will move in a unique future trajectory. Further, this approach tells us little about the transition between frames and how phenotypic transformations may occur. This is where studies of cortical development merge with comparative analyses.

Studies of the development of the nervous system can strengthen our inferences regarding how phenotypic transitions occur by providing a number of possible mechanisms for this process. However, like the use of the comparative approach, using a developmental approach in isolation to understand brain evolution is problematic. While a number of recent studies provide insight into potential mechanisms that could be involved in some aspect of cortical organization, such as regulating cortical sheet size, they do not demonstrate that such a mechanism is actually being employed in a naturally evolving system. Thus, only by combining both the comparative approach and developmental approach can we appreciate the types of changes that have occurred in different lineages, predict how these transitions may have happened, and validate these predictions by manipulating some aspect of development and determining if the resulting phenotype is consistent with a type of neocortical organization that would naturally occur, as validated through comparative studies.

In this article, we begin by exploring what constitutes a cortical field and discuss homologous features of cortical organization across mammals. Next, we discuss the importance of distinguishing homology from instances of homoplasy when making comparisons across species. Because the concepts regarding what constitutes a cortical field are changing in light of new studies on molecular development, in the second section of this article we discuss some of the molecular aspects of cortical field development, and describe both intrinsic and extrinsic contributions to cortical development, and the role of peripheral morphology and behavior in shaping the cortical field throughout the life of an individual. Then, we discuss the evolution of the neocortex and outline the types of systems level modifications that have been made to evolving brains. Finally, we speculate on the idea that the neocortex evolves to be flexible, and that genetically based adaptations of the brain and body may initially have been activity-dependent features of organization that were present only under unique and consistent environmental conditions.

23.2 What is a Cortical Field? Homology, Homoplasy, and Analogy

A cortical field is considered to be the principal organizational feature of the cortex, and most neuroscientists would contend that the addition of cortical fields to the neocortex is what endows greater degrees of neural and behavioral complexity to mammals. Indeed, most would agree that the neocortex, in general, and cortical fields, in particular, are the essence of the mammalian brain; the feature that distinguishes mammals from other vertebrates. We raise the question of what is a cortical field because this issue is particularly important for the study of cortical evolution. If one is interested in the evolution of the neocortex and the addition of cortical fields, then defining homologous cortical fields across mammals is critical. Specifically, it is important to determine which features of the cortical field are most usefully compared across species, and ultimately to appreciate how these features change during evolution.

Although concepts regarding what constitutes a cortical field are changing in light of new studies on the molecular development of the neocortex, in adult mammals, a cortical field is determined by a number of well-defined anatomical, histochemical, and electrophysiological criteria. These criteria were previously outlined by Kaas (1982), and although not exhaustive, have enabled investigators to subdivide the neocortex in a variety of mammals with a high degree of success. Some of these criteria include a complete representation of the contralateral sensory surface (or visual field for visual cortical areas), a unique architectonic appearance, and a distinctive pattern of connectivity. Other criteria include utilization of some subset of neurotransmitters, or the presence of particular behavioral deficits when the area is lesioned. Because errors can be made in subdividing the neocortex when any single criteria is used in isolation, using a combination of criteria to subdivide the

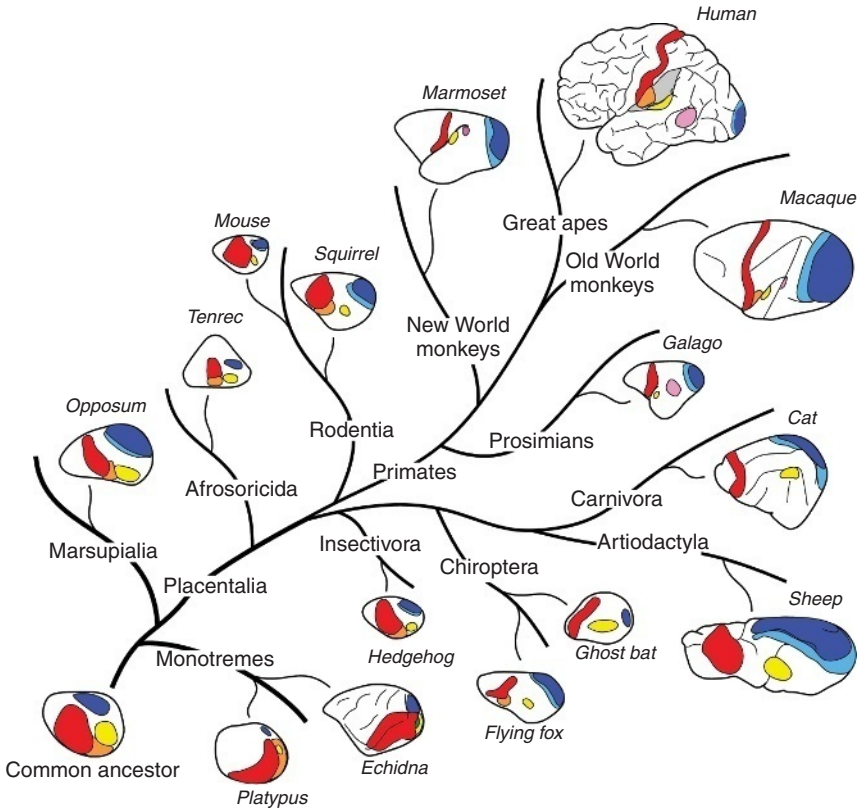

Figure 1 A phylogenetic tree depicting the relationships between major mammalian lineages. The cortex of each mammal contains a constellation of cortical fields that have been identified in all mammals examined. These cortical areas were likely inherited from a common ancestor, and therefore are homologous. Although the organization of the neocortex of the common ancestor is not known, a cladistic analysis allows one to infer the organization of unknown forms, such as the common ancestor. Dark blue primary visual area; light blue second visual area; red primary somatosensory area; orange second somatosensory area; yellow primary auditory area; pink middle temporal visual area. Redrawn from Krubitzer, L. and Kahn, D. 2003. Nature vs. nurture: An old idea with a new twist. *Prog. Neurobiol.* 70, 33 52.

neocortex allows for more accurate comparisons of cortical organization across mammals.

Using these criteria, it has been determined that in some mammals, such as mice, the number of areas that compose the neocortex is relatively small, on the order of 7–12 cortical fields. In other mammals, such as macaque monkeys, the number of cortical fields is larger, on the order of 30–50 cortical areas (see Kaas, 1988, 1993, for review). This increase in the number of cortical fields in some lineages, at least in part, is the neural basis of complex behaviors such as sophisticated communication (language in humans), learning, and cognition. While the number of cortical fields is highly variable in mammals, several cortical fields are common to all species (see Krubitzer, 1995; Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005). These fields include the primary sensory areas (primary visual area, V1; primary somatosensory area, S1; and primary auditory area, A1), second sensory areas (secondary visual area, V2; secondary somatosensory area, S2; secondary auditory area, A2, and rostral auditory area, R), as well as motor areas such as primary motor area, M1 (Figure 1). These fields are homologous because they have been identified in all mammals examined, and it is likely that these cortical areas arose early in mammalian evolution and were inherited from a common ancestor in all lineages, rather than having evolved independently in each group. As such, a number of features of organization are similar across groups of mammals including similarities in topographic organization, aspects of cortical architecture, and thalamocortical and corticocortical connections. Later in this article we will discuss the types of modifications made to this homologous plan of organization and how these modifications might have arisen in evolution.

A broad comparative analysis also indicates that some features of cortical organization look strikingly similar in different mammals, but this similarity is not due to inheritance from a common ancestor. Rather, these features are homoplaseous, and have independently evolved in each mammal.

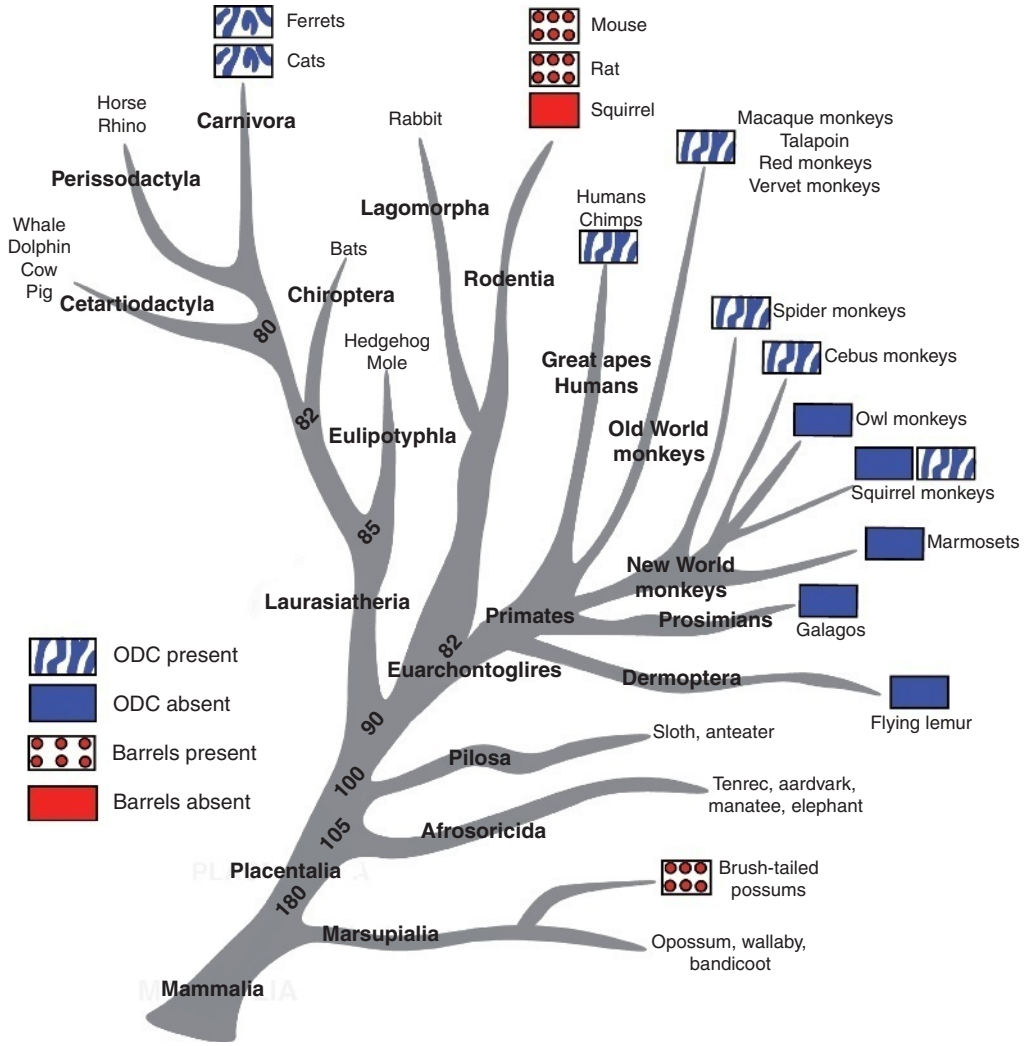

Figure 2 Homoplasy-independent evolution: a phylogenetic tree depicting the relationships between major mammalian lineages and the emergence of independently evolved features of cortical organization. Because the emergence of barrels in mice and rats arose independently from those in brush-tailed possums, they are considered as homoplaseous rather than homologous. Likewise, the presence of ODCs in ferrets and cats arose independently from those in some primate lineages. The fact that such similarities in organization emerge in different lineages despite over 90 million years of independent evolution indicates that the evolution of the neocortex is highly constrained. It also indicates that although the features themselves are homoplaseous, their presence could reflect the presence of homologous developmental mechanisms. Phylogenetic relationships based on Murphy, W. J., Pevzner, P. A., and O'Brien, S. J. 2005. Mammalian phylogenetics comes of age. *Trends Genet.* 20, 631 639.

An excellent example of a homoplaseous feature of the neocortex is the barrel field in the rat and mouse, and the brush-tailed possum (Figure 2; Weller and Haight, 1973; Weller, 1993). An out group comparison indicates that no intervening group of mammals has barrel cortex. Thus, the most parsimonious explanation for their presence in each group is that they have evolved independently in rodents and brush-tailed possums. Another example of homoplasy is the presence of ocular dominance columns (ODCs) in carnivores and some primates. ODCs are present in great apes and humans (Tigges and Tigges, 1979; Horton and Hedley-Whyte, 1984), Old World monkeys (e.g., LeVay *et al.*, 1975; Florence and Kaas, 1992), and a few species of New World monkeys (e.g., Florence *et al.*, 1986; Rosa *et al.*, 1992). They are absent in other New World monkeys, prosimians, and dermopotera (Figure 2), and in all other clades except carnivores (e.g., Löwel and Singer, 1987; Law *et al.*, 1988). This out-group comparison indicates that ODCs arose in primates after the divergence of New and Old World monkeys from prosimians (approximately 70 Mya), and that ODCs were lost in some New World species. The presence of ODCs in only two species of carnivores suggests that ODCs arose

independently in carnivores and primates, since the lineage that leads to carnivores diverged from that leading to primates over 90 Mya, and no intervening groups possess ODCs. What is remarkable about ODCs and the barrel cortex is that despite 90–180 million years of independent evolution, the arrangement of these modules looks very similar in carnivores and primates, and in rodents and brush-tailed possum respectively.

When making cross-species comparisons, there is often an assumption that homologous fields perform the same function or are analogous. However, this may not be the case. For example, over the years, a solid case for the presence of V1 in a variety of species has been established. All data indicate that V1 resides on the caudal pole of occipital cortex, contains a complete, first-order representation of the visual hemifield, receives connections from the dorsal division of the lateral geniculate nucleus (LGNd) of the thalamus, and has a striated appearance in tissue that has been sectioned perpendicular to the cortical layers and stained for Nissl substance. In cortex that has been sectioned tangentially and stained for myelin, V1 appears as a densely myelinated wedge at the caudal pole of the neocortex. Given these identifying features, V1 is proposed to be homologous across all mammals, and to form a basic component of a visual processing network in the mammalian neocortex. But what of analogy? Does it naturally follow that V1 as a homologous cortical area has a similar function or set of functions across groups of mammals?

The answer is 'no'. If we examine V1 in the mouse and compare it to V1 in the macaque monkey, several differences emerge. Most notable are the addition of modules to V1, such as orientation and ODCs, the addition of visual cortical fields, and the concomitant change in cortical connections in monkeys. Thus, V1 in monkeys and mice varies substantially in organization, and intrinsic and extrinsic connectivity. To illustrate this concept we have drawn a simple circuit containing three separate nodes (cortical fields A, B, and C in Figure 3). These nodes have a homologous pattern of interconnection across mammals (connections 1, 2, and 3 in Figure 3). In some groups of mammals, the nodes have been further subdivided to mimic the generation of modules (Figure 3). In addition, new nodes, representing new cortical areas, have been added to the network (D, Figure 3), which result in the addition of new connections and a potential re-weighting of existing connections between homologous nodes. This example shows that because of the emergence of new organizational features

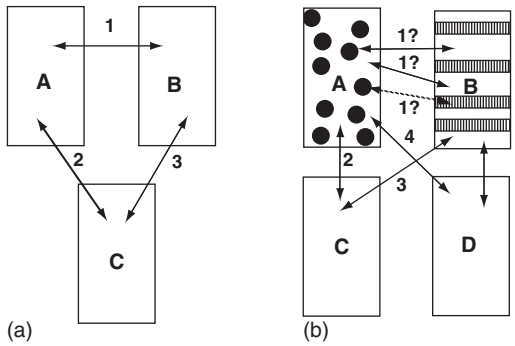

Figure 3 A hypothetical processing network (a) originally consisting of three cortical fields (A, B, and C) with a set of interconnections (1, 2, and 3). The evolution of this network (b) includes the addition of a new cortical field (D), the emergence of modules within existing cortical fields (circles in A and stripes in B), the emergence of new connections (4), and the re-weighting of existing connections (compare thick vs. thin line of connection 2 in (a) and (b)). These types of changes that naturally occur in evolution, indicate that homologous cortical fields may not be analogous since the interconnection relationships change and intrinsic processing modules emerge.

(modules), new inputs, and a re-weighting of retained connections, homologous cortical fields may not have the same function.

In answer to the question posed at the beginning of this section 'what is a cortical field?', we believe that it may be fruitful to consider cortical fields, at least in part, as homologous patterns of interconnection upon the cortical sheet. These patterns appear to be quite robust across species, and are associated with the emergence of specific architecture and neural properties in the developing nervous system. While maintaining their global relationships, these patterns shift, or 'float' upon the cortical sheet within the life of an individual (particularly during development), and to a greater extent, within and across species over time.

23.3 The Development of Cortical Fields

It has been appreciated for some time that both genes and the environment, as broadly defined, contribute to the development and the organization of the neocortex. How each of these factors contributes to development is couched in the long-standing 'nature vs. nurture' debate (see Krubitzer and Kahn, 2003 for review). Fortunately, the issue of the inherent, genetic contribution to the cortical phenotype has recently crystallized into hypotheses which are amenable to vigorous experimentation regarding the temporal and spatial distribution of genes and proteins that occur in development, and give rise to aspects of cortical organization including

cortical field location, size, and connectivity. The 'nurture' side of the debate has also become more experimentally tractable, and questions regarding the activity-dependent cellular mechanisms that alter aspects of development including the expression of genes, regulation of synaptic morphology and function, and dendritic and axon growth are now being examined. The problem is that in some instances it is difficult to draw a distinct line between genetic and epigenetic contributions to the phenotype, and the two become intricately intertwined.

23.3.1 Nature: The Contribution of Genes to Cortical Field Development

Understanding how genes control cortical field development can be broken into three broad categories. First, there are several genes that are intrinsic to the neocortex which control specific aspects of cortical development. The expression of these genes occurs in the normal developing system, and their action is independent of neural activity. Second, the expression of some genes in the central nervous system is induced by activity and requires feedback from the developing system to become activated. Finally, there are genes that regulate aspects of the body plan and peripheral morphology that contribute substantially to aspects of cortical organization.

23.3.1.1 Activity-independent genes intrinsic to the neocortex Recent work indicates that genes intrinsic to the neocortex, or the developing ventricular zone, control a number of aspects of cortical development, all of which have a large impact on the organization and function of the neocortex in the adult phenotype. Some examples include the regulation of the size of the cortical sheet, cortical field coordinates in the rostrocaudal and mediolateral axis, and thalamocortical connectivity.

In terms of the overall size of the cortical sheet, studies on cell cycle kinetics of neocortical progenitor cells in the ventricular zone indicate that the size of the cortical sheet is intrinsically regulated and that there are a number of plausible ways in which this regulation can occur. In general terms, the number of cells in the developing ventricular zone can be increased by extending the length of time that cells undergo symmetric divisions, and/or the rate at which cell divisions occur. A comparative analysis of small-brained mammals, such as mice, and large-brained mammals, such as macaque monkeys, indicates that cortical neurogenesis is both prolonged and accelerated in macaque monkeys compared to mice (Kornack and Rakic, 1998; Kornack, 2000). Several hypotheses regarding the specific genes and proteins involved in this process and the types of alterations to the kinetics of division have recently been proposed. For example, 'beta-catenin' is an intracellular protein that is expressed in neuroepithelial precursor cells during neurogenesis (Chenn and Walsh, 2002). In transgenic mice that over express a form of this protein, the size of the neocortex increases dramatically. This massive increase in the size of the cortical sheet is due to an increase in the proportion of progenitor cells that re-enter the cell cycle and continue mitotic division. Another gene proposed to alter cell cycle kinetics is *Brain Factor-1* (*BF-1* or *Foxg1*). This gene is expressed in telencephalic progenitor cells (Tao and Lai, 1992), and regulates cell proliferation and differentiation in the developing neocortex (Hanashima *et al*., 2002). *BF-1* is regulated by *FGF2*, which is also involved in regulating cortical sheet size by determining the number of cycles of division that progenitor cells undergo during cortical neurogenesis. For example, injections of FGF2 into the ventricle of embryonic rats results in a substantial increase in cortical volume (Vaccarino *et al*., 1999), and FGF2 knockouts have smaller neocorticies (Raballo *et al*., 2000). These studies indicate that the disproportionate size of the neocortex in different lineages could be regulated in several ways by different genes that affect the kinetics and timing of cell division in the ventricular zone.

Related studies of cell cycle kinetics in monkeys indicate that primary areas, such as V1, may be specified very early in development, during neurogenesis. For example, in primates, V1 is characterized by an increase in cell density and laminar complexity compared to other cortical areas, and compared to other mammals. In development, the rate of production cells in the ventricular zone is higher in the region where V1 will ultimately reside than in other regions (DeHay *et al*., 1993). Differences in laminar histogenesis for different regions of the ventricular zone have also been observed in mice (Polleux *et al*., 1997). These studies indicate that areal differences arise very early in neocortical development, well before thalamic innervation of the neocortex occurs.

In addition to intrinsic mechanisms that operate during cortical neurogenesis to specify cortical fields, recent work indicates that somewhat later in cortical development, the transcription factors *Emx2* and *Pax6* are involved in the expression and

patterning of downstream genes in the rostrocaudal axis of the neocortex, and potentially even cortical field size. For example, experiments in which these genes are deleted result in shifts of downstream genes such as *Cad8* and *Cad6* either rostrally (for *Emx2* deletion) or caudally (for *Pax6* deletion; Bishop *et al*., 2000). In addition to the observed changes in gene expression, *Emx2* and *Pax6* mutants also exhibit alterations in thalamocortical connectivity. In experiments in which *Emx2* is deleted and the neocortex is rostralized (e.g., rostral cortical fields are shifted caudally), cortex at the caudal pole that would normally receive thalamic input from the LGN receives inputs from the ventral posterior nucleus (VP) (which normally projects to somatosensory cortex rostral to this region; Bishop *et al*., 2000). Furthermore, mice in which *Emx2* is overexpressed have a significantly larger V1 than in normal animals (i.e., cortex has been caudalized; Hamasaki *et al*., 2004).

In terms of connectivity, some of the cadherins appear to regulate thalamocortical connectivity. For example, *Cad6*, *8*, and *11* are expressed in unique subsets of thalamic afferents (Suzuki *et al*., 1997; Korematsu and Redies, 1997). Further, *Cad6* is co-localized with the synaptic marker, synaptotagmin, and is correlated with the formation of synaptic connectivity between a source and its target in the developing nervous system (Inoue *et al*., 1998). The ephrins have also been proposed to play a role in thalamocortical development. While their presence in locations extrinsic to the neocortex, such as the ventral telencephalon, serves a role in gross topographic guidance, they appear to intrinsically mediate the refinement of thalamocortical connectivity within a cortical field (see Vanderhaeghen and Polleux, 2004 for review). For the development of cortical connections, recent work has demonstrated that FGF2, which may be regulated by *Emx2*, is involved in guiding (modulating) corticocortical connections (Huffman *et al*., 2004). Thus, the transcription factor *Emx2* controls a genetic cascade involved in structure formation, location, and connections.

It is important to note that evolutionarily, this type of regulation of events imposes formidable constraints on the developing and evolving nervous system. Given the constraints imposed by such a contingent system, it seems inevitable that very small changes in the timing and spatial distribution via base substitutions, recombination, and transposition, for example, of any one of the genes involved in these aspects of cortical field development can have a very large effect on the phenotype.

As mentioned earlier, a recent perspective on how cortical fields should be defined is to consider the subdivisions or areas of the neocortex from a spatiotemporal perspective. In this view, cortex is examined over time as a series of coordinated patterns of gene expression which are thought to be involved in generating features of the neocortex that will ultimately be realized in the adult, such as cortical layering, architecture, transmitter utilization, and connectivity. While this perspective is certainly important from both a developmental and evolutionary perspective, it may not be appropriate to define a cortical field in terms of the patterns of gene expression exhibited early in development for two reasons. First, the direct relationship between a functionally defined cortical field and some pattern or patterns of gene expression has yet to be established. Second, in the neocortex, early patterns of gene expression often represent potential, while the adult form directly generates the behavior that is the target of selection.

23.3.1.2 Activity-dependent regulation of genes that control aspects of cellular morphology, connection, and function In addition to the genes we described above, a number of studies describe intracellular, molecular mechanisms that are driven and regulated by neural activity, and generate changes in the temporal expression of genes within a cell employing these mechanisms. Altering the expression of genes can change aspects of synaptic morphology. For example, recent work demonstrates that increases in intracellular calcium, due to changes in neuronal activity, trigger a cascade of events, including the activation of the cAMP pathway and phosphorelation of CREB, which binds to the regulatory region of a gene and induces transcription of genes (see Finkbeiner and Greenberg, 1998; West *et al*., 2001 for review). There are several different types of molecules which are regulated by activity, and which in turn are involved in synaptic modeling during development. One of these is a class of proteins called neurotrophins. These proteins are relevant to the discussion above because their levels and secretion are regulated by activity, they are expressed in synapses, and they regulate morphological changes in both the pre- and postsynaptic elements (McAllister *et al*., 1995, 1999; Lein *et al*., 2000; McAllister, 2001 for review). Neurotrophins such as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophic factor 4/5 (NT4/5) play a number of important roles in nervous system development

including mediation of rates of neuronal survival (see Levi-Montalcini, 1987; Miller and Kaplan, 2001 for review), induction of cell migration out of the ventricular zone (Borghesani *et al*., 2002), regulation of the extent of axon outgrowth (Segal *et al*., 1995), enhancement of dendritic outgrowth, and stimulation of protein synthesis in dendrites (Aakalu *et al*., 2001).

Another group of molecules recently identified by Shatz and colleagues (Corriveau *et al*., 1998; Huh *et al*., 2000) are the class I major histocompatibility complex (class I MHC) antigens. The expression of class I MHC is reduced in the developing cat LGN with the application of tetrototoxin (TTX) via intraocular injections given *in utero* (Corriveau *et al*., 1998). TTX blocks neural activity by deactivating sodium channels. In cats that are monocularly deprived during the critical period, class I MHC expression is reduced in the eye-specific layers of the LGN that were deprived. Further, in mice lacking class I MHC, refinement of retinogeniculate connections is incomplete (Huh *et al*., 2000). Thus, as in the above example for BDNF, activity controls the expression of these molecules, which in turn alters aspects of synaptic development.

While the above descriptions are brief and the intracellular processes that are modified by activity are not completely known, there are a number of potential intracellular mechanisms and molecules involved in nervous system construction whose action is modulated by activity. In the beginning of this section on development, we suggested that the boundary between genetic and activity-dependent contributions is somewhat blurred. This is the case for the scenario described above in which activity regulates gene expression, which in turn regulates aspects of nervous system construction and function. This type of activity-dependent regulation depends on calcium sensitive intracellular mechanisms that may be genetically determined and intrinsic to the composition of the cell. If this is the case, then the ability of the developing organism to respond to environmental fluctuations may be genetically specified and selected for in evolution, but the resulting phenotype would only be expressed in a particular environment (Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005). If the environment is stable, the specific phenotypic characteristic generated would be stable, and in essence would masquerade as an evolutionary (heritable) phenomenon.

23.3.1.3 Genes extrinsic to the neocortex but intrinsic to the organism contribute to aspects of cortical development and organization All mammals have a conserved body plan that includes forelimbs with distal appendages, hind limbs with distal appendages, a trunk, neck, head, face, snout, two eyes, two ears, one nose, and one mouth. Interestingly, this basic plan has been conserved in all vertebrates, due to genetic constraints, and like the neocortex, has been modified in a very limited fashion. Homeodomain genes, such as T-box genes and Hox genes, are involved in specification of the body plan; they arose early in the evolution of living organisms, and are highly conserved across taxa from arthropods to vertebrates (e.g., Patel, 2003; Boncinelli *et al*., 1994; Schilling and Knight, 2001; Banerjee-Basu and Baxevanis, 2001; Showell *et al*., 2004).

Despite the restrictions these genes place on the evolving body, morphological diversity of the limbs, head, and face abound. For example, limbs have been modified into wings (bats), flippers (dolphins), hoofs (ungulates), claws (cats), and hands (primates). For the head and face, alterations have been made to the location of the eyes on the head, the size, location, and mobility of the pinna, and the presence of vibrissae, follicles on a nose, or specialized oral structures. At a finer level of organization, the receptor arrays associated with a specialized morphology and behavior also undergoes modifications. However, like those of the body and brain, they are generally limited in number and include:

1. alterations in the location of receptors,
2. alterations in the density of receptors,
3. alterations in the number of receptors,
4. addition of new receptors, and
5. sensitivity of receptors.

Specific examples of some of these modifications would include the disproportionate amount and density of cutaneous receptors on the glabrous digit tips of the hands of primates, the concentration of cones at the fovea of primates and visual streak in rabbits (Hughes, 1977), the differential expansion of particular portions of the basilar membrane devoted to ultrasonic frequencies in echolocating bats (Ramprashad *et al*., 1979), and the addition of electrosensory receptors in the bill of a platypus (Scheich *et al*., 1986; Manger and Pettigrew, 1996), to name a few.

Not only does the actual structure of the body part contribute to features of cortical organization, but also how these body parts are utilized and modified for exploration is equally important. For example, for the somatosensory system, primates tactually explore objects with their glabrous hands, elephants with their distal trunk, muriad rodents with their vibrissae, the star nosed mole with the

many follicles of the nose, and the naked mole rat with their teeth (see Catania, 2005). Thus, body parts and associated receptor arrays that are used repeatedly and uniquely have large amounts of cortical space devoted to their representation in both sensory and motor cortex. Indeed, without exception, behaviorally relevant, specialized sensory receptor surfaces occupy a greater amount of cortical space than less relevant surfaces. This is observed at the sensory systems level in cortical domain allocation, and at the level of the individual cortical field (cortical magnification). A cortical domain is the amount of space allotted to a particular sensory system, and this differs for different mammals, even those with approximately the same size cortical sheet. For example the amount of cortical territory devoted to processing visual inputs is greater in the highly visual squirrel than in the mouse (Figure 4; Rosa and Krubitzer, 1999). In terms of cortical field magnification, the amount of cortex devoted to processing inputs from the fovea is greatly enlarged in V1 of primates compared to the amount of cortex devoted to processing inputs from the rest of the eye. For the somatosensory cortex, in S1 and other

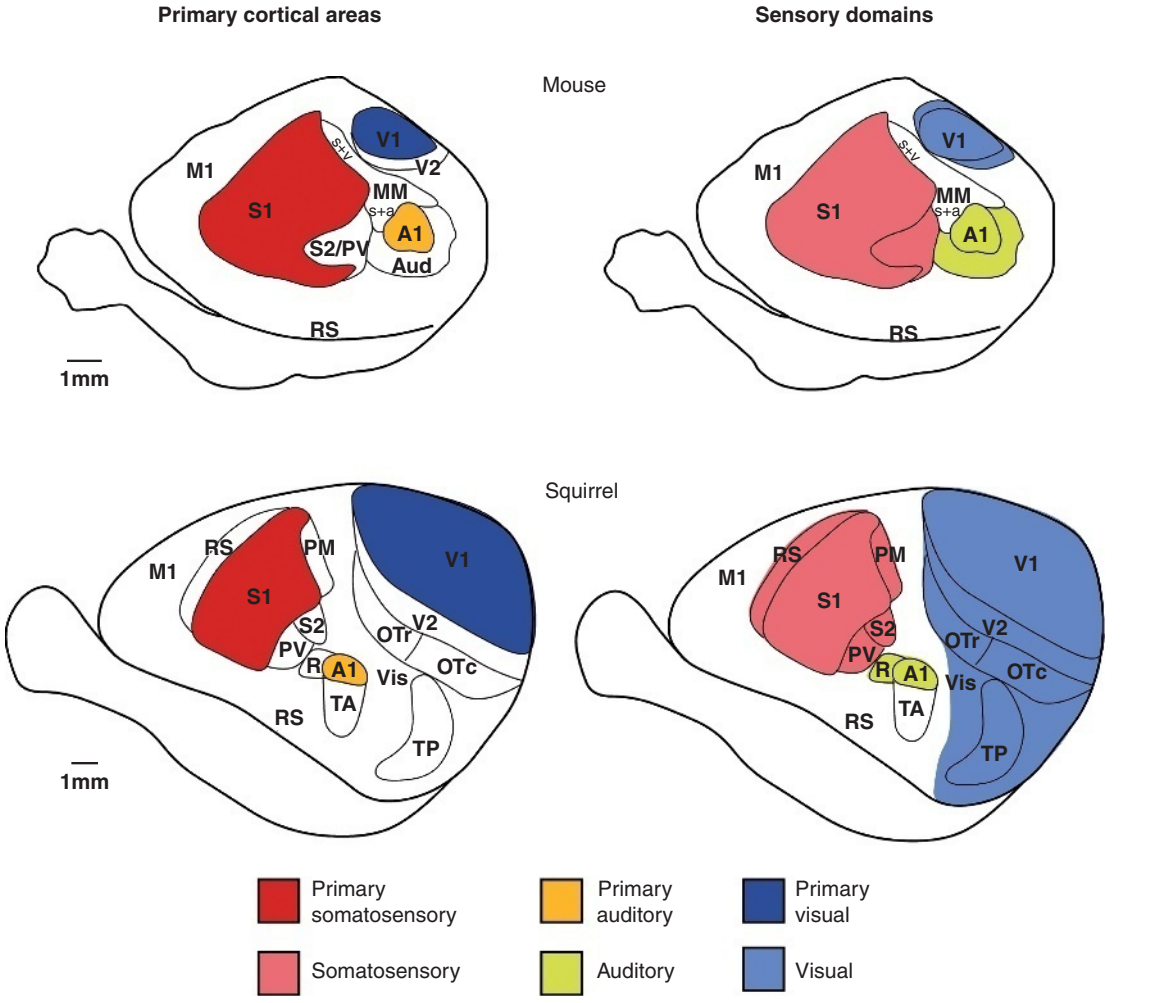

Figure 4 The organization of primary cortical areas and sensory domain allocation in the mouse (top) and squirrel (bottom). Each of these rodents occupies a particular niche and relies on different sensory systems for survival. The mouse is a terrestrial rodent that explores and navigates with its vibrissae, while the squirrel is an arboreal rodent that relies heavily on vision. Differences in cortical organization are observed at both the level of the cortical field and cortical domain. In the mouse, the primary somatosensory area is relatively large and occupies a good deal of cortex, while in the squirrel the primary visual area is relatively large compared to other primary sensory fields. Sensory domains, or the amount of cortex devoted to processing inputs from a particular sensory system, are also distributed differently in each species. In mice, the somatosensory domain is relatively large, while in the squirrel, the visual domain is extremely large and occupies at least one third of the entire cortical sheet. a, auditory; A1, primary auditory area; Aud, auditory; M1, primary motor area; MM, multimodal; OTc, caudal occipital-temporal cortex; OTr, rostral occipital-temporal cortex; PM, parietal medial area; PV, parietal ventral area; RS, rhinal sulcus; s, somatosensory; S1, primary somatosensory area; S2, secondary somatosensory area; v, visual; V1, primary visual area; V2, secondary visual area; Vis, visual.

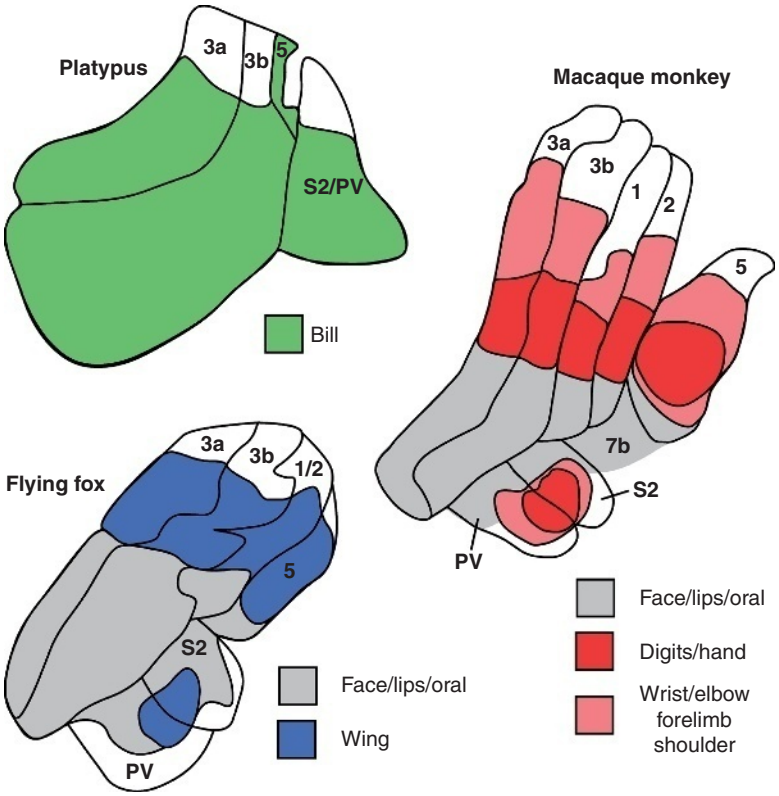

Figure 5 Cortical magnification of behaviorally relevant body parts within the somatosensory cortex of different mammals. In the duck-billed platypus, the bill representation (green) dominates all three somatosensory fields identified (R or area 3a, 3b, or S1, and S2/PV). In the highly dexterous macaque monkey, the representation of the glabrous digits (dark red), forelimb (light red), and oral structures (gray) dominate all somatosensory fields identified. In some fields, such as area 5, the magnification of the hand and forelimb dominates almost the entire field. Finally, in the flying fox, the wing (blue) and oral structures (gray) dominate all somatosensory areas identified.

cortical fields, the hand and mouth representations are magnified in primates, the wing and mouth representations are magnified in the flying fox, and the bill representation is magnified in the platypus (Figure 5; see Krubitzer and Disbrow, 2005 for review). As noted earlier, these specialized receptor surfaces are interfaced with the stimulus to be explored via specialized motor sequences. Thus, the motor system and the behaviors that allow for this interface are an integral part of sensory reception and cortical organization.

Since there is clearly an important relationship between cortical organization, peripheral morphology, and use, it is important to understand how body morphology evolves and how variability in body morphology is achieved in different lineages. Interestingly, the questions regarding diversification of the body plan in mammals are the same as those that arise when considering diversity in neocortical organization. Given the rather large constraints imposed on a basic plan of organization by these homeodomain genes, how can morphological diversity arise? It has been suggested that while the protein coding sequence of these homeodomain genes is relatively static across lineages, divergence in the regulatory portion of the gene can account for much of the morphological diversity observed in mammal body plans (Cretekos *et al*., 2001). Thus, slight differences in the temporal and spatial patterning of genes generates large modifications in body plan organization. For example, the expression of a gene involved in the specification of the body plan (Hoxd9–13) was compared in two mammals with strikingly different forelimb morphology, the short-tailed fruit bat and the mouse (Figure 6; Chen *et al*., 2005). Comparison of the distribution of Hoxd9–13 in bats and mice revealed that there were significant differences in the expression of this gene in the distal forelimb (dfl), but not the hindlimb, in later stages of limb development. Specifically, the anterior expression boundary of Hoxd9–13 in the bat is shifted posteriorly in the mouse (Figure 6). Thus, phenotypic diversity, or the transition from one phenotype to another that occurs in evolution, could be accomplished by subtle shifts in the expression of genes involved in

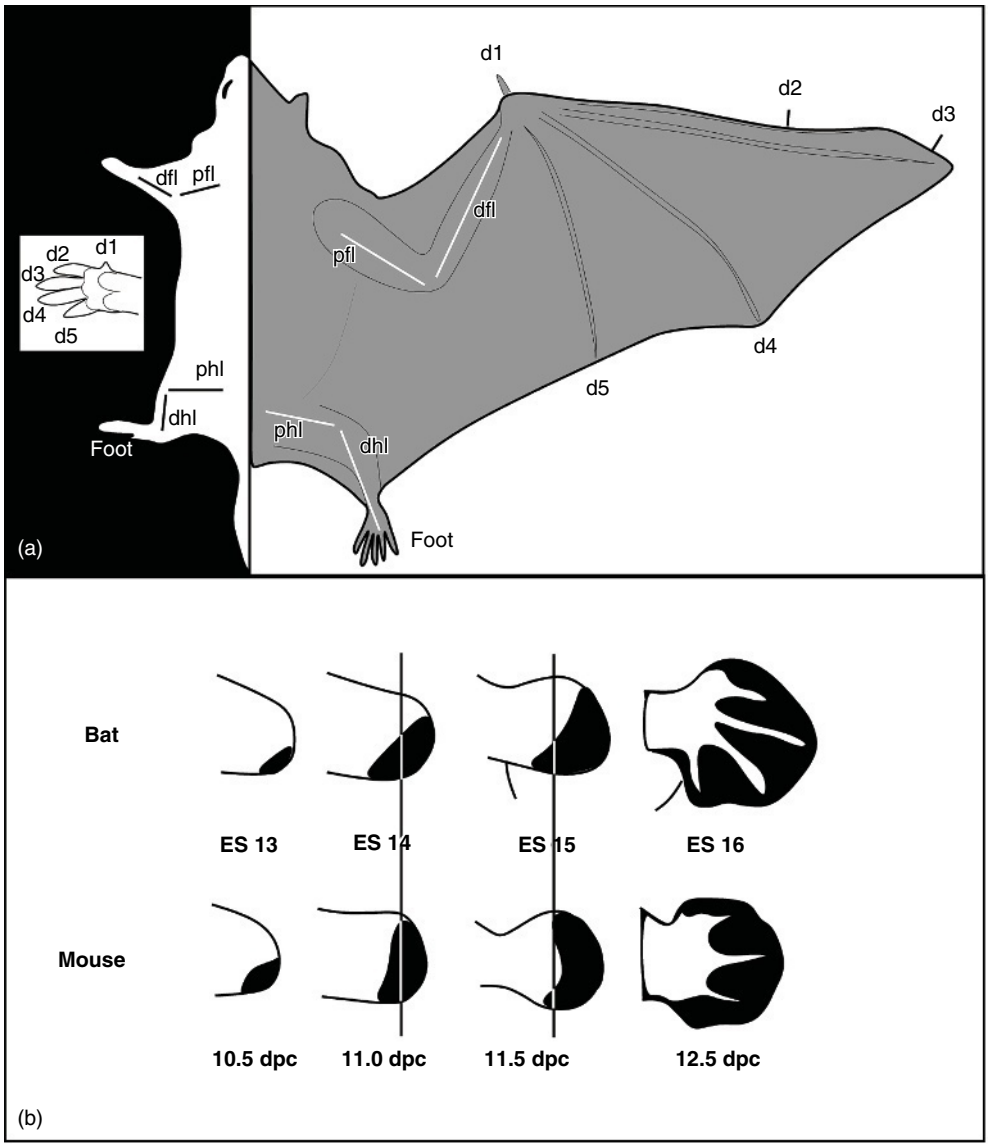

Figure 6 a, The body plan in mice and bats has a similar structural organization. Major body axis such as proximal and distal forelimbs and hind limbs (pfl, dfl, phl, and dhl), as well as individual digits (d1 d5), can be identified in both animals. However, modifications have evolved in each lineage in the form of the forepaw of a mouse and the wing of a bat. b, The expression pattern of Hoxd13 in the developing forelimb of the bat and mouse. The extent of the expression differences in bats and mice is evident during particular phases of limb development (bat ES 14, ES 15; mouse 11 dpc, 11.5 dpc), and such differences in homeodomain gene expression patterns could, at least in part, account for variations in forelimb morphology observed in each species. Such differences in expression are not noted for the hindlimb. dfl, distal forelimb; dhl, distal hindlimb; dpc, days post coitus; ES, embryonic stage; pfl, proximal forelimb; phl, proximal hindlimb. a, Modified from Cretekos, C. J., Rasweiler, J. J., and Behringer, R. R. 2001. Comparative studies on limb morphogenesis in mice and bats: A functional genetic approach towards a molecular understanding of diversity in organ formation. *Reprod. Fertil. Dev.* 13, 691 695. b, Modified from Chen, C. H., Cretekos, C. J., Rasweiler, J. J. T., and Behringer, R. R. 2005. Hoxd13 expression in the developing limbs of the short-tailed fruit bat, *Carollia perspicillata*. *Evol. Dev.* 7, 130 141.

major aspects of body and brain development. It should be noted that alterations in the temporal and spatial dynamics of gene expression have been known to account for variation of body segmentation in insects for some time (see Davis and Patel, 2002). It is only relatively recently that these well-established ideas from work on insects have been used to understand the evolution of the mammalian nervous system.

The case of body plan organization is another example where the boundary between intrinsic genetic contributions to the phenotype and activity dependent or environmental contributions are often difficult to draw. As Figures 4 and 5 illustrate,

specialized body morphology and use affect cortical domain allocation and sensory field magnification. The genes, which are involved in setting up the body plan organization, do not exclusively determine the final morphology of a particular body part, nor the resultant cortical organization. Indeed, several extrinsic factors related to the development of a body part contribute to the organization of the neocortex. For example, use directly affects the skeletal morphology, which in turn affects cortical organization. Several studies have shown that alterations in mastication behavior in development, often brought about by changes in diet, have a direct effect on craniofacial morphology (He, 2004), skull dimensions (Katsaros *et al*., 2002), mandibular morphology (Bresin, 2001), and bone density (Davies *et al*., 2005). The types of diet that produce such alterations during development are associated with hard versus soft food sources and the presence or absence of particular nutrients. Other extrinsic factors, which directly contribute to the development of body morphology and indirectly to cortical organization, are factors such as temperature, humidity, salinity, diet (see Johnston and Gottlieb, 1990 for review) and even gravity (e.g., Singh *et al*., 2005). The observation that body plan morphology can be altered by epigenetic factors is analogous to the observations made for the neocortex. That is, despite the very large constraints imposed by regulatory genes on fundamental aspects of body morphology or cortical organization, a large degree of phenotypic variability is still possible, and alterations to the body plan can indirectly alter cortical organization.

23.3.2 Nurture: How Activity Contributes to the System Level Aspects of Cortical Development and Organization

The relationship between the cortical domain, cortical field magnification, peripheral morphology, and use in the adult mammalian neocortex has important implications for developmental and adult plasticity, and evolution. In terms of development, it seems clear that peripheral morphology, sensory receptor organization, and the specialized motor programs that are part of efficient sensory reception, play a very large role in determining a number of aspects of cortical organization that are observed in adult mammals. Several series of recent experiments in our laboratory in which peripheral sensory receptor arrays have been physically excised or activity has been modified throughout development underscore this point. For example, in a recent study *Monodelphis domestica* were bilaterally enucleated well before the retinal ganglion cells reached the diencephalon and before the thalamocortical afferents reached the neocortex (Kahn and Krubitzer, 2002). Using electrophysiological, anatomical, and architectonic analyses in these animals after they reached adulthood, we found large shifts in sensory domain allocation, in that all of cortex that would normally be occupied by the visual system was occupied by the auditory and somatosensory system (Figures 7a and 7b). Interestingly, architectonically defined area 17 was still present, although reduced in size, and major thalamic projections from the LGN were preserved. However, there were also alterations in thalamic projections in that area 17 or 'V1' received additional input from the VP nucleus, the medial geniculate (MG) nucleus, and nuclei in the anterior group (Kahn *et al*., 2006). Further, corticocortical connections were altered in that area 17 received inputs from S1, A1, and frontal cortex. These patterns of thalamocortical and corticocortical connections are not observed in normal *Monodelphis* (Kahn *et al*., 2000).

Related experiments in congenitally deaf mice revealed much the same results (Hunt *et al*., 2005, 2006). These experiments were somewhat more subtle in that the sensory receptor array was not removed, but the ability to transduce auditory stimuli was eliminated in these animals throughout development. As with the blinded animals, congenitally deaf mice had large alterations in sensory domain allocation and alterations in cortical and thalamocortical connections (Figures 7c and 7d). All of cortex that would normally process auditory inputs contained neurons responsive to visual and somatic stimulation (Hunt *et al*. 2006). A surprising observation was that this lack of sensory driven activity resulted in alterations in connectivity at very early stages of sensory processing. In addition to its normal targets, the retina also projected to the MG nucleus and middle layers of the superior colliculus, structures generally associated with auditory processing (Hunt *et al*., 2005).

In adult mammals, plasticity within cortical fields has been observed, but the magnitude of the reorganization is much less pronounced than that observed in developing animals. The studies that examined the relationship between sensory experience and cortical map reorganization detailed the precise conditions under which plasticity will occur and described the map changes that were generated under those conditions. For example, studies in which monkeys were trained on digit discrimination tasks demonstrated a direct relationship between increased discrimination performance and an increase in the cortical space in

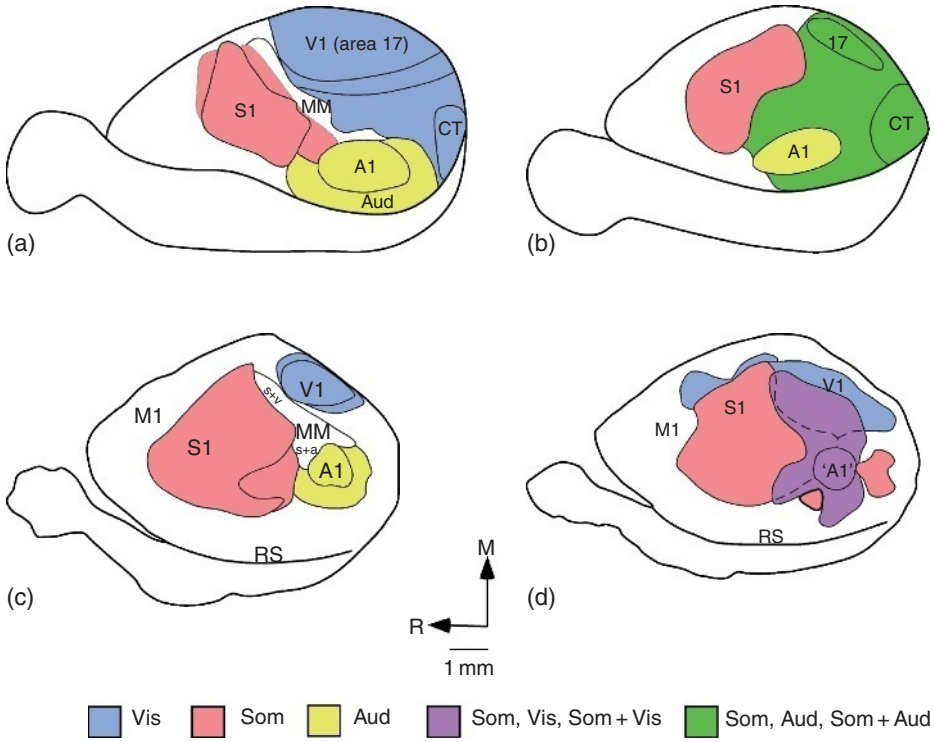

Figure 7 The organization of neocortex in normal opossums (a), opossums bilaterally enucleated very early in development (b), normal mice (c), and congenitally deaf mice (d). In the normal animals, both cortical fields and cortical domains are illustrated. In the bilaterally enucleated opossum, all of cortex that would normally be involved in visual processing, contains neurons responsive to somatic, auditory, or both somatic and auditory stimulation (green). In the congenitally deaf mouse, the cochlea is still present and a reduced eighth nerve exists, but no auditory driven activity is present. In this mouse all of cortex that would normally be devoted to processing auditory inputs contains neurons responsive to somatic, visual, or both somatic + visual stimulation. In both of these animals, the cross modal plasticity is extremely large such that all of cortex that is deprived of normal inputs is responsive to new types of sensory stimulation. In both mice and opossums, the cortical areas deprived of their normal inputs can still be identified architectonically, but at least in the opossum, the fields are smaller than in normal animals. a, auditory; A1, primary auditory area; Aud, auditory; M1, primary motor area; MM, multimodal; RS, rhinal sulcus; s, somatosensory; S1, primary somatosensory area; Som, somatosensory; v, visual; V1, primary visual area; Vis, visual. b, Modified from Kahn, D. M. and Krubitzer, L. 2002. Massive cross-modal cortical plasticity and the emergence of a new cortical area in developmentally blind mammals. *Proc. Natl. Acad. Sci. USA* 99, 11429 11434. d, Data from Hunt, D. L., Yamoah, E. N., and Krubitzer, L. 2006. Multisensory plasticity in congenitally deaf mice: How are cortical areas specified? *Neuroscience* 139, 1507 1524.

S1 (area 3b) devoted to the trained digit, while no expansion of adjacent nontrained digits was observed (Figure 8a; Recanzone *et al.*, 1992a, 1992b). Further, a requisite of the expansion was that the animal must attend to the task; repeated passive stimulation of the digit alone did not result in an expansion. Similar results have been observed for the auditory and motor cortex. In the auditory system, discrimination training of particular frequencies leads to an expansion of the cortical space devoted to that frequency (Figure 8b; Recanzone *et al.*, 1993). Likewise, training in a motor control task that involves particular hand movements, results in an expansion of those movement representations in motor cortex (Nudo *et al.*, 1996). These studies are important because they are the first to demonstrate a direct relationship between alterations in the neocortex with learning, and thus, the neural substrate for behavioral fluidity within the life of the individual.

The studies of developmental and adult plasticity demonstrate that peripheral morphology, sensory driven activity, and in normal circumstances, the behaviors associated with sensory reception play a large role in generating aspects of cortical organization including sensory domain assignment, cortical field size, the amount of space devoted to representing a particular body part or sensory receptor surface, and cortical and subcortical connectivity. These alterations are independent of the genes intrinsically expressed in the neocortex, which restricts the avenues along which evolution can travel. Thus, despite these restrictions, a fair amount of functional and anatomical fluidity is possible both within the life of an individual and in species over the course of evolution.

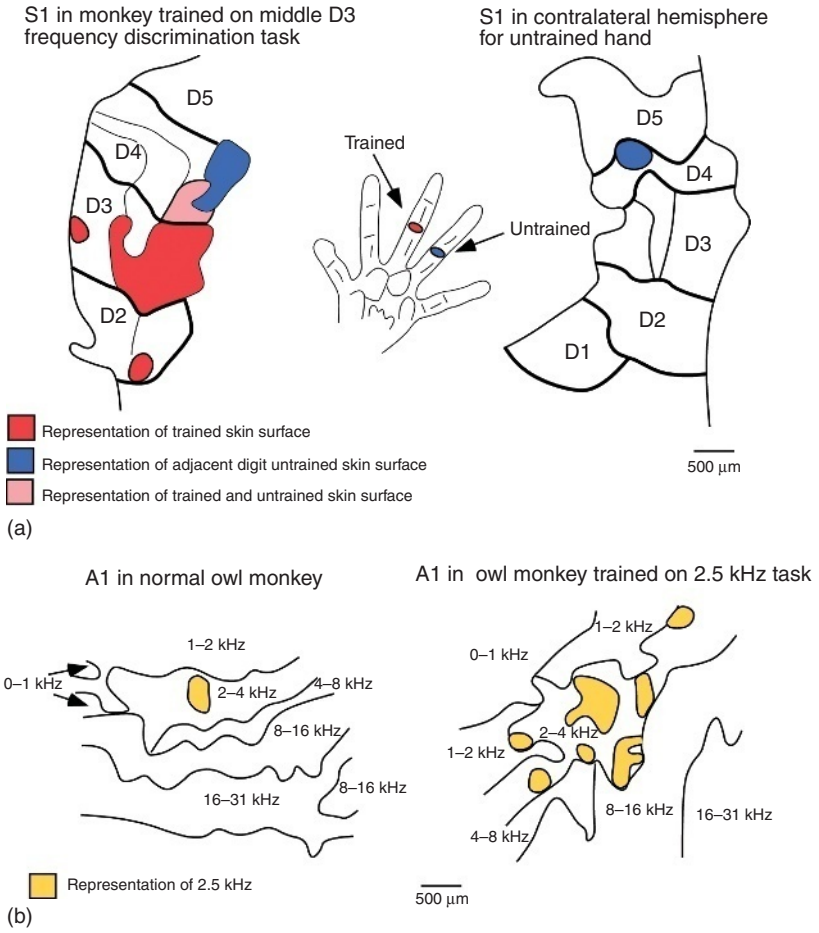

Figure 8 Cortical plasticity in adult owl monkeys following: a, somatosensory and b, auditory training. In the somatosensory cortex, training on somatosensory discrimination tasks increases the animal's ability to detect differences between two different stimuli, and this improvement in discriminatory ability is associated with an increase in the amount of neocortex devoted to representing the skin of the trained digit (red). In this case, the middle glabrous D3 was trained, and the contralateral S1 representing that portion of D3 (red) had an expanded representation compared to nontrained digits (blue). This plasticity was not observed in the hemisphere ipsilateral to the trained hand. Indeed, the portion of the cortex that represents the same location on the skin of the hand opposite to that trained was so small it was not found. A similar result was observed for the primary auditory cortex (A1). In owl monkeys trained on a 2.5 kHz discrimination task, the amount of cortex devoted to representing this frequency was expanded (b, yellow in left panel). A1, primary auditory area; S1, primary somatosensory area. a, Modified from Recanzone *et al.* (1992a, 1992b). b, Modified from Recanzone, G. H., Schreiner, C. E., and Merzenich, M. M. 1993. Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J. Neurosci.* 13, 87 103.

23.4 The Evolution of Cortical Fields

Earlier in this article we described the basic plan of cortical organization that all mammals possess, likely due to inheritance from a common ancestor (homology). Despite the large alterations that can occur in peripheral morphology, use, and lifestyle, the basic aspects of organization and connectivity of these fields are highly stable across lineages. However, there are modifications to this plan of organization, and a comparative analysis reveals that, at least at the systems level, these modifications take a similar form. In this section, we will describe some of the alterations that have been made to the cortical sheet in general, and to cortical fields in particular. We then postulate how some of these changes may have arisen in evolution, based in part on the information we have gained regarding the developmental mechanisms that construct cortical fields and their connectivities.

23.4.1 Changes in the Size of the Cortical Sheet

In addition to considering the cortical field in isolation, it is also necessary to consider general features of the brain as a whole that vary in predictable ways across species, which in turn have a large impact on the internal organization of the neocortex and the cortical field. The most

obvious feature is a change in the size of the brain and the size of the cortical sheet. Observations in a variety of mammalian brains indicate that there are two distinct types of changes in cortical sheet size, one in which the entire brain and its parts, including the neocortex, increase in size proportionately, and one in which there is a disproportionate expansion of the neocortex relative to the size of the rest of the brain.

Proportional changes in the overall size of the brain can result in an absolute increase in the size of the cortical sheet and the size of cortical fields. For instance, marsupials range in size from 4g to 67kg. Like the body, the range in brain size in marsupials is extreme. The marsupials we have examined in our laboratory include the dunnart (marsupial mouse, *Sminthopsis crassicaudata*), striped possum (*Dactylopsila trivirgata*), quoll (*Dasyurus hallucatus*), and short-tailed opossum (*Monodelphis domestica*; see Huffman *et al.*, 1999). In all but the striped possum, the most remarkable difference in the brains of these animals is that of absolute size. For example, the quoll and dunnart are both Polyprotononts from the family *Dasyuridae*. They differ substantially in body size with the dunnart weighing an average of 10g, and the quoll weighing an average of 750g. However, both are terrestrial hunters, occupy a similar niche, and have similar sensory specializations related to their predatory lifestyles (i.e., well-developed visual system). Examination of the neocortex of each animal demonstrates a clear difference in absolute size. However, much of the organization in terms of relative location and size of primary cortical fields are remarkably similar. This is best illustrated when the quoll brain is scaled to that of the dunnart. This scaling of brain size to body size and neocortex size relative to the rest of the brain is observed in other orders of mammals as well. For example, in a wonderful comparative analysis by Campos and Welker (1976), the neocortex of the capybara and guinea pig were compared. These investigators demonstrated that the size and relative location of primary cortical fields in the very large capybara compared to the much smaller guinea pig scales with the size of the body and the size of the brain as a whole (Figure 9).

The idea that the size of a cortical field scales linearly with brain size must be qualified. Comparative analysis has also shown that with dramatic specializations in the sensory epithelium, concomitant changes occur in the amount of neocortex devoted to that specialized sensory system, and the sizes of primary areas associated with that sensory system increase. Thus, if cortical sheet size is held constant and the internal organization of two highly derived species is compared, then differences in the allotment of neocortex and cortical field size can be readily observed.

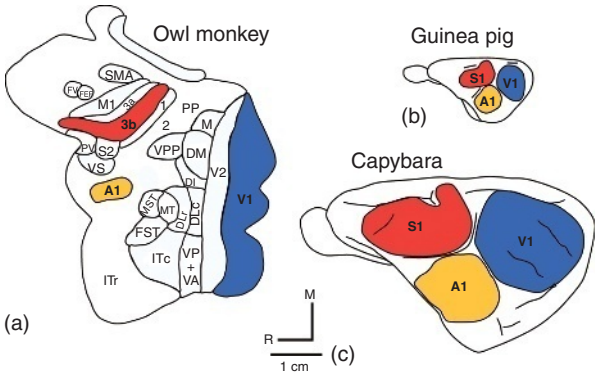

Figure 9 The organization of primary cortical fields in the: a, owl monkey; b, guinea pig; and c, capybara drawn to scale. In some species, the size of the brain has increased with body size, and the neocortex has increased in size proportionately with the rest of the brain (capybara). In this case, cortical field size has scaled linearly and the organization of the neocortex is much like that of other smaller rodents such as the guinea pig. In mammals, such as the owl monkey, whose body size is about ten times smaller than that of the capybara, the neocortex has enlarged disproportionately to the rest of the brain, although its absolute size approximates that of the guinea pig. With this disproportionate increase, the size of primary fields is reduced and more cortical fields are present. A1, primary auditory area; DLc, caudal division of dorsolateral visual complex; DLr, rostral division of dorsolateral visual complex; DM, dorsomedial visual area; FEF, frontal eye field; FST, fundal superior temporal area; FV, frontal ventral eye movement field; ITc, caudal division of inferotemporal cortex; ITr, rostral division of inferotemporal cortex; M, medial visual area; M1, primary motor area; MST, medial superior temporal area; MT, middle temporal visual area; PP, posterior parietal cortex; PV, parietal ventral area; S1, primary somatosensory area; SMA, supplementary motor area; V1, primary visual area; V2, secondary visual area; VA, ventral anterior area; VP, ventral posterior nucleus; VPP, ventral posterior parietal area; VS, ventral somatosensory area. a, Adapted from Krubitzer, L. and Kaas, J. H. 1993. The dorsomedial visual area of owl monkeys: Connections, myeloarchitecture, and homologies in other primates. *J. Comp. Neurol.* 334, 497 528. b and c, Modified from Campos, G. B. and Welker, W. I. 1976. Comparisons between brains of a large and a small hystricomorph rodent: Capybara, Hydrochoerus and guinea pig, Cavia; neocortical projection regions and measurements of brain subdivisions. *Brain Behav. Evol.* 13, 243 266.

The second type of size change that can occur is a disproportionate increase in the size of the neocortex compared to the rest of the brain. This results in a change in the pattern of neocortical organization. As in proportional increases in brain size, a disproportionate increase results in an absolute increase in the size of homologous cortical fields; however, the increase is less extreme than in the former type of size change. Furthermore, with a disproportionate

increase an additional organizational change to the neocortex is observed in that the number of cortical fields increases (Figure 9). This is nicely illustrated by comparing species that have different sized bodies, a similar absolute neocortical size, but a different neocortical size relative to brain and body size. For instance, although the capybara is well over 50 times the size of the owl monkey (50–70 kg vs. 1 kg), the neocortex of the owl monkey is disproportionately expanded, and its absolute size approximates that of the capybara. Examination of the neocortex of both species reveals very different types of organization. In the capybara, V1, A1, and S1 are large and compose much of the neocortex. In the owl monkey, V1, A1, and S1 are smaller than in the capybara, but many more cortical fields are present (Figure 9).

The question of how a disproportionate increase in neocortical size results in an increase in cortical field number is difficult to answer. It is possible that an increase in cortical field number, with an increase in the size of the neocortex relative to the rest of the brain, is due to a physical mismatch in the target (cortical sheet) and the projection zone (dorsal thalamus), or to a mismatch in the molecular coordinates between the thalamus and the cortex. This mismatch may result in new combinations of thalamocortical connections projecting to the expanded cortical sheet, in addition to the retained, highly restricted thalmocortical patterns of the primary and second sensory fields.

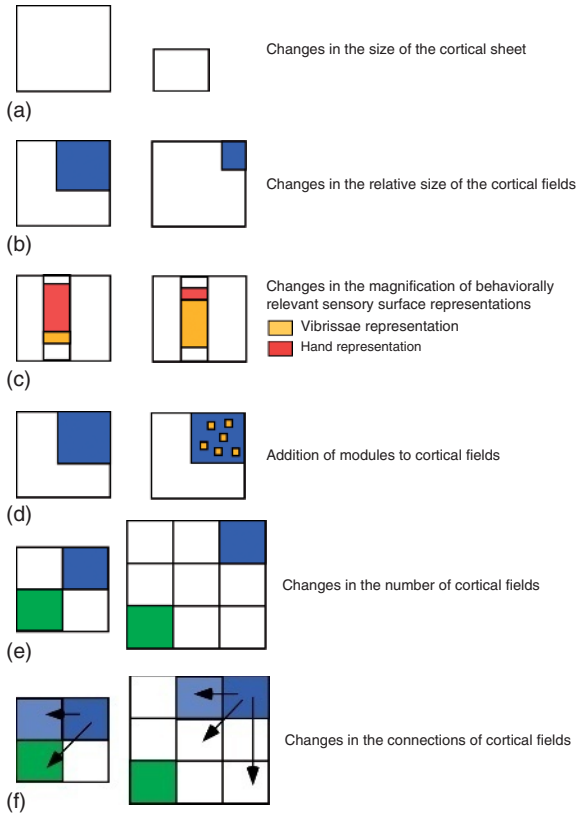

Figure 10 Modifications to the neocortex: a schematic representing the types of systems level changes that have evolved in different mammals. These changes, although few in number, presumably account for the wide range of behavioral differences observed in different lineages. Modified from Krubitzer, L. and Kaas, J. 2005. The evolution of the neocortex in mammals: How is phenotypic diversity generated? *Curr. Opin. Neurobiol.* 15, 444 453.

23.4.2 What Features of the Cortical Field Have Changed during Evolution?

In addition to changes in the size of the cortical sheet, several types of modifications have been made to the evolving neocortex (Figure 10). These modifications have been well documented (Krubitzer, 1995; Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005) and include:

1. changes in the relative size and internal organization of cortical fields,
2. changes in lamination of cortical fields,
3. changes in cell types,
4. changes in cortical thickness,
5. changes in the connections of cortical fields,
6. changes in the number of cortical fields,
7. the addition of modules to cortical fields, and
8. changes in the size of the cortical sheet (see above).

Interestingly, the brevity of this list of possible systems level modifications that brains have undergone or potentially could undergo suggests that it must be extremely difficult to modify the neocortex in evolution. Indeed, while we cannot predict the exact changes that may occur in future brains, we could predict with a fair amount of certainty what would not happen, and the types of changes that one would likely see. The observation that the types of modifications that have been made to the brain are limited indicates that these systems level modifications can generate a tremendous amount of phenotypic variability in terms of behavior.

23.4.3 The Module and Cortical Field Evolution

The module has been described in sensory cortex for a variety of different mammals (Figure 11). Modules are smaller units of organization that reside within a classically defined cortical field, and they have a long and dynamic history. Mountcastle (1957) described the first module, termed the cortical

column, almost 50 years ago (also see Mountcastle, 1978). He described the cortical column as a fundamental unit of cortical organization composed of a vertical group of cells extending through all of the cortical layers. This unit should not be considered as a fixed structure, but as a continuum with set dimensions, and no absolute boundaries. The modern concept of the module is different than its original conception in that it refers to different configurations of horizontal or tangential cell groups that do have fixed boundaries, and do not necessarily traverse all cortical layers. We have defined modules as "small architectonic, neuroanatomical, and physiological territories that can be distinguished from other tissue within the classically defined cortical field" (Manger *et al*., 1998).

Modules have been observed in a number of different cortical fields in different mammals and examples include barrels in rodent S1, blobs in V1 of primates, stripes in S1 of the star-nosed mole, ocular dominance bands in V1 of primates, and cytochrome oxidase (CO) bands in V2 of primates, to name a few (Figure 11). Although modules are a common feature of cortical organization that most mammals share, in most instances they are homoplaseous. The similarity of size and structure of modules across mammals argues that large constraints must be placed on evolving nervous systems. While evolution has been likened to a 'tinkerer', the bag of tools used to generate new phenotypes and the genetic material available for construction is highly limited. Thus, while the particular module itself may be homoplaseous, its presence may be due to homologous developmental programs (coordinated patterns of genetic interactions) that unravel in a particular molecular, neural, and sensory environment.

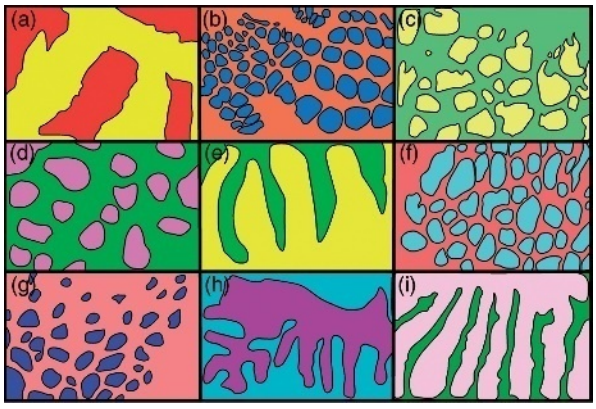

Figure 11 A schematic representing the many types of modules that have been identified in different sensory cortical areas in different mammals. While independently evolved or homoplaseous, the similarity in structure, shape, and size indicates that there are similar constraints imposed on the evolving and developing nervous system. a, Myelin bands in V2 of squirrel monkeys; b, barrel cortex in S1 of rats; c, modules in insular cortex of dolphins; d, clusters in entorhinal cortex in macaque monkeys; e, ODCs in V1 of talapoin monkeys; f, clusters in entorhinal cortex of humans; g, barrel cortex in S1 of brush-tailed possums; h, electrosensory/mechanosensory bands in S1 of platypus; i, rhinarium bands in S1 of the star-nosed moles. Modified from Manger, P., Sum, M., Szymanski, M., Ridgway, S., and Krubitzer, L. 1998. Modular subdivisions of dolphin insular cortex: Does evolutionary history repeat itself. *J. Cog. Neurosci.* 10, 153 156.

The identification of modules within cortical fields has implications for how a cortical field is defined. The traditional, and still dominant, view of cortical organization holds that the neocortex is compartmentalized into highly discrete cortical areas. However, the evidence for modular organization in cortical fields calls into question the traditional view of neocortical compartmentalization. Modules meet most of the criteria that generally are used to define a cortical field in that they are architectonically or histochemically distinct, have a unique set of connections, and contain neurons that are functionally distinct. When considered together, they form a complete representation of the sensory epithelium. An apt comparison between traditional and modern views of cortical fields is illustrated well for V1 and V2 of squirrel monkey neocortex (Figure 12). Until relatively recently, V1 and V2 were described as discrete, homogeneous representations of the visual hemifield with a distinct architectonic appearance and pattern of connectivity. The use of new histochemical staining techniques, optical imaging techniques, and fine-grained electrophysiological exploration of these fields has provided a very different view compared to traditional views. Rather than appearing as homogenous regions of cortex, both V1 and V2 have been further divided into modules. V1 is composed of blobs, interblobs, orientation columns, and ODCs. V2 is composed of thick and thin CO dense bands as well as interbands, and contains multiple representations of the visual hemifield.

Electrophysiological recording experiments of V2 in cebus monkeys and optical imaging experiments in macaque monkeys indicate that there is a re-representation of the same portions of the visual hemifield in these different bands (Rosa *et al*., 1988; Roe and Ts'o, 1995). Therefore, there is more than one map of the visual field in V2, and the separate maps are architectonically, histochemically, and connectionally distinct. These results suggest that 'chunking' V2 into one large, coherent field may

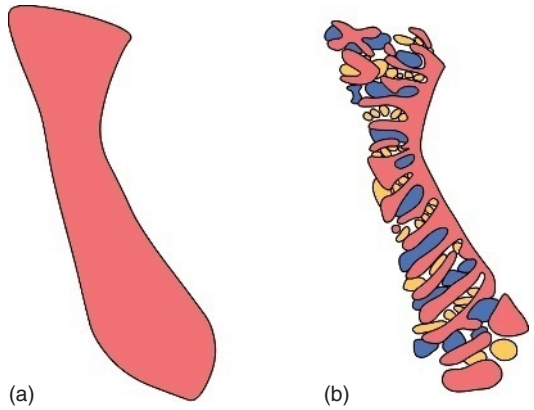

Figure 12 A schematic representing the: a, traditional and b, modern view of the organization of V2 in monkeys. Traditionally, V2 was considered to be a single, homogenous field adjacent to the rostral border of V1. Anatomical and functional studies (Rosa *et al.*, 1988; Roe and Ts'o, 1995) of the organization of V2 have since determined that it is modularly organized, and that there appear to be three independent representations of the visual field within this traditional area, associated with different histochemically identified stripes. These different strips, or bands in V2, have different patterns of connectivity. Thus, a new interpretation of this region of cortex is that three separate, completely interdigitated fields exist within the traditional V2. Adapted from Krubitzer, L. and Kaas, J. H. 1990. Convergence of processing channels in the extrastriate cortex of monkeys. *Vis. Neurosci.* 5, 609 613.

not be appropriate. Rather, V2 in primates could be considered as three separate, interdigitated fields (Figure 12).

In terms of modular organization and the evolution of cortical fields, we have proposed previously (Krubitzer, 1995; Krubitzer and Kahn, 2003) that modules reflect a stage in cortical field evolution within a lineage; that 'snapshot' alluded to in the introduction of this article. As noted earlier, we believe that a cortical field represents, at least in part, some patterns of connectivity on the cortical sheet. Within the life of an individual (particularly during development), and across species over time, this pattern of connectivity can shift such that the position of homologous fields is geographically displaced (Figure 13). Further, there are discontinuities within a cortical field (modules) that may represent an invasion of new inputs, discorrelated with existing inputs. This could represent fields completely embedded within other fields, as we believe is the case for V2. Over time, if selected for, these inputs coalesce and form partially invaginated regions, which may ultimately completely coalesce to form a new cortical field (Figure 13; see Krubitzer, 1995; Krubitzer and Kahn, 2003 for full explanation). Thus, the different modular and nonmodular organization of cortical fields within different sensory systems in different mammals represents different stages of this process in each lineage.

23.4.4 What Constrains Cortical Evolution?

There are three observations from comparative studies which indicate that neocortical evolution must be highly constrained. The first is the very presence of a common constellation of cortical fields, which was outlined in Section 23.2. That these fields and aspects of their connectivity and function can be modified substantially is without question. However, what is notable is that they have never been completely lost, even in highly derived mammals, such as the blind mole rat, which has micro-ophthalmic eyes covered by skin and a highly degraded retinofugal pathway (Klauer *et al.*, 1997; David-Gray *et al.*, 1998). The reduced visual system in blind mole rats is only involved in the circadian system. Yet, despite the lack of use of this system for visual functions, the geniculo-cortical pathway is still intact, and area 17 or V1, as architectonically defined, is still present and resides in the far rostral pole of the neocortex. The second observation is the very limited types of systems level changes that have been made to the brain, as outlined above. This suggests that the neocortex is not altered in a random fashion. The final, related observation is the instance of homoplasy. The fact that remarkably similar modules have formed, despite hundreds of millions of years of independent evolution, indicates that considerable constraints are placed on evolving nervous systems and that modularity is a part of this process.

What imposes constraints of the evolving neocortex? Primarily, genes constrain evolution and limit the types of phenotypic modifications that are possible, and these constraints are due to both pleitropy and contingency. Genetic pleitropy, or the fact that a single gene controls a number of activities in development, leads to functional integration, and as a result, it exerts a restriction on the number of possible changes that could be effected by any particular gene. Genetic contingencies restrict neural development and evolution in that any genetically mediated event is most often dependent on one or more prior genetic events and in turn may instruct some combination of downstream genetic events. Thus, it is rather difficult to substantially modify an organism by extreme genetic manipulations. This suggests that small genetic alterations can generate large phenotypic

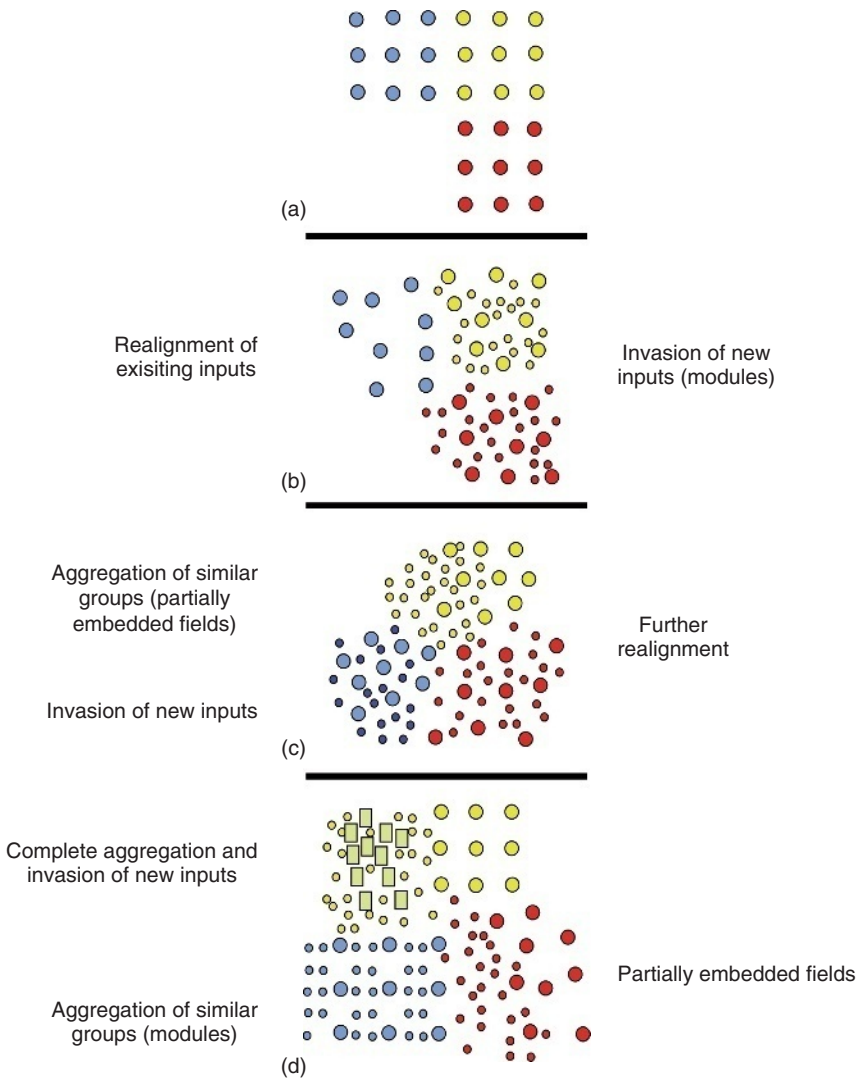

Figure 13 A theory representing the relationship between modules and the evolution of cortical fields. a, represents a hypothetical state of the neocortex with different colored circles representing a cortical field, or some pattern of thalmocortical interconnections within a field. An invasion of new inputs to existing fields (b, small red and yellow dots) results in a modular organization within these fields and a realignment of existing inputs. Modularly organized inputs may aggregate to form a partially embedded field (small yellow dots in (c)), causing a further realignment of fields, and new inputs may invade existing fields (small blue dots). Inputs that initiated within a cortical field and formed a modular arrangement (yellow dots), may completely aggregate to form a new field, and new inputs may invade this field (yellow squares). We propose that this is how cortical fields evolve and that each figure (a d) illustrates snapshots or frozen frames that we observe in extant mammals. Modified from Krubitzer, L. 1995. The organization of neocortex in mammals: Are species differences really so different? *Trends Neurosci.* 18, 408 417.

modifications and that phenotypic change can be accomplished in the absence of nonactivity-dependent genetic change.

In addition to genetic forces, there are also substantial constraints imposed on evolving nervous systems by the environment in which an animal operates. When we discuss the nervous system, we rarely talk about physics, but the physical parameters of any environment are set and quantifiable. For example, nervous systems must contend with gravity, self-movement, and the movement of objects and other animals in time and in the three dimensions of our universe. The physical parameters of a stimulus are also important, and include the presence or absence of photons, the rate at which a stimulus travels and bends through space, the diffusion of molecules through different media, and the perturbations of molecules in different media, such as changes in air pressure. Although the amount and patterns of a physical stimulus that impinge on any given mammalian sensory receptor array may be distributed

differently in different terrestrial and aquatic environments, and in diurnal versus nocturnal mammals, the actual physical unit that is transduced, such as a photon, is invariant and therefore serves to anchor the evolutionary boat. While it seems clear that genes and their highly coordinated activities constrain a system, it is important to keep in mind that within a population of individuals, both the spatial and temporal expression of genes involved in the processes described above are normally distributed. This natural variability allows for some degree of flexibility within a relatively fixed genetic environment. Energy, while absolute, is variably distributed within any environment such that the amount and pattern of photons falling on a retina, for example, is different in different ecospheres. While we have noted above that both genes and the physical parameters of the environment constrain the development and evolution of mammalian neocortex, and ultimately behavior, it should be noted that the combinatorial possibilities of these two fixed parameters can generate a high number of degrees of freedom for potential phenotypic outcomes despite these constraints.

Despite these constraints, it is clear that sensory driven activity and the animal's own movement within an environment can generate a large amount of phenotypic variability. We have discussed the types of systems level changes that can occur with variable use and under particular environmental conditions in the developing and adult nervous system. But, how do such alterations become genetically encoded within a population and ultimately evolve?

At first reading, the idea that acquired traits can somehow evolve seems to smack of Lamarkianism. However, the notion that a living organism's ability to respond to environmental fluctuations has a genetic basis is relatively well established and compatible with Darwinian selection. This idea was formulated over a century ago by Baldwin (1886, 1902), and termed the Baldwin effect. The Baldwin effect is the ability of an animal to respond optimally to a particular environment. This effect could hold true for behaviors as well as anatomical features or aspects of functional organization of the neocortex. Thus, the Baldwin effect is the idea that genes for plasticity evolve, and that the phenotype that is optimal for a given environment could become genetically encoded and evolve if the genes that encode for plasticity and those for the actual phenotypic feature in question covary (Figure 14). This characteristic would then be selected for and be displayed even in the absence of the original environmental stimulus that induced it. This phenomenon was experimentally tested by Waddington and termed genetic assimilation (Waddington, 1959, 1961).

A related process has recently been described as 'evolvability'. Evolvability is the ability of an organism to generate heritable, selectable phenotypic variation (Kirschner and Gerhart, 1998). These authors propose that selection for evolvability has occurred and has three components. At the level of the individual, the ability to be flexible would contribute directly to physiological fitness. At a group level, individuals within the group would be buffered against the lethal effects of mutation. Finally, at the level of the clade, such an ability would allow the clade to radiate into new (emptied) environments. Recently, experimental support for the notion that evolvability is a selected trait has been put forward by Earl and Deems (2004). They find evidence that the rate at which genetic change in the form of recombination, substitutions, and transpositions occurs is variable in different lineages and is genetically encoded.

Taken together, it appears that activity can regulate gene expression which, in turn, can regulate anatomical and functional characteristics of the developing nervous system within an individual lifetime. This process, or the ability to respond to some external stimulus, is optimal in some individuals and can be selected for (the Baldwin effect). In a particular environment, an optimal trait can become genetically encoded in a population and evolve if there is a strong correlation between phenotypic and genotypic space (genetic assimilation). Finally, the ability to respond optimally and to assimilate, while maintaining a fundamental plan of organization, is a variable trait itself, and is the target of selection (evolvability).

23.5 Conclusions

How should we view the evolution of the cortical field? While a cortical field has been previously proposed to be a fixed, genetically determined structure that occupies some area on the cortical sheet, a comparative analysis highlights the dynamic nature of a cortical field within the life of an individual and over generations within and across lineages. We believe that the cortical field is an event or a process, not an entity that is easily captured. While genes and the physical environment impose severe constraints on this process, neural activity within the developing organism generated by the highly constrained physical

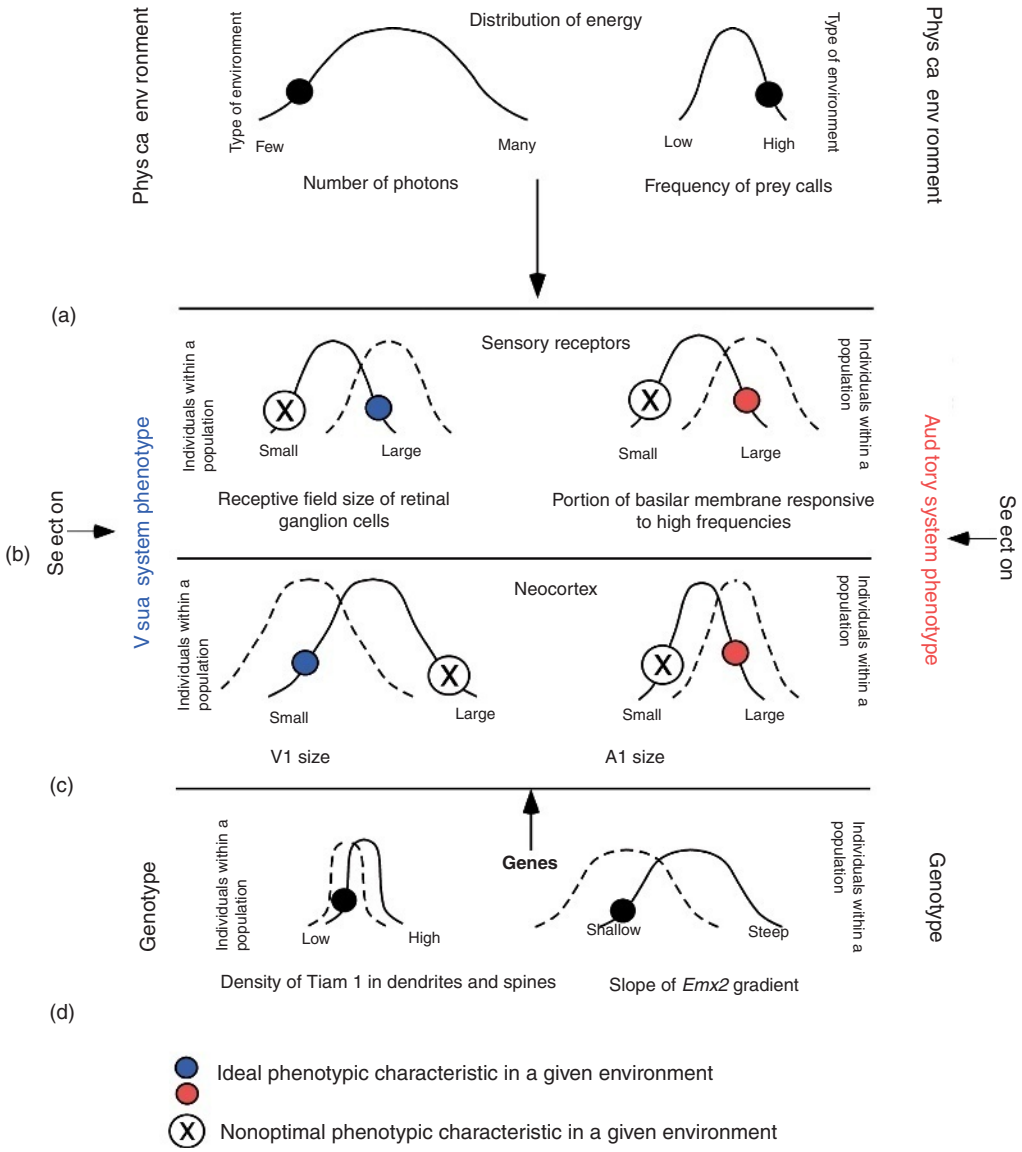

Figure 14 A schematic illustrating the Baldwin effect and genetic assimilation, and how features of cortical organization that are initially activity dependent, become encoded by genes and evolve. Within a particular environment (a), light levels may be low, and prey call frequency may be high (black dots on the distributions in a). The optimal sensory receptor phenotype (b), receptive fields size of ganglion cells distribution of frequency on the basilar membrane (blue and red dots respectively) are normally distributed within a population. For the neocortex (c), the optimal phenotype for this environment would be a small V1 and a large A1 (blue and red dots respectively). These size differences of cortical fields are normally distributed within a population. Finally, particular genes which are normally distributed in a population (d) control aspects of cortical field organization either directly via *Emx2*, or indirectly through activity-dependent mechanism (e.g., Tiam 1). Although natural selection acts on the phenotype, the genes that control for the particular phenotype in question as well as plasticity may co-vary, and thus allow activity-dependent contributions to the phenotype to become genetically encoded and evolve. This type of selection could shift the distribution (dashed lines) of genes that both enable plasticity (activity dependent), as well as those directly determine the characteristic (e.g., *Emx2* and size of cortical fields). A1, primary auditory area; V1, primary visual area. Modified from Krubitzer, L. and Kaas, J. 2005. The evolution of the neocortex in mammals: How is phenotypic diversity generated? *Curr. Opin. Neurobiol.* 15, 444 453.

parameters of the environment, and the movement of the organism itself in time and space, serves to loosen these constraints. An extant mammal represents only a snapshot in this process. This snapshot may give the impression that a cortical field is static, when, in reality, we have simply caught a frozen moment in the continually moving picture of life.

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24 The Evolution of the Dorsal Thalamus in Mammals

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Glossary

layers and subnuclei	Parts of nuclei sometimes differ somewhat in histological characteristics, connections, and neuron response characteristics to the extent that they are recognized as subnuclei or layers, while having enough features in common to include them in a nucleus. Sometimes subnuclei are called nuclei.
nuclear complex	Adjoining nuclei of related functions are sometimes grouped into a complex such as the pulvinar complex. In some instances, the nuclei of the complex may have differentiated from a single ancestral nucleus.
nucleus	A collection of neurons and other cells in the thalamus that are united by a common function. Nuclei have been historically identified in brain sections as groups of neurons that differ from surrounding thalamus in the packing of neurons, cell types, and other histological characteristics. Nuclei should also differ in connections and, of course, the response properties of their neurons.
thalamus	A part of the forebrain between the cerebral cortex and midbrain. This review concerns the dorsal thalamus, the division that is largest in mammals, and projects to neocortex.

24 Introduction

While the region of the diencephalon called the thalamus includes the ventral thalamus, the hypothalamus, and the epithalamus, authors commonly use the term to refer to the dorsal thalamus only, the topic of this review. The dorsal thalamus of mammals is a collection of nuclei in the diencephalon with neurons that project to neocortex. If the neocortex is removed, the projection neurons die, leaving the nuclei of the dorsal thalamus severely degenerated, while the nuclei of the ventral thalamus, hypothalamus, and epithalamus remain intact or slowly respond to changes in the dorsal thalamus (Rose and Woolsey, 1943). In this way, the dorsal thalamus can be experimentally distinguished from other parts of thalamus. This is not to say that all of the neurons of the dorsal thalamus project to neocortex, as there are many intrinsic neurons as well, and a number of neurons project to the striatum (Jones, 1985), a major target of some of the nuclei of the dorsal thalamus of the reptilian ancestors of mammals. The major steps in the evolution of the thalamus in vertebrates, and the transition from the thalamus of reptiles to that of mammals, have been discussed in this series and elsewhere (Butler, 1994; Puelles, 2001; see Evolution of the Nervous System in Reptiles). Therefore, this review focuses on the specializations of nuclei of the mammalian thalamus as the various branches of the mammalian radiation lead to the over 4500 extant species (Wilson and Reeder, 1993). Of course, there have been few or no observations on the thalamus of most of these species, so the concentration is necessarily on the thalamic nuclei of the few well-studied taxa.

Jones (1985) defined a thalamus nucleus as "a circumscribed region of cytoarchitecture receiving a particular set of afferent connections and projecting within the borders of a particular field or fields." To elaborate on this definition, a nucleus is a collection of

neurons and other cells that are unified by participating in a common function or set of functions. In order to do this, neurons within a nucleus require a unique set of inputs and outputs, and a great number of other specializations are possible as well, including neurons with distinctive morphological and histochemical properties. Nissl stains may reveal nuclei distinguished by neurons of distinctive sizes and staining properties, and this has been the traditional approach toward defining and identifying nuclei. In current investigations, nuclei are often identified with more assurance by differences in the expression of neurotransmitters and other components of neurons. Nevertheless, a major problem in comparative studies of thalamic organization is in reliably distinguishing nuclei. Identifying nuclei that are poorly or differently differentiated in various taxa can be difficult and result in errors. Regions of the thalamus can be misidentified as nuclei or misnamed, a problem confounded by the lack of a standard nomenclature. Homologous nuclei are not only given different names in the thalamus of birds, reptiles, and mammals, but different names in different mammals, or even in the same mammal by investigators that favor either one or another name. Another problem is distinguishing parts of nuclei from subnuclei. For example, the ventroposterior medial (VPM) nucleus is histologically distinguishable from the ventroposterior lateral (VPL) nucleus, but both are parts of the same functional unit, the ventral posterior nucleus, that contains a systematic representation of the cutaneous receptors of the contralateral half of the body. The ventroposterior (VP) nucleus also goes by several other names, including the ventrobasal nucleus. Finally, there is the problem of identifying nuclei that are present in some mammals and absent in others. There is generally a reluctance to identify any brain structure as new (Striedter, 2005), because it is difficult to determine if an easily identified structure in some taxa is not present in some cryptic form in other taxa. A similar dilemma exists with regard to the evolution of cortical fields, but it has gradually become clear that some areas of primate neocortex, for example, have no apparent homologues in other mammals (e.g., middle temporal (MT) visual area of primates; see Kaas and Preuss, 1993). It seems likely that some thalamic nuclei have evolved in some branches of the mammalian radiation but not in others. The evidence has become very strong that early mammals had few cortical areas, and the number of areas has increased independently in several lines of mammalian evolution by adding new areas. A comparable pattern of evolution must have occurred for the dorsal thalamus of mammals, with the thalamus adding new nuclei as the cortex added areas, although not necessarily in a matching matter. But less is certain about the thalamus, as the organization of the mammalian thalamus has been less intensively investigated. Thus, this review starts by considering the well-defined thalamic nuclei and how they vary across taxa, and then addresses the issue of increased complexity and new nuclei. We start with the visual relay nucleus, the dorsal lateral geniculate nucleus (LGN) (called dorsal to distinguish it from the ventral lateral (VL) geniculate nucleus of the ventral thalamus), often simply identified as the LGN. As the neuroanatomist Rose (1971) noted, "In the dorsal thalamus itself our anatomical and functional knowledge is at its best when it concerns the projection nuclei of the great afferent systems." The situation remains much the same today (see The Evolution of the Basal Ganglia in Mammals and Other Vertebrates).

24.2 The Lateral Geniculate Nucleus

The dorsal LGN is a thalamic structure common to all mammals. The LGN receives inputs from the ganglion cells of the retinas of both eyes, and has neurons that project to primary visual cortex (area 17 or striate cortex). This is a pattern that has been retained from the reptilian ancestors of mammals: extant reptiles such as turtles have a small but distinct dorsal LGN with retinal input and projections to dorsal cortex, the homologue of mammalian neocortex (Hall and Ebner, 1970b; Hall *et al.*, 1977; Ulinski, 1986; Zhu *et al.*, 2005). The LGN is located on the lateral margin of the thalamus, where it is innervated by axons coursing in the optic tract as other axons and collaterals of axons continue on to the superior colliculus of the midbrain. Across mammals, the LGN differs greatly in histological appearance, from a scattered group of neurons that is only marginally distinct from the adjoining thalamus, to a well-segregated and variously laminated structure.

A common form of the LGN, found in some members of most of the major branches of the mammalian radiation, is a rather undifferentiated nucleus with no obvious substructure, such as the LGN of a hedgehog (Figure 1). This nucleus is characterized by a nearly uniform distribution of neurons that are not very different in appearance and distribution from those in adjoining parts of the thalamus. Nevertheless, there is a concealed lamination, as retinal projections from the ipsilateral eye occupy a dorsocentral oval in the nucleus

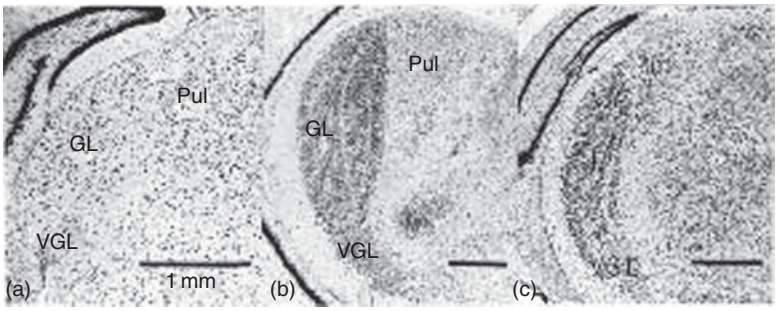

Figure 1 A coronal brain section stained for cells (Nissl preparation) through the dorsal lateral geniculate nucleus (GL), the ventral lateral geniculate nucleus (VGL), and the pulvinar (Pul) of, a, hedgehog, a small insectivore; b, a tree squirrel; and c, a tree shrew. Note that the nuclei are poorly differentiated from each other in the hedgehog, but well differentiated from each other in the squirrel and the tree shrew. Note also that the dorsal lateral geniculate nucleus has visible but different types of layers in the squirrel and tree shrew, and that no visible layers are seen in the nucleus in the hedgehog. The pulvinar is also more differentiated in squirrels and tree shrews. Scale bar: 1 mm.

surrounded by terminations from the contralateral eye (Hall and Ebner, 1970a). Thus, the LGN of hedgehogs appears to have a middle layer with inputs from the ipsilateral eye, and adjoining layers with inputs from the contralateral eye. The layer for the ipsilateral eye does not extend into the most ventral third of the nucleus because that is where the monocular visual field of peripheral vision of the contralateral eye is represented (Kaas *et al.*, 1972). A similar LGN is found in Afrotherian tenrecs (Künzle, 1988), North American opossums of the marsupial radiation (Royce *et al.*, 1976), some rodents such as rats (Reese, 1988), rabbits (Holcombe and Guillery, 1984), pangolins (Lee *et al.*, 1991), and echidnas and platypuses of the monotreme radiation (Campbell and Hayhow, 1971, 1972). Such a distribution of a poorly differentiated LGN with perhaps three cryptic layers argues that this type of LGN was present in early mammals and was retained in many branches of the mammalian radiation. As most early mammals were small and likely nocturnal (Kielan-Jaworowska *et al.*, 2004; Rose and Archibald, 2005; however, see Martin, 2006), a large, highly differentiated visual thalamus would not be expected, and the present-day mammals with a poorly differentiated visual thalamus are those mammals that are not highly visual.

In contrast to the mammals noted above, the LGNs of the highly visual mammals that have been studied have an architectonic appearance that is distinct from the adjoining thalamus, and typically have several layers of two or more types. Thus, squirrels, a highly visual rodent, have a large LGN of darkly staining neurons that has three visible layers. These layers are separated from each other by cell-poor septa (Figure 1), with one of these layers subdivided by a segregation of retinal inputs into a middle layer of ipsilateral retinal inputs, and two adjoining layers of contralateral retinal inputs, making five layers in all (Kaas *et al.*, 1972). As another visually dominated mammal, tree shrews, are small, squirrel-like mammals that are closely related to primates (Kaas, 2002), tree shrews have a clearly laminated LGN, but in a different pattern of six layers that are separated by cell-poor septa (Figure 1). The layers are further distinguished by inputs from either the ipsilateral or the contralateral eye, histochemical characteristics, and innervation by different types of retinal ganglion cells, including the ON and OFF and W-cell pathways (Conway and Schiller, 1983; Conley *et al.*, 1984; Diamond *et al.*, 1993). ON ganglion cells are those that respond to an increase of light in the excitatory receptive field (light onset), while OFF ganglion cells respond to the dimming of light (light offset). The W-cell pathway includes ganglion cells with thin axons and slow conduction, possibly homologous with the K pathway of primates (see below).

Other visual mammals also have complexly differentiated LGNs but of various types. Sanderson *et al.* (1984, 1987) have described the LGN of some of the diprotodont marsupials, an advanced order including kangaroos, wombats, koalas, and varieties of possums. The LGN of these marsupials includes a visibly laminated segment with three cytoarchitectonic regions, some of which subdivide further, and a segment without visible lamination, which is subdivided by regions of inputs from either the ipsilateral or contralateral eye. Across species, the number of eye-specific layers varies from 8 to 11. The LGN of different ungulates has been described as consisting of three to five layers (see for review, Sanderson *et al.*, 1984; Clarke *et al.*,

1988). Carnivores are generally described as having two dorsal layers: layer A for the contralateral eye, layer A1 for the ipsilateral eye, and three ventral C (smaller cell) layers (Kaas *et al.*, 1972, 1973). In addition, mink and weasels of Mustelidae taxon of carnivores have duplicated their A and A1 layers with one A and one A1 layer for ON retinal ganglion cells (those responding to light onset) and one A and one A1 layer for OFF ganglion cells (those responding to light offset) (Sanderson, 1974; LeVay and McConnell, 1982). The projections from the ON and OFF ganglion cells are mixed in the A and A1 layers of other carnivores. The echolocating bats typically have poorly differentiated visual systems with a simple LGN, while the crepuscular fruit-eating megabats have large eyes and an LGN of five or six layers (see Kaas and Preuss, 1993, for review). The LGN of gliding lemurs, considered close relatives of primates, appears to have six layers (Kaas *et al.*, 1978; Kaas and Preuss, 1993).

Primates are highly visual mammals, and this is reflected in the LGN (Kaas *et al.*, 1978). The basic lamination pattern (Figure 2) consists of two parvocellular layers (one for each eye), with inputs from a class of retinal ganglion cells (P cells) that are specialized for detailed object vision and color, and two magnocellular layers (one for each eye), with inputs from the M-cell class of ganglion cells that are important for motion detection and vision in dim light. In addition, small koniocellular neurons are sometimes recognized as scattered within the septal zones between layers or as forming distinct layers. In prosimian primates, two thick koniocellular layers are generally recognized (Figure 3), while thinner distributions of K cells are only sometimes recognized as layers. In addition to the layers noted above, the parvocellular layers subdivide to form four or more parvocellular layers or sublayers in some taxa of anthropoid primates (Kaas *et al.*, 1978). In general, the magnocellular and koniocellular layers are well developed in nocturnal primates, while the parvocellular layers are well developed in diurnal primates. Thus, the laminar pattern is complex and variable across primate taxa.

Other features of the LGN also vary across mammals. In general, primary visual cortex is the main target of LGN projection neurons, with very few projections to other visual areas. But this too is one of the variable features. In cats and at least some other carnivores, a major projection of one class of LGN neurons is to the second visual area, V2, and projections from another class of LGN cells extend across four visual areas (Stone, 1983). Some of the LGN neurons in primates also project to

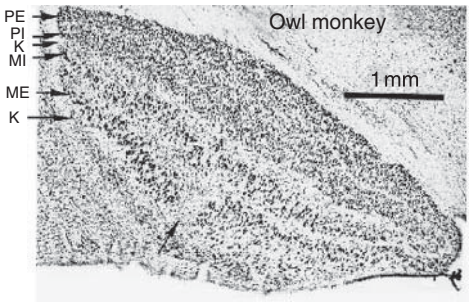

Figure 2 A parasagittal brain section through the dorsal LGN of an owl monkey. Owl monkeys are the only nocturnal monkeys, and this is reflected in the proportionately small parvocellular layers. Note that the external parvocellular layer (PE) is only marginally segregated by a cell-poor septum from the internal parvocellular layer (PI). Nevertheless, it is apparent that PE extends to the rostral pole of the nucleus (right) while PI does not, as PE represents the complete contralateral visual hemifield via the contralateral eye, while PI represents the slightly smaller binocular hemifield via the ipsilateral eye. The neurons of the magnocellular layers, MI (internal) and ME (external), are noticeably larger. ME, with input from the contralateral eye, also extends to the rostral pole of the nucleus, while MI, with input from the ipsilateral eye, does not. Koniocellular (K) layers of the smallest neurons are not commonly reorganized in anthropoid primates, although two K layers are widely recognized in nocturnal prosimian primates. In anthropoid primates, the two K layers noted here are well differentiated, and expanded in owl monkeys, suggesting that K layers are especially important for vision in dim light. The arrow pointing to the middle of ME indicates a small discontinuity in the layer that corresponds to the receptor-free oval of the optic disk (nerve head) of the contralateral eye (a discontinuity also occurs in PE of nearby brain sections). Such discontinuities are only apparent in mammals with good visual acuity. Modified from Kaas, J. H., Guillery, R. W., and Allman, J. M. 1973. The representation of the optic disc in the dorsal lateral geniculate nucleus: A comparative study. *J. Comp. Neurol.* 147, 163 180.

nonprimary visual areas in primates (Stepniewska *et al.*, 1999), including to visual areas such as the MT visual area that appear to be unique to primates. Thus, as new visual areas emerged in early primates or the immediate ancestors of primates, LGN projection patterns were altered to include these visual areas.

In summary, the poorly differentiated LGN with little substructure of early mammals appears to have differentiated in several ways in a number of branches of the mammalian radiation. There have been independent increases in numbers of cell layers. These layers appear to be segregating inputs from functionally different classes of retinal ganglion cells, inputs from the superior colliculus, and inputs from the ipsilateral and contralateral eyes. Some of the resulting segregations are similar, although independently derived, such as layers for ON and OFF ganglion cells in some carnivores

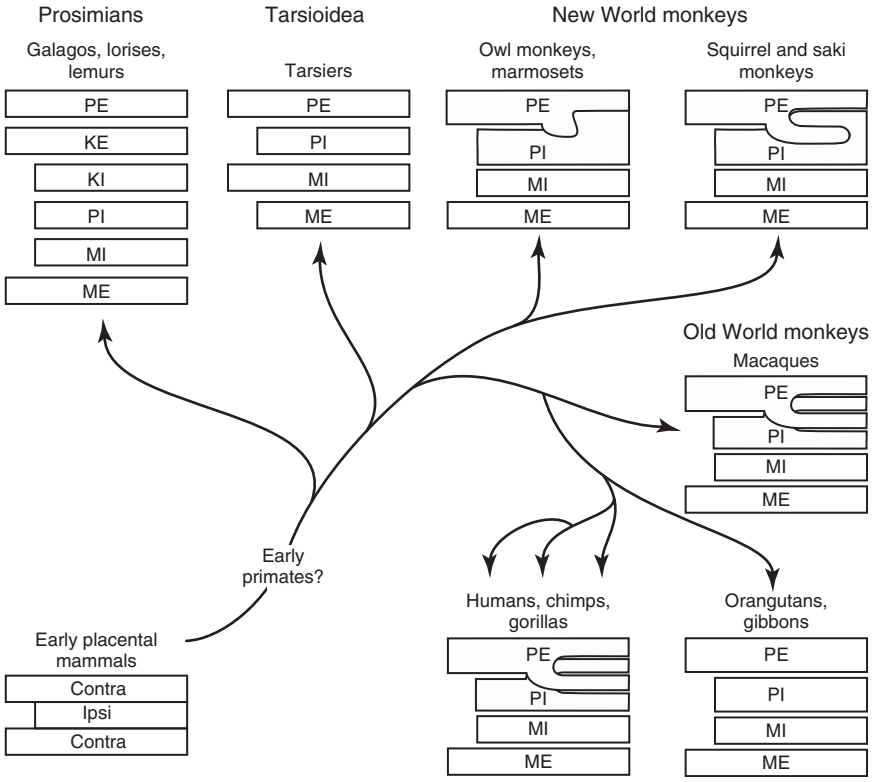

Figure 3 A schematic of the lamination pattern of the dorsal LGNs of primate taxa. Layers are labeled by the type of neurons that they contain (P, parvocellular layers; M, magnocellular layers; K, koniocellular layers) and their internal (I) or external (E) position in the nucleus. Short layers receive inputs from the ipsilateral eye and long layers from the contralateral eye. In some taxa, P layers subdivide and interdigitate, while in owl monkeys and marmosets, there is only a hint of this. Spaces between layers mean that the layers are well separated by septa. Note that the lamination pattern varies across primate taxa. Modified from Kaas, J. H., Huerta, M. F., Weber, J. T., and Harting, J. K. 1978. Patterns of retinal terminations and laminar organization of the lateral geniculate nucleus of primates. *J. Comp. Neurol.* 182, 517 554.

and in tree shrews. Other types of segregations of neurons into layers may be unique, as there is much structural diversity. What this means in terms of acquired functions is not completely clear, but highly visual mammals have more distinct layers and more layers, and nocturnal mammals emphasize different types of layers than do diurnal mammals. It is also important to remember that changes were not always in the direction of increased differentiation. The LGNs of the microphthalmic blind mole rats, for example, have greatly regressed (Cooper *et al.*, 1993).

24.3 The Visual Pulvinar

The pulvinar in primates is a complex of nuclei that are largely, but not completely, visual in function. Because this part of the thalamus was so prominent in primates, most early comparative neuroscientists considered the pulvinar to be unique to primates, and a homologous region was not recognized in other mammals. Instead, this part of the visual thalamus was thought to be the homologue of another part of the primate thalamus, the lateral posterior nucleus, which in primates is generally associated with the somatosensory system. Le Gros Clark (1932) was one of the first to recognize that "the pars posterior of the lateral nucleus is the homologue of at least part of what, in higher primates, is termed the pulvinar." Although evidence accumulated in support of considering parts of the primate pulvinar complex homologous with parts of the lateral posterior complex in other mammals, the use of the term lateral posterior to refer to the pulvinar in nonprimate mammals has usually, but not always, continued. To add to the confusion, both terms have been used in the same mammal to refer to different parts of the pulvinar complex, as is currently done in cats. This confusion of terms has undoubtedly hindered comparative studies of the organization, and studies of the visual thalamus in mammals. Here the term visual

pulvinar is used in all mammals to designate those parts of the thalamus that receive inputs from the superior colliculus and/or are reciprocally connected with visual areas of cortex.

The reptilian homologue of the mammalian pulvinar complex, if any, is somewhat uncertain. In both reptiles and birds, the optic tectum (the homologue of the superior colliculus) projects to a well-defined nucleus rotundus of the dorsal thalamus (see Belekhova *et al.*, 2003; for review). For some time, a homology of the mammalian pulvinar complex with nucleus rotundus has been widely accepted, but recently nucleus rotundus has been compared to the intralaminar complex of mammals (see Belekhova *et al.*, 2003, for review). While the issue remains unresolved, the bulk of the evidence appears to favor the long-standing view of a rotundus–pulvinar homology. In support of this view, Major *et al.* (2000) have provided evidence that the tectorotundal and tectopulvinar pathways are homologous to the level of even involving the same cell subtypes.

In early mammals, the pulvinar complex appears to have consisted of a rather poorly differentiated group of cells in the posterior thalamus, just medial to the LGN, much as in present-day hedgehogs (Figure 1a). In such mammals, this poorly differentiated group of neurons receives inputs from the superior colliculus and projects to visual cortex, including primary visual cortex and the second area, V2. Quite possibly two or three regions or nuclei can be distinguished based on differences in cortical and superior colliculus connections (Crain and Hall, 1980). Overall, this caudal portion of the thalamus receives superior colliculus inputs and projects to visual cortex in all mammals studied, but the size and histological differentiation of the region differ considerably. Thus, a large pulvinar nucleus is easily identified histologically in both squirrels and tree shrews (Figure 1), and has expanded and become more differentiated than in most mammals. Moreover, in both tree shrews and squirrels, the pulvinar is not a single nucleus, but a complex of several nuclei differing histochemically and in connections. Thus, in tree shrews the pulvinar complex consists of four nuclei (Figure 4a) (Lyon *et al.*, 2003a, 2003b). A large central nucleus (Pc) receives a retinotopic pattern of projections from the superior colliculus and projects to primary visual cortex and adjoining visual areas. A smaller dorsal nucleus (Pd) receives diffuse projections from the superior colliculus, stains darkly for acetylcholinesterase, and projects to higher-order visual areas of the temporal lobe. A small ventral nucleus (Pv) expresses high levels of the antigen for the Cat-301 antibody that identify large neurons with rapidly conducting axons, is interconnected with primary visual cortex and adjoining visual areas, while having few or no inputs from the superior colliculus. A small posterior division of the pulvinar complex (Pp) appears to receive diffuse inputs from the superior colliculus while projecting to higher-order visual areas in temporal cortex. The large pulvinar of squirrels (Figure 1c) is subdivided into at least three nuclei (Figure 4b), two with superior colliculus inputs, but of different types, and one without. All three divisions differ in their connections with cortical visual areas (see Lyon *et al.*, 2003b, for review).

The pulvinar complex has been extensively studied in domestic cats, which have divisions of a lateral posterior complex as well as a pulvinar (Figure 4c). A large nucleus receives inputs from the superior colliculus while projecting to temporal visual areas. This nucleus expressed high levels of acetylcholinesterase and the neurotransmitter, substance P. Another large nucleus is interconnected with primary and secondary visual areas, and a third nucleus projects to visual areas of temporal cortex. Monkeys have even more subdivisions of the pulvinar complex, which is large and dominates the posterior thalamus (Figure 4d). The classical inferior pulvinar consists of four nuclei differing in histochemical characteristics and patterns of cortical connections. Two of these nuclei have dense inputs from the superior colliculus. The large lateral pulvinar has two divisions, differing in connections and patterns of retinotopic organization. The traditional medial pulvinar and anterior pulvinar have nonvisual connections, and are not subdivisions of the visual pulvinar.

The point of this brief and limited survey is that mammals in different lines of evolution have independently increased the differentiation and complexity of the pulvinar complex. The pulvinar of early mammals was probably a relatively undifferentiated mass of cells in the posterior thalamus that included one or two zones with superior colliculus inputs, and possibly a zone without such inputs, but with visual cortex connections. This ancestral condition is reflected in the posterior thalamus of many extant mammals with poorly developed visual systems. However, in some lines of descent, more nuclei appeared, but in different patterns and arrangements. As a result, some or most pulvinar nuclei will only be present in some taxa. However, it may be possible to homologize a few specific pulvinar nuclei across taxa. For example, a region of the pulvinar complex that receives the class of superior colliculus projections that

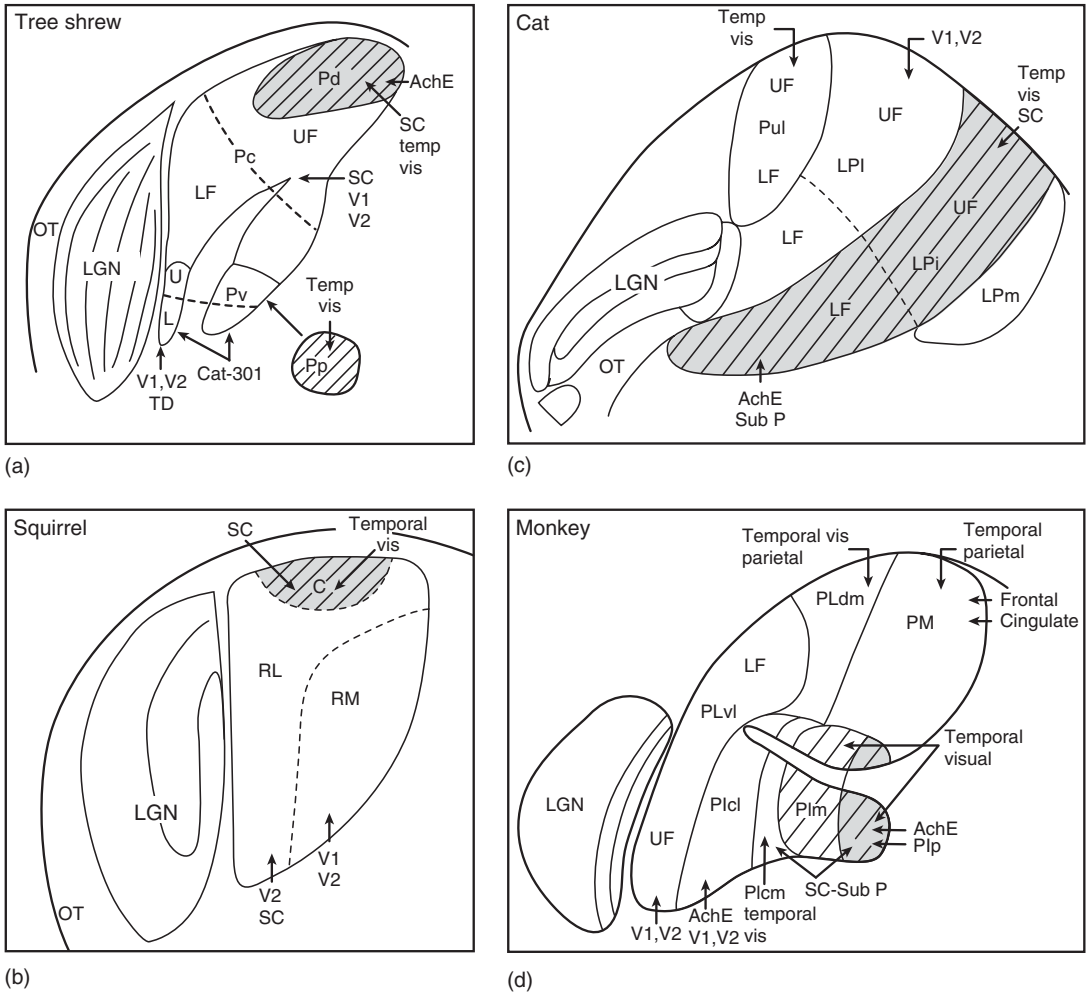

Figure 4 The organization of the pulvinar complex in four mammalian taxa. The drawings are of coronal brain sections through the caudal thalamus where the lateral border is defined by the optic tract (OT). a, In tree shrews, the four subdivisions or nuclei of the pulvinar complex include a large central pulvinar (Pc) with inputs from the superior colliculus (SC) and projections to visual areas V1 and V2, as well as a temporal dorsal (TD) visual area. Pc forms a systematic representation of the contralateral visual hemifield with the upper field (UF) represented dorsal to the lower field (LF). A dashed line approximates the zero horizontal meridian. The ventral pulvinar (Pv) also contains representations of the upper (U) and lower (L) hemifields, while expressing the antigen for the Cat-301 antibody and projecting to V1, V2, and TD. A posterior pulvinar (Pp) lies behind Pv and Pc, as indicated by an arrow, and projects to visual cortex of the temporal lobe. A dorsal pulvinar (Pd) expresses acetylcholinesterase (AchE), receives diffuse inputs from the SC, and projects to temporal visual cortex. b, In squirrels, only rostrolateral (RL), rostromedial (RM), and caudal (C) divisions of the pulvinar have been distinguished by differing connection patterns (abbreviations as in (a). c, In cats, one division of the pulvinar complex is called the pulvinar (Pul), while three others are called lateral (LPl), intermediate (LPi), or medial (LPm) nuclei of the lateral posterior complex. Note that three nuclei form separate representations of the visual hemifield, that nuclei differ in connections, and that LPi is distinguished by expressing AchE and the neurotransmitter, substance P (Sub P) that is expressed in one class of inputs from the SC but not others. d, Monkeys and other primates have a pulvinar complex that includes central lateral (PIcl), central medial (PIcm), medial (PIm), and posterior (PIp) nuclei of the inferior pulvinar, and ventrolateral (PLvl) and dorsomedial (PLdm) nuclei of the lateral pulvinar. The medial pulvinar (PM) is not completely visual and the anterior pulvinar (not shown) is somatosensory in function. Note that nuclei differ in connections and expression of AchE and Sub P. PLvl has the most precise representation of the contralateral visual hemifield, but other representations are in nuclei of the inferior pulvinar. Reproduced from Lyon, D. C., Jain, N., and Kaas, J. H. 2003b. The visual pulvinar in tree shrews. II: Projections of four nuclei to areas of visual cortex. *J. Comp. Neurol.* 467, 607 627, with permission.

express substance P may be revealed by further study to be homologous in a wide range of mammals (Hutsler and Chalupa, 1991; Stepniewska *et al*., 2000). A broader survey of extant mammals with well-developed visual systems could usefully extend our appreciation of the variety of specializations that have emerged in pulvinar organization, and comparisons within and across taxa could provide an understanding of how specializations emerged.

24.4 The Somatosensory Thalamus: The Ventroposterior Complex and the Adjoining Posterior Complex

In all studied mammals, a VP nucleus can be identified by its characteristic position in the ventral thalamus, its inputs from the dorsal column and trigeminal somatosensory brainstem nuclei, and its projections to primary somatosensory cortex, S1 (area 3b) (Jones, 1985; Kaas and Pons, 1988). Commonly, investigators have divided VP into VPM and VPL. However, these represent only major divisions of VP, as VPM receives inputs from the trigeminal nuclei for the face, oral cavity, and head, while VPL receives inputs from the gracile nucleus for the lower body (hindlimb and tail) and the cuneate nucleus for the upper body (forelimb and trunk). Note, by the same logic, the trigeminal, cuneate, and gracile 'nuclei' are subnuclei. These inputs to VP via the medial lemniscus provide cutaneous receptor information about touch on the skin and hair movement. Other somatosensory afferents involving a larger range of modalities including touch, pain, and temperature course in the spinothalamic pathway (and the equivalent component of the trigeminal complex) to terminate in and around VP, sometimes including a segregated nucleus on the ventral margin of VP, the VP inferior (VPI) nucleus. Another collection of small brainstem subnuclei, including the external cuneate nucleus, relays proprioceptive information, mainly from muscle spindle receptors, to the region of VP in the thalamus. In some mammals (see below), the proprioceptive inputs are now recognized as terminating within a separate cell group on the dorsorostral margin of VP, termed in primates the VP superior (VPS) nucleus (Krubitzer and Kaas, 1992).

Adjoining VP along its dorsocaudal margin, a poorly differentiated region termed the posterior nucleus or posterior group contains neurons that respond to somatosensory, but also auditory and visual stimuli. In addition, in primates, an anterior pulvinar nucleus is recognized just dorsal to part of VP. This nucleus is interconnected with subdivisions of somatosensory cortex while receiving little or no other sources of sensory inputs (Kaas and Pons, 1988). Finally, a taste nucleus (with much broader functions), the parvocellular ventroposterior medial nucleus (VPMpc), receives gustatory, tactile, and visceral information from the brainstem, and projects to somatosensory and adjoining cortex (Kaas *et al.*, 2006b). At least some of these subdivisions of the mammalian somatosensory thalamus seem to be common to all mammals, and therefore they must have originated with or before the first mammals and diversified in various ways.

The stem reptilian amniotes that gave rise to modern reptiles, birds, and mammals likely had features of the dorsal thalamus that have been retained in all three groups. In present-day reptiles and birds, somatosensory inputs relayed from the dorsal column nuclei reach a nucleus and a perinuclear region in the dorsal thalamus. These inputs in turn project to the rostral part of the dorsal cortex of reptiles or Wulst of birds (Wild, 1997; Medina and Reiner, 2000), forming much of the evidence for the conclusion that a homologue of at least the mammalian VP nucleus was present in the common ancestor. Stem reptiles may also have had some segregation of proprioceptive and spinothalamic inputs in the thalamus, but this seems uncertain. Gustatory and related sensory inputs may have been segregated in the ventral thalamus, but the dorsal thalamus and the pallium were probably not the important processing centers for gustatory inputs (Finger, 1997; Pritchard and Norgren, 2004).

The mammalian VP nucleus can be identified by its ventral position and histological characteristics in all examined extant mammals. In some mammals, such as opossums (Pubols and Pubols, 1966; Donoghue and Ebner, 1981; Wild, 1997) and hedgehogs (Erickson *et al.*, 1967), the neurons stain somewhat darker in Nissl preparations, and they are grouped to form an identifiable nucleus, but the nucleus is only marginally different from the adjoining thalamus (see Ebbesson *et al.*, 1972 for review). The VPM subnucleus forms the largest component of the VP nucleus in most mammals. This observation suggests that, in many taxa of extant mammals, the important somatosensory information came from the whiskers of the face, the nose, and the tactile receptors of the mouth. A comparative analysis suggests that VP in early mammals was poorly differentiated, and was dominated by a large representation of the face and oral cavity within the medial division of the nucleus. As with most studied extant mammals, VP of early mammals projected not only to primary somatosensory cortex, S1, but also to a second somatosensory area, S2, and possibly to a more recently defined somatosensory area, the parietal ventral area, PV (Kaas, 2004). This basic VP has been modified in mammalian evolution in many ways to accommodate changes in somatosensory receptor distributions and functions.

One of the variables in the organization of VP is in the proportions of the nucleus that are devoted to representing different body parts. For example, rats, mice, and many other rodents devote much of VP to representing the inputs from receptors

associated with their facial vibrissae, as they use these vibrissae extensively as they explore their tactile world (Woolsey *et al.*, 1974). In a similar manner, the star-nosed mole, with its unique fleshy appendages of the nose as its major tactile organ, has much of VP devoted to the receptors of those nose appendages (Catania and Kaas, 1996). In the naked mole rat, a rodent that uses its enlarged front teeth, the incisors, to carry and manipulate food, young, and other objects, approximately one-third of VP represents the teeth (Catania and Remple, 2005). Primates (Kaas *et al.*, 1984) and raccoons (Welker and Johnson, 1965) have disproportionately large representations of their glabrous forepaws, as they use this skin surface to explore the environment. In addition, spider monkeys have a prehensile tail with a sensitive glabrous ventral surface near the tip, and this tail has a large representation in VP (Pubols, 1968). Bats have an enlarged representation of the tactile receptors of the wing that are used to guide flight (see Somatosensory Specializations of Flying Mammals). Thus, the proportion of VP devoted to representing different body parts has been adjusted in both different and sometimes parallel ways in the various branches of mammalian evolution (Welker, 1973).

Another feature of VP that varies across species is the degree to which subnuclei are histologically obvious. In species where VP is histologically more distinct from the adjacent thalamus, cell-poor septa of fibers are typically present between highly innervated positions of the receptor sheet (skin) that are discontinuous but represented in VP next to each other. In primates, the glabrous foot, hand, and the face are separated from each other in VP by fiber bands into subnuclei for the head (VPM), hand, and foot (Kaas *et al.*, 1984). Often a subnucleus can be distinguished for the tail as well, and in some instances thin septa separating the representations of the digits in the hand representation can be detected. Fiber septa separating the representation of individual digits are even more obvious in VP of raccoons (Welker and Johnson, 1965; Wiener *et al.*, 1987a). The star-nosed mole has fiber bands that separate subnuclei in VP for each of the 11 long appendages on each side of the nose (Kaas and Catania, 2002). Rats and mice have visible subnuclei (barreloids) for each of the long sensory mystacial vibrissae on the side of the face (Akers and Killackey, 1979; Haidarliu and Ahissar, 2001). Other instances of visible subnuclei in VP have been reviewed by Welker (1973), and they appear to reflect a developmental mechanism that tends to segregate groups of neurons with different patterns of activation (Kaas, 1982; Kaas and Catania, 2002).

The connections of VP have also been altered in evolution. For example, the primitive mammalian pattern of cortical projections was to S1 and S2 (Kaas, 2004). The projections to S2 were lost in anthropoid primates, while a dense projection emerged in area 1 along the caudal border of area 3b (S1). A sparse but notable projection of VP to area 2, just caudal to area 1, developed in macaque monkeys and perhaps other catarrhine primates (Kaas, 2004). While VP independently activates S1 (area 3b) and S2 in most mammals, including prosimian primates, VP activates area 3b (S1 proper) and modulates areas 1 and 2 in catarrhine primates, reflecting the important roles of area 1 and 2 in these primates, and increased emphasis on higher-order processing in S2.

The VPI nucleus is a nucleus that has been described as only distinct in the primate brain (Jones, 1985), although it is also quite distinct in raccoons (Herron, 1983). In these mammals, VPI is composed of small, pale-staining neurons grouped just ventral to VP (Figure 5). VPI receives inputs from the spinothalamic somatosensory pathway and projects densely to the second somatosensory area, S2, as well as less densely to other somatosensory areas, including S1 (area 3b). Because similar connections exist for the cell-poor fiber septa that subdivide VP into subnuclei, and these septa have small pale-staining neurons, it seems likely that VPI is a nucleus that became largely segregated from VP in primates and raccoons, but much less so or not at all in other mammals (Krubitzer and Kaas, 1992). Thus, VPI may be an example of a new thalamic structure that arose independently via the process of differentiation and segregation in primates and raccoons from a mixed-cell population in ancestral VP. In both primates and raccoons, this birth of a new nucleus may be related to the increased use of the hand in skilled movements (Iwaniuk and Whishaw, 1999).

The VPS nucleus is another somatosensory nucleus with an uncertain history in mammalian evolution. In early studies of the primate thalamus, VPS was often included in VP. However, the recognition of VPS as a separate nucleus (Kaas *et al.*, 1984) was motivated by the evidence that it receives inputs from a brainstem relay of deep body (muscle spindle) receptors rather than cutaneous receptors. VPS forms a separate somatotopic representation of body receptors, but of deep rather than cutaneous receptors, as in VP. VPS is histologically distinct from VP in prosimians and New World monkeys, but not as distinct in Old World monkeys. VPS projects to area 3a and 2 in monkeys, rather than

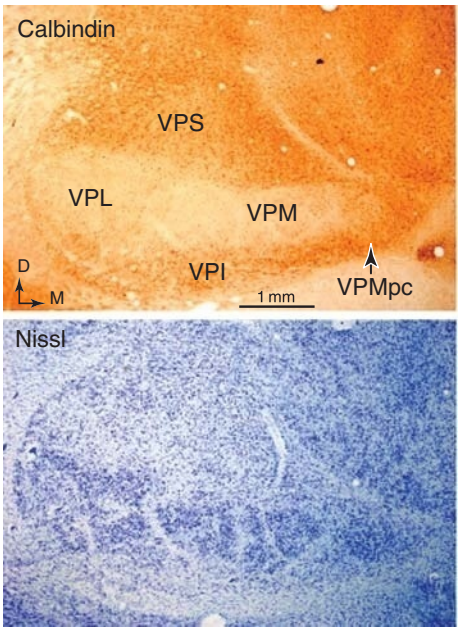

Figure 5 Above: Coronal brain section through the ventroposterior complex of a galago, a prosimian primate, which has been processed for the calcium-binding protein, calbindin. The ventroposterior, VP, nucleus, has two traditional subdivisions, the VPL and VPM, separated by a cell-poor septum. In this preparation, VP (VPL plus VPM) stands out as a light calbindin-poor region. Just ventral to VP, the ventroposterior inferior nucleus expresses a moderate amount of calbindin, while more is expressed in the taste nucleus of the thalamus, the VPMpc. Just above VP, the ventroposterior superior nucleus expresses moderate amounts of calbindin. Thus, calbindin levels usefully distinguish nuclei within the ventroposterior complex. Arrows indicate medial (M) and dorsal (D) in the photomicrograph. Below: An adjacent brain section processed for Nissl substance to reveal cell bodies. Note the cell-poor septa that divide VP into subnuclei.

area 3b and 1, as does VP (Kaas, 2004). While VPS has only been recognized in primates, the same nucleus likely exists in most mammals, and may have been present in the reptilian ancestors of mammals, given that they likely had distinct brainstem nuclei for the relay of muscle spindle information to the cerebellum and to the thalamus (Butler and Hodos, 2005). More significantly, a VPS-like nucleus or subnucleus has been identified in a range of mammalian taxa, including cats, raccoons, opossums, rats, and squirrels (Wiener *et al.*, 1987b; Gould *et al.*, 1989). The VPS-like nucleus is variously considered part of VP, part of the motor thalamus, or part of the posterior group of nuclei adjoining VP, but this kinesthetic or proprioceptive nucleus is always located on the dorsorostral border of VP, and is highly likely to be homologous across mammals, rather than independently derived.

The existence of VPS as a relay of deep receptor information raises the issue of what is included in the posterior nuclear group or complex. In rodents and cats, the part of the thalamus just dorsal to VP is commonly subdivided into a medial posterior nucleus (POM) and a lateral posterior nucleus (POL). The VPS relay nucleus in these mammals appears to occupy the ventral parts of these nuclei (Gould *et al.*, 1989), suggesting that some revision in nuclear boundaries and terminology is justified. Historically, the concept of a posterior group emerged from architectonic studies of the thalamus of sheep, cats, and rabbits by Rose (Rose and Woolsey, 1949). The complex includes parts of the thalamus dorsal and caudal to VP, and between VP and the medial geniculate complex. The region has poorly defined boundaries, and nuclear boundaries. Part of the region contains neurons that are responsive to more than one modality, often to both somatosensory and auditory, but sometimes visual stimuli, and the region has connections with nonprimary regions of sensory cortex (Jones, 1985). As nuclei of the posterior complex are difficult to identify and delimit, it seems likely that nuclei have been misidentified and misnamed across taxa. Thus, it is difficult to speculate on the evolution of the complex. As noted above, part of the complex probably includes the VPS of nonprimate mammals. Another part may well include the anterior (oral) pulvinar of primates. The anterior pulvinar is part of the somatosensory rather than the visual thalamus, and probably, it should not be grouped with the nuclei of the visual pulvinar. The anterior pulvinar receives inputs from somatosensory areas of cortex and projects back to somatosensory areas of cortex (Kaas and Pons, 1988). It has only been described in primates, and it has no known homologue in other mammals. The location of the anterior pulvinar on the mediodorsal (MD) border of VP, and the connections of the anterior pulvinar with somatosensory cortex, suggest that part of the posterior complex of other mammals is homologous with the anterior pulvinar of primates. The anterior pulvinar could also correspond to part of the lateral posterior region that is identified in most mammals, although most of the lateral posterior region corresponds to the pulvinar complex in mammals where the visual pulvinar is not recognized, or only part of the complex is recognized as the pulvinar.

24.5 The Auditory Thalamus: The Medial Geniculate Complex

The medial geniculate complex is another part of the mammalian thalamus that was retained from

reptilian ancestors. The reptilian homologue, nucleus medialis (also known as nucleus reuniens), has a core of more densely packed neurons, and a shell of more diffusely distributed neurons, already suggesting the existence of a nuclear complex rather than a single nucleus. The medial geniculate of reptiles resembles that of mammals in having its activating auditory input relayed from the auditory midbrain (the inferior colliculus in mammals), but differs from the mammalian medial geniculate by having a medial rather than a lateral location in the thalamus, and by projecting to the striatum and ventral pallium rather than to dorsal cortex, the homologue of neocortex. Puelles (2001) suggests that most of the auditory complex in the thalamus of reptiles migrated laterally in ancestral mammals, while keeping the auditory input from the midbrain developing a new collateral projection to the emerging neocortex, thereby forming several auditory fields. Parts of the auditory complex of the reptilian thalamus that did not migrate all the way may have led to the emergence of some of the intralaminar nuclei of mammals.

Three nuclei are widely recognized in the medial geniculate complex (Figure 6) of mammals: (1) the ventral medial geniculate (MGv); (2) the dorsal medial geniculate (MGd); and (3) the magnocellular medial geniculate (MGm), also termed the internal nucleus, as the neurons in the nucleus are not particularly large in mammals where the complex is not well differentiated (Jones, 1985). The ventral nucleus projects to one or more primary or primary-like areas

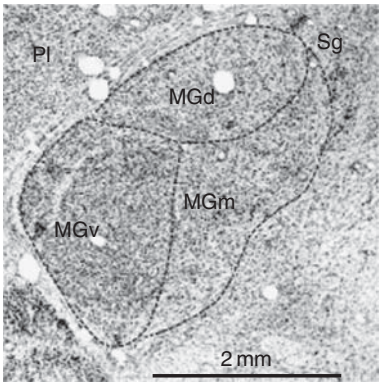

Figure 6 The medial geniculate complex of a macaque monkey. The coronal brain section has been stained for Nissl substance to reveal cell bodies. The ventral nucleus (MGv) is ventrolateral and composed of tightly packed small neurons. The dorsal nucleus (MGd) and the medial or magnocellular nucleus (MGm) have less densely packed neurons, and MGm has larger neurons. The suprageniculate nucleus (Sg) also has auditory functions, while the adjoining inferior pulvinar (PI) is visual. Some investigators have defined further subdivisions of the medial geniculate complex in cats and monkeys (see text).

of a core region of auditory cortex in topographic patterns that preserve tonotopic organization in this cortex. As high-frequency hearing emerged with mammals, a major change from reptiles was the representation of high frequencies in MGv. The dorsal and magnocellular nuclei project more broadly to auditory cortex, to the secondary areas of the auditory belt and parabelt, with little input to the primary core (see Hackett *et al.*, 1998, for review). The divisions of the medial geniculate nucleus are distinct and well differentiated from each other and the adjoining thalamus in well-studied cats and monkeys, but much less so in hedgehogs and opossums (Winer *et al.*, 1988), supporting the contention that these nuclei became more differentiated independently in several lines of mammalian evolution. It is likely that in all mammals, the ventral nucleus is characterized by a dense distribution of the calcium-binding protein, parvalbumin, while the adjoining dorsal and magnocellular nuclei express the calcium-binding protein, calbindin (Cruikshank *et al.*, 2001). These two proteins distinguish other thalamic nuclei as well: parvalbumin distributions are high in the core nuclei that activate cortical areas, and calbindin distributions are high in the nuclei that largely modulate cortical activity.

As for other thalamic nuclei, those of the medial geniculate complex vary in differentiation and relative size in members of different taxa. As one would expect from the auditory needs of echolocating bats, the medial geniculate complex in these bats is the largest nuclear complex of the dorsal thalamus, occupying three-fifths of the rostrocaudal extent of the thalamus (Radtke-Schuller, 2004). The MGv occupies about 40% of the complex, somewhat less than 50% or more found in most mammals. The three divisions of the medial geniculate complex do vary in proportions relative to the total size of the complex in various mammals (reviewed by Radtke-Schuller, 2004). MGv occupies more of the complex in species where primary auditory cortex is prominent, while a relatively large MGd is found in species with expanded nonprimary cortical areas, as in macaque monkeys (Kaas and Hackett, 1998).

Of course, the medial geniculate complex in echolocating bats is also specialized in that the echo frequencies have a disproportionately large representation in MGv (Wenstrup, 1999). The ultrasonic communication calls of mice also appear to have an enlarged thalamic representation (Hofstetter and Ehret, 1992). Another distinctive feature of echolocating bats is that many neurons in both the MGv and MGd have complex sensitivities to sound frequencies, often having responses that are facilitated by combinations of frequencies

in the range of the biosonar signals (Wenstrup, 1999). However, the most remarkable specializations of the auditory thalamus of echolocating bats may be the existence of a direct auditory pathway from the suprageniculate nucleus to the unique frontal auditory area of cortex just rostral to motor cortex (Kobler *et al.*, 1987). While only three divisions of the medial geniculate complex have been considered here, the complex of cats and monkeys appears to have more subdivisions (Winer *et al.*, 2001; de la Mothe *et al.*, 2006), suggesting the independent evolution of new divisions.

24.6 The Motor Thalamus: The Ventral Lateral Complex and the Ventral Anterior Complex

The VL complex and the ventral anterior (VA) complex occupy the rostral pole of the thalamus just rostral to the somatosensory nuclei, VP, VPS, and VPI. The VL and VA nuclei are highly variable in size and differentiation, forming a small, poorly differentiated portion of the thalamus in many mammals such as hedgehogs, opossums, and even rats, while being large and architectonically subdivided into several nuclei in anthropoid primates. In addition to VL and VA, a ventromedial nucleus (VM) is often distinguished.

Much of the motor thalamus is defined by inputs from the deep nuclei of the cerebellum and from the substantia nigra and globus pallidus of the basal ganglia. The cerebellum and the basal ganglia, together with the motor cortex, are the main structures that regulate motor behavior. Thus, they provide important sources of information to the motor thalamus, which in turn projects to motor cortex. The cerebellothalamic afferents tend to project predominantly to VLp, a posterior division of the ventrolateral complex, while basal ganglia (pallidal) projections terminate more rostrally in the VLa, the anterior division of the ventrolateral complex, and in the caudal aspect of the VA complex (Ilinsky and Kultas-Ilinsky, 1984; Sakai *et al.*, 2000), although it is uncertain to what degree this segregation of inputs applies to all mammals. In tenrecs, a small insectivore from Madagascar with little thalamic differentiation, the region identified as VM receives a prominent input from the cerebellum (Künzle, 1998), but cerebellar input to VM is considerably less in rats (Herkenham, 1979).

In most mammals, VLp projects mainly to primary motor cortex, while VLa and VA project mainly to premotor cortical areas (Jones, 1985). The notable exception is in mammals that appear to have no motor cortex. Thus, in opossums and perhaps other marsupials (see Beck *et al.*, 1996, for review), there is no evidence for a primary motor area, M1, or any other motor area rostral to primary somatosensory cortex, S1. Instead, S1 is bordered rostrally by a narrow somatosensory area that resembles area 3a of cats and primates. All of the corticospinal projections in opossums originate from S1, the narrow 3a-like area, and S2 (Cabana and Martin, 1984). In other mammals, S1 and the narrow 3a-like strip of opossums have motor functions and electrical stimulation of these areas evokes motor movements. When this was noted by investigators, the S1 area was conceptualized as a sensorimotor amalgam in marsupials (Lende, 1963). Alternatively, this area in opossums can be considered to be simply S1, as it has the identifying features of S1, including sensory architecture, a characteristic somatotopic organization, bordering somatosensory areas, and input from the VP nucleus (Beck *et al.*, 1996).

However, S1 of opossums apparently receives inputs from both VP and VL (Killackey and Ebner, 1973), although these nuclei are poorly differentiated in opossums and difficult to separate. This apparent distinction between metatherian and placental mammals (where a primary motor area, M1, with inputs from VL has been consistently identified) suggests that a motor thalamus existed in early therian mammals, but separate motor cortex targets only evolved in eutherian (placental) mammals (note that projections from VL to parts of the rostral margin of parts of S1 have been reported for some eutherian mammals, but technical difficulties in obtaining relevant data make this uncertain). Arguing against the hypothesis that motor cortex emerged with placental mammals is the questionable evidence that protherian mammals (monotremes) do have a separate motor cortex (Ulinski, 1984; Krubitzer *et al.*, 1995). If so, it might be more parsimonious to argue that opossums and perhaps other marsupials have regressed from the ancestral plan, and have lost a separate primary motor area, M1. This uncertainty relates to the issue of whether reptilian ancestors of mammals had a motor thalamus. Although a region of the thalamus of birds and reptiles has been suggested as a homologue of the mammalian motor thalamus, the evidence has been described as inconclusive (Medina *et al.*, 1997; Medina and Reiner, 2000).

An unusual feature of hedgehogs is that the VL nucleus projects bilaterally to motor cortex (Dinopoulos, 1993). While crossed thalamocortical projections have been reported for midline nuclei in a range of mammals, they have not been described for VL of other mammals, including opossums

(Donoghue and Ebner, 1981) and echidnas (Ulinski, 1984). Given this apparently unique feature of the motor thalamus of hedgehogs, the most logical conclusion is that the immediate ancestors of hedgehogs developed a crossed projection that earlier ancestors did not have. However, Dinopoulos (1993) suggested instead that hedgehogs have retained a primitive trait, as their forebrain appears to be primitive in general and such connections exist in postnatal rats that are lost during development (Minciacchi and Granato, 1989). Thus, hedgehogs might have retained a primitive crossed thalamocortical projection from VL to motor cortex that is preserved in other mammals only as a transient projection early in development.

In anthropoid primates, VL and VA are large and architectonically divided into several nuclei or subnuclei (Stepniewska *et al.*, 1994). Thus, VL is commonly divided into four regions, with names varying across authors, and VA is divided into a parvocellular and a magnocellular division. This complexity likely relates to the changes that have taken place in the organization of motor cortex of primates, where dorsal and ventral premotor areas, a supplementary motor area, and a presupplementary motor area have been identified in dorsolateral frontal cortex. Such complexity has not been identified in nonprimate mammals (Kaas, 2004). Although prosimian galagos also have these additional premotor areas (Wu *et al.*, 2000), and the motor thalamus is large and subdivided, the subdivisions are architectonically much less distinct than in monkeys (Fang *et al.*, 2006).

In summary, the motor thalamus of mammals appears to be unusual in that it seems to have emerged as a subdivision or subdivisions of the thalamus before a separate motor area or motor areas existed. Alternatively, motor areas of cortex could have been lost in evolution while thalamic areas were preserved. In addition, the complexity in terms of subdivisions substantially increased at both cortical and thalamic levels in primates, but the architectonic distinctiveness of thalamic subdivisions especially increased with the advent of anthropoid primates.

24.7 The Anterior and Lateral Dorsal Nuclei

Three anterior nuclei and the associated lateral dorsal (LD) nucleus are well differentiated and histologically distinct in the anterior thalamus of most mammals. The anterodorsal (AD) nucleus is characterized by tightly packed, darkly stained cells in Nissl preparations, while the adjoining anteroventral (AV) nucleus is less darkly stained, but usually separated from AD by a cell-poor septum. The anteromedial (AM) nucleus resembles AV, but has a more VM position. The LD nucleus, lateral and dorsal to AD and AV, has pale-staining cells in Nissl preparations. The four nuclei are functionally related in that they project to different parts of limbic cortex on the medial wall of the cerebral hemisphere, and receive inputs from the hippocampal formation either directly from the subicular complex or indirectly via the mammillary nuclei of the hypothalamus (Price, 1995). The limbic connections of the anterior group suggest that these nuclei have roles in basic, ancient functions. Yet, this collection of nuclei has not been identified in reptiles. Butler and Hodos (2005) postulate that the elaboration of this complex involved a shift from a multisensory relay nucleus in the thalamus to a cortical–thalamic–cortical circuit resulting from an increased role of limbic cortex in learning and memory. In any case, the four nuclei vary in development across taxa in a way that suggests that their functions vary in importance. Monotremes appear to have only a single anterior nucleus (Butler and Hodos, 2005), possibly reflecting the state of early mammals, while opossums have all three anterior nuclei (Bodian, 1939). Other variations in the anterior nuclei across mammalian taxa have been reviewed by Jones (1985). In general, there seems to be an association between the architectonic representation of the nuclei and that of limbic cortex. The AM nucleus is sometimes indistinctly separated from the AV nucleus, and the AV nucleus is greatly enlarged in many primates. The AD nucleus is sometimes subdivided into regions of larger and smaller cells, and it is disproportionately large in some mammals such as echolocating bats.

24.8 The Mediodorsal and Intralaminar Thalamic Nuclei

A number of other nuclei of the mammalian thalamus deserve at least brief comment. The MD nucleus is a large group of cells in the AM thalamus that is often subdivided based on regional differences in cell size and packing. The nucleus or complex receives inputs from olfactory cortex, the amygdala, and entorhinal cortex while projecting to regions of frontal cortex (Price, 1995). An olfactory pathway through the thalamus to dorsal pallium appears to be widespread in reptiles and birds (Veenman *et al.*, 1997), suggesting that the reptilian ancestors of mammals had the homologue of an MD nucleus. An MD nucleus has been identified with

connections to frontal cortex in monotremes (Welker and Lende, 1980), marsupial opossums (Tobias and Ebner, 1973; Benjamin and Golden, 1985), and a wide range of placental mammals (see Benjamin and Golden, 1985, for review). One difference across mammals is that olfactory cortex projects to all of MD in opossums, but only to the medial part in placental mammals, which is further distinguished by the magnocellular division of MD in monkeys. These findings suggest that MD in early mammals functioned to relay olfactory information to frontal cortex. In some lines of evolution, especially in primates, the olfactory functions became less dominant, while MD differentiated into two or more subnuclei with olfactory and other functions.

The cell groups within the internal medullary lamina of the dorsal thalamus are called the intralaminar nuclei. The 'centre médian' is of special interest as it is a well-differentiated component of the primate thalamus, but not an obvious nucleus in most other mammals. The 'centre médian' has been suggested as one of the new components of the primate thalamus (Jones and Rubenstein, 2004). Historically, Le Gros Clark (1932) proposed that the 'centre médian' nucleus evolved from part of the intralaminar group, but the origin and primitive form of the nucleus are unclear. Veenman *et al.* (1997) propose, on the basis of immunohistochemical evidence and connection patterns, that the intralaminar, midline, and MD nuclei of the mammalian thalamus evolved from dorsal thalamic groups in reptilian ancestors that also gave rise to a dorsal thalamic zone in birds.

24.9 Conclusions

We have seen that there are both consistent and variable features of the mammalian dorsal thalamus. The most notable consistent feature is that a number of thalamic nuclei, such as the dorsal LGN, the medial geniculate complex, the pulvinar, and the VP nucleus, can be identified in most or all mammals studied, and likely were retained from the immediate reptilian ancestors of mammals. However, the architectonic differentiation of these nuclei was not marked, judging from the poor differentiation of thalamic nuclei in a range of extant mammals. Variable features, reflecting evolutionary changes in various lines of descent, include: increases (and, more rarely, decreases) in the absolute and relative sizes of some nuclei relative to others, changes in the proportion of nuclei devoted to some inputs over others (e.g., representation of inputs from face or hand in VP), the formation of layers and subnuclei in nuclei, alterations in the inputs and outputs of nuclei, and the emergence of new nuclei. Similar evolutionary changes have taken place in the more extensively studied neocortex (Krubitzer and Kaas, 2005).

It seems likely that few or no thalamic nuclei have been lost in mammalian evolution. Even the subterranean blind mole-rat has an identifiable, but small, dorsal LGN (Cooper *et al.*, 1993). In contrast, new nuclei appear to have emerged in many lines of mammalian evolution. A clear example is the pulvinar complex, where different nuclei have emerged in carnivore and primate lines. By using the expression of genes during development to help identify thalamic nuclei, Jones and Rubenstein (2004) listed six nuclei found in monkeys that are not found in mice, and this is certainly an underestimate. On the other hand, the patterns of gene expression suggested that some nuclei that are well differentiated in the thalamus of monkeys, such as the suprageniculate/limitans, do have a poorly differentiated homologue in the thalamus of mice. It seems likely that, as new expanses of neocortex subdivided into new areas in evolution, as is the case with much of posterior parietal cortex of anthropoid primates (Kaas *et al.*, 2006a), new thalamic nuclei with projections to the new cortical areas emerged. However, the increase in the number of cortical areas and thalamic nuclei was most likely not on a one-to-one basis, as the neocortex generally seems to have more subdivisions than does the dorsal thalamus. As an example of a new nucleus, the medial nucleus of the inferior pulvinar (IPm) of anthropoid primates projects densely to the MT visual area, but neither MT nor IPm appears to exist outside the primate order. However, in some instances, the complexity of the thalamus may exceed that of the cortex, as in opossums where the thalamic motor nucleus, VL, and the thalamic somatosensory nucleus, VP, both project to primary somatosensory cortex, S1. Also, there is no evidence of a separate primary motor area, M1, although a separate and distinct M1 with inputs from VL is characteristic of all placental mammals studied.

If new nuclei of the thalamus emerged, from where did they come? The prevailing notion is that poorly differentiated nuclei gradually differentiated to two or more nuclei in some lines of descent. This hypothesis has been stated repeatedly as an explanation for how new cortical areas emerged (Kaas, 1982; Krubitzer and Kahn, 2003; Krubitzer and Kaas, 2005). In some sense, one could argue that the new areas are not really new, and two or more nuclei in one taxon can be identified as a field homology with a single nucleus in other taxa. Alternatively, new nuclei could emerge as the result

of a change in gene expression, perhaps as a consequence of gene duplication (Allman and Kaas, 1971a, 1971b; Fukuchi-Shimogori and Grove, 2001, 2003). Finally, it is important to stress that the evolution of the thalamus is not well understood, as the great diversity of thalamic organization that must exist in mammalian radiation has been largely unexplored. For practical reasons, research efforts have focused on a few well-studied species. Clearly, there is much to learn, and many opportunities for the adventurous, comparative neuroscientist to make new discoveries of substance.

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25 The Evolution of the Basal Ganglia in Mammals and Other Vertebrates

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Glossary

agnathans Jawless fish, including the extant lamprey and hagfish.

amniotes Collective term for vertebrate groups whose members develop inside an amniotic sac, and which is a synonym for birds, reptiles, and mammals.

anamniotes Collective term for vertebrate groups whose members do not develop inside an amniotic sac, and which is a synonym for fish and amphibians.

basal ganglia A subcortical telencephalic region that in mammals includes two cell groups, the caudate and putamen (together referred to as the striatum), and a third cell group known as the globus pallidus.

caudate putamen The input subdivision of the basal ganglia (together referred to as the striatum).

cerebral cortex The part of the mammalian pallium that is organized into six layers and mediates higher order learning, perception, and motor control.

chondroicthyans Cartilaginous fish, such as skates, rays, sharks, and chimeras.

direct pathway The striatal output pathway to the internal pallidal segment and the substantia nigra, which facilitates desired movement of enkephalin. Opiate neuropeptide characteristically present in the GABAergic spiny striatal neurons of the so called indirect striatal output neurons that project to the external pallidal segment.

globus pallidus The output subdivision of the basal ganglia.

indirect pathway The striatal output pathway to the external pallidal segment, which inhibits unwanted movement.

neostriatum Outdated synonym for the caudate putamen that reflects the repudiated notion that the caudate putamen appeared after the globus pallidus in evolution.

paleostriatum Outdated synonym for the globus pallidus that reflects the repudiated notion that the globus pallidus appeared before the caudate putamen in evolution.

pallidum Short hand term referring to the globus pallidus.

pallium Term referring to the part of the telencephalon (cerebrum) that lies above and/or around the basal ganglia.

sauropsids Collective term for birds and reptiles.

striatum Short hand term for the input part of the basal ganglia, also known as the caudate putamen in mammals.

subpallium Term referring to the part of the telencephalon that lies below the pallium.

substance P Tachykinin neuropeptide characteristically present in the GABAergic spiny striatal neurons of the so called direct striatal output neurons that project to the internal pallidal segment and the substantia nigra.

substantia nigra Cell group in the midbrain tegmentum, in mammals consisting of two parts, a pars compacta rich in dopaminergic neurons and a pars reticulata rich in GABAergic neurons.

subthalamic nucleus	Cell group in the lower thalamus of mammals that receives input from the external pallidal segment and projects to the internal pallidal segment
tetrapods	Collective term referring to the limbed vertebrate groups, and it includes amphibians, reptiles, birds, and mammals.

25.1 Introduction

The basal ganglia is a subcortical telencephalic region that includes two cell groups, the caudate and putamen (together referred to as the striatum), and a third cell group known as the globus pallidus. Whereas additional cell groups such as the nucleus accumbens, the olfactory tubercle, and the ventral pallidum are sometimes considered to be part of the basal ganglia (Heimer *et al*., 1985, 1997; Reiner *et al*., 1998), for present purposes these will be considered the limbic or ventral basal ganglia and regarded as a separate entity from the basal ganglia. Understanding of the organization and function of the basal ganglia of mammals has increased tremendously in the past 100 years. When the term basal ganglia first became commonplace in the late 1800s, it loosely referred to various subcortical telencephalic cell groups (i.e., the basal nuclei) that included the amygdala and claustrum (and in some cases the thalamus), as well as what are now regarded as the basal ganglia (Parent, 1986). Without reliable methods for tracing brain connectivity, knowledge of the basal ganglia largely consisted of efforts to identify the same cell groups in diverse species, with some inferences made about a role in motor function from clinical findings (Vogt, 1911; Wilson, 1912). This early view suggested that the basal ganglia and cerebral cortex exerted separate control on motor function: the motor cortex via projections to brainstem and spinal cord via the pyramidal tract, and the basal ganglia via nonpyramidal circuits. Thus, motor control was thought to be effected by pyramidal and extrapyramidal motor systems.

With the advent of silver staining of degenerating fibers in the 1950s, it was soon recognized that the caudate and putamen receive extensive cortical input and have extensive projections to the globus pallidus and substantia nigra (Carman *et al*., 1963; Nauta and Mehler, 1966; Parent, 1986). The globus pallidus, in turn, was found to project to thalamic cell groups projecting to motor cortices (Nauta and Mehler, 1966). These findings indicated that the role of the basal ganglia in motor control involved cortical input and was mediated by a return projection to motor cortex. Neurochemical methods developed in the 1960s revealed a major nigral input to striatum that used dopamine (DA) as a neurotransmitter and led to the discovery that loss of this input was the basis of Parkinson's disease (Carlsson, 1959; Carlsson *et al*., 1962; Dahlstrom and Fuxe, 1964). Understanding of the cellular makeup of the basal ganglia, the neurotransmitters used by its neurons, and delineation of its circuitry at a cellular level accelerated greatly with the application of immunohistochemistry in the 1970s, and of *in situ* hybridization histochemistry in the 1980s, to the study of the basal ganglia (Parent, 1986; Reiner and Anderson, 1990; Gerfen, 1992; Graybiel, 1990). Finally, over the past 10 years, great advances have been made in identifying the genes controlling the regional identity and development of the striatum, globus pallidus, and cerebral cortex. These genes include *Dlx1* and *Dlx2*, which specify the striatum and pallidum, and *Nkx2.1*, which specifies the globus pallidus (Rubenstein *et al*., 1994; Smith-Fernandez *et al*., 1998; Puelles *et al*., 2000). Other genes control pallial identity, such as *Emx1*, *Emx2*, and *Tbr1*, and are expressed at high levels in developing cerebral cortex (Rubenstein *et al*., 1994; Smith-Fernandez *et al*., 1998; Puelles *et al*., 2000). Projection neurons of the cerebral cortex and other pallial telencephalic areas characteristically use glutamate as their neurotransmitter, whereas those of the subpallium (to which the basal ganglia belongs) characteristically are GABAergic (Swanson and Petrovich, 1998).

This accumulation of data, together with insights from physiology, pharmacology, and molecular biology, led to circuit level models of basal ganglia function that relate the major aspects of basal ganglia cytology and circuitry to normal and pathological basal ganglia function (Albin *et al*., 1989; Crossman, 1990; DeLong, 1990). In these models, the striato-pallidal output circuitry is recognized as being organized into two channels. One arises from substance P (SP)-containing GABAergic striatal neurons that project to the large GABAergic neurons of the internal segment of globus pallidus (GPi) that promote movement. The second output arises from enkephalinergic (ENK^+) GABAergic striatal neurons that project to the large GABAergic neurons of the external segment of globus pallidus (GPe) that inhibit unwanted movement (Figure 1). The GPe neurons have indirect outputs to GPi neurons via the subthalamic nucleus (STN) and for this reason the SP^+ striatal projection to the GPi is called the direct pathway and the ENK^+ striatal projection to GPi via the GPe–STN connection is called the indirect pathway. Identification of the cell types of the basal ganglia by their neurotransmitter

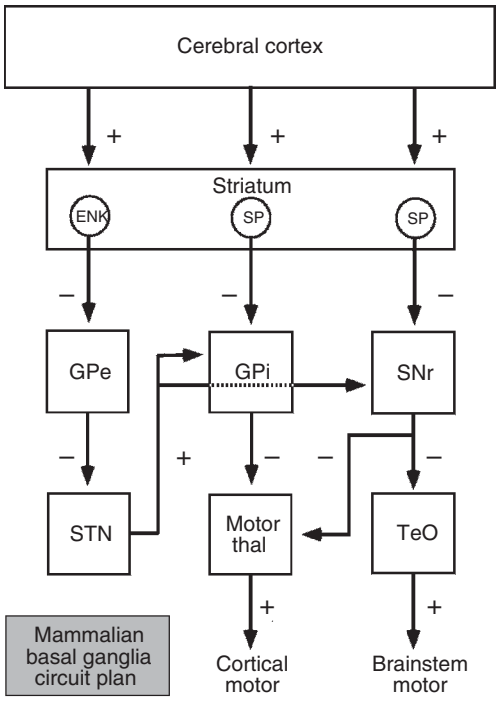

Figure 1 Circuit diagram illustrating the basic direct indirect pathway organization of basal ganglia functional circuitry in mammals. The plus and minus signs indicate whether the specific projections of the basal ganglia circuitry are glutamatergic excitatory (+) or GABAergic inhibitory ( ). ENK, enkephalinergic neurons; GPe, external globus pallidus segment; GPi, internal globus pallidus segment; SNr, substantia nigra pars reticulata; SP, substance P-containing neurons; STN, subthalamic nucleus; TeO, optic tectum; Motor thal, motor thalamus.

content and the genes controlling their identity led to the realization that the interneurons of both the striatum and the cerebral cortex, which tend to be GABAergic, migrate in from the Nkx2.1-expressing zone from which the globus pallidus also forms (Marin *et al.*, 2000; Marin and Rubenstein, 2001). For striatum, these include three major neurochemically distinct types of interneurons: large cholinergic neurons, large GABAergic neurons co-containing parvalbumin (PARV), and small GABAergic neurons co-containing the neuropeptides somatostatin and neuropeptide Y and the nitric oxide (NO)-synthesizing enzyme NO synthetase (Kawaguchi *et al.*, 1995).

Before the advent of modern neuroanatomical hodological methods, several investigators formulated a theory of basal ganglia and overall telencephalic evolution based strictly on the size, position, and cytological appearance of the major telencephalic regions (Edinger *et al.*, 1903; Edinger, 1908; Ariëns-Kappers *et al.*, 1936). This theory resulted in terminologies for both mammalian and nonmammalian telencephalons that have proven enduring, even in the face of their thorough repudiation by modern data. In this traditional terminology, the globus pallidus in mammals is alternatively referred to as the paleostriatum, whereas the caudate–putamen is called the neostriatum. These terms stem from the notion that the parts of the telencephalon had evolved in serial order during vertebrate evolution: the globus pallidus in jawed fish, the neostriatum in amphibians, and the primitive cerebral cortex in reptiles (Ariëns-Kappers *et al.*, 1936). Mammals were thought to have elaborated cerebral cortex into neocortex, whereas birds were thought to have elaborated the basal ganglia by addition of a new territory known as the hyperstriatum. This view of telencephalic evolution has been refuted by modern neuroanatomical, molecular biological, and neurochemical studies, but vestiges of it have survived in the frequent use of the terms paleostriatum and neostriatum to refer to the globus pallidus and caudate–putamen in mammals and in the telencephalic terminology that was formerly used in birds (Reiner *et al.*, 2004). Older views of telencephalic evolution also promoted the idea that a process of encephalization had occurred during vertebrate evolution, with more rostral structures taking over functions carried out by more caudal regions (Ariëns-Kappers *et al.*, 1936; Herrick, 1948, 1956). In the case of telencephalic evolution, the cerebral cortex was thought to take over behaviors that had been carried out by the basal ganglia in a stereotyped fashion in the stem amniote common ancestors of mammals, birds, and reptiles, and still were carried out by the basal ganglia in modern birds and reptiles. The implication of this for mammals was the expectation that the cerebral cortex and basal ganglia size should be dissociated or inversely related.

The neuroanatomical tools that have been used to clarify the cellular neurochemistry and connectivity of the basal ganglia of mammals and determine the genetic control of regional identity in the developing mammalian telencephalon have been used to study telencephalic organization and development in members of other vertebrate groups as well (Parent, 1986; Reiner *et al.*, 1998; Marin *et al.*, 1998a, 1998b). These studies have dramatically revised our understanding of basal ganglia evolution and have profound implications for the traditional view of basal ganglia evolution that still is all too often promulgated in neuroanatomy textbooks. In the following paragraphs, I discuss evidence showing that both a striatum and a pallidum have been basal ganglia constituents since early in vertebrate evolution. I also examine data emphasizing the functional interrelatedness of cerebral cortex and basal ganglia, with both enlarging in parallel during brain expansion in the mammalian radiation.

25.2 Anamniote Basal Ganglia Evolution

25.2.1 Agnathans

There are two extant groups of jawless fish: hagfish and lamprey. Modern taxonomic studies suggest lamprey and hagfish to be only distantly related, with lamprey being a sister group of jawed vertebrates (Forey and Janvier, 1994). Telencephalic organization in these two agnathan groups reflects their taxonomic distance. (see Structure of Brains of Primitive Vertebrates (tunicates, amphioxus, lampreys) and the Basic Features of the Vertebrate Brain) Neurochemical studies clearly identify a ventral telencephalic region rich in SP^+ perikarya in lamprey (Table 1; Figure 2; Nozaki and Gorbman, 1986; Auclair *et al*., 2004). This region receives a dopaminergic input from the midbrain and has a return projection to these dopaminergic neurons (Pierre *et al*., 1994; Pombal *et al*., 1997a). This region also expresses lamprey homologues of *Dlx1/2* (Murakami *et al*., 2001; Neidert *et al*., 2001) and contains some cholinergic interneurons (Pombal *et al*., 2001). For these reasons, this region appears to be homologous to the mammalian striatum. Although a globus pallidus in lamprey has not been demonstrated unequivocally (Nieuwenhuys and Nicholson, 1998; Murakami *et al*., 2001), SP^+ woolly fibers ventrolateral to the striatum in a field of GABAergic neurons within a region that has been called the ventral pallium delineate a field that may be pallidal (Pombal *et al*., 1997b). Alternatively, SP^+ woolly fibers ventromedial to the striatum define a field that may be pallidal (Figure 2; Nozaki and Gorbman, 1986). By contrast, SP and ENK immunolabeling fails to unequivocally identify a striatum or pallidum in hagfish (Wicht and Northcutt, 1994). Although lampreys clearly possess a striatum, the region is small and neuron sparse and the midbrain dopaminergic input is meager. Telencephalic inputs or descending projections of the lamprey striatum have not been investigated and not much is known of lamprey basal ganglia functional circuitry (Table 1).

25.2.2 Chondroicthyans

Cartilaginous fish possess simple tubular paired telencephalic hemispheres, as do lobe-finned fish and amphibians, and the ventrolateral sector of the telencephalon contains both a striatum and a globus pallidus, by neurochemical and hodological criteria (Table 1; Figure 2; Reiner and Carraway, 1985; Northcutt *et al*., 1988; Reiner *et al*., 1998). The striatal sector is located nearest the ventricle and is cell sparse, but contains SP^+ and ENK^+ neurons that give rise to projections to a cell plate lying external to the striatal field (Figure 2). This cell plate appears comparable to the globus pallidus, both because of this striatal input and because the neurons of the pallidal field contain the neurotensin-related hexapeptide LANT6 (Lys^8–Asn^9–neurotensin$^{8–13}$), which is present in mammalian pallidal neurons (Northcutt *et al*., 1988; Reiner and Carraway, 1985, 1987; Reiner, 1987a; Rodriguez-Moldes *et al*., 1993). In this pallidal field, the SP^+ and ENK^+ inputs overlap, indicating that GPi- and GPe-type neurons are intermingled. Moreover, the striatum receives a dopaminergic input from the midbrain and these dopaminergic neurons receive a return projection from SP^+ striatal neurons (Meredith and Smeets, 1987; Northcutt *et al*., 1988; Smeets and Reiner, 1994; Stuesse *et al*., 1994). The pallium occupies the dorsolateral sector of the telencephalon in cartilaginous fish, its development is controlled by homologues of some of the same genes controlling pallial development in mammals (Derobert *et al*., 2002), and this region is larger and more complex in the more advanced cartilaginous fish (Northcutt, 1981a; Northcutt *et al*., 1988). Although not experimentally demonstrated, it seems likely that *Dlx* homologues control subpallium development in cartilaginous fish, given their expression in lamprey and bony fish subpallium (Murakami *et al*., 2001; Neidert *et al*., 2001; Stock *et al*., 1996) and their demonstrated existence in cartilaginous fish (Stock, 2005). Interneuron populations of the striatum in cartilaginous fish have not been extensively studied, but appear to be sparse at best (Reiner *et al*., 1998).

25.2.3 Osteicthyes – Ray-Finned versus Lobe-Finned Fish Divergence

Bony fish diverged into two very different groups early in their evolution (Nieuwenhuys, 1966; Northcutt, 1981a). One group, the lobe-finned fish, possesses the same paired tubular evaginated telencephalons as cartilaginous fish and includes the ancestors of amphibians. The other group, the ray-finned fish, evolved a very different telencephalic morphology, referred to as the everted telencephalon (see Evolution of the Nervous System in Fishes). In both groups, a basal ganglia has been demonstrated by modern hodological, neurochemical, and developmental criteria (Table 1; Figure 2; Reiner *et al*., 1998). For lobe-finned fish, the topography and cytology of the basal ganglia resemble those in cartilaginous fish (Reiner and Northcutt, 1987), whereas the peculiarities of the ray-finned fish telencephalon have rendered basal ganglia

Table 1 Summary of the major features of basal ganglia organization and whether those features are present in the major extant vertebrate groups possessing an evaginated telencephalon

Animal group	*Striatum*	*Pallidum*	*SP^+ striato-pallidal pathway*	*ENK^+ striato-pallidal pathway*	*SP^+ striato-nigral pathway*	*DA^+ nigro-striatal pathway*	*Glu^+ thalamo-striatal pathway*	*BG pretecto-tectal pathway*	*Glu^+ cortico-striatal pathway*	*GPe and GPi neuron location*	*GPe- STN-GPi pathway*	*GPi-thalamo-cortical pathway*
Lamprey	Present	Present	Present	Present	Present	Modest	Unknown	Unknown	Unlikely	Unknown	Unknown	Unlikely
Cartilaginous fish	Present	Present	Present	Present	Present	Modest	Unknown	Unknown	Unlikely	Intermixed	Unknown	Unlikely
Lobe-finned bony fish	Present	Present	Present	Present	Present	Modest	Unknown	Unknown	Unlikely	Intermixed	Unknown	Unlikely
Amphibians	Present	Present	Present	Present	Present	Modest	Present	Present	Negligible	Intermixed	Unknown	Absent
Reptiles	Present	Present	Present	Present	Present	Prominent	Present	Present	Present	Intermixed	Likely present	Likely present
Birds	Present	Present	Present	Present	Present	Prominent	Present	Present	Present	Intermixed	Present	Present
Mammals	Present	Present	Present	Present	Present	Prominent	Present	Indistinct	Present	Segregated	Present	Present

The table emphasizes that basal ganglia evolution was highly conservative among anamniotes, with the major changes occurring at the anamniote amniote transition. A few additional changes have occurred in the evolutionary transition from stem amniotes to mammals. BG, basal ganglia; DA, dopaminergic; ENK^+, enkephalinergic; Glu^+, glutamatergic; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; SP^+, substance P-containing; STN, subthalamic nucleus.

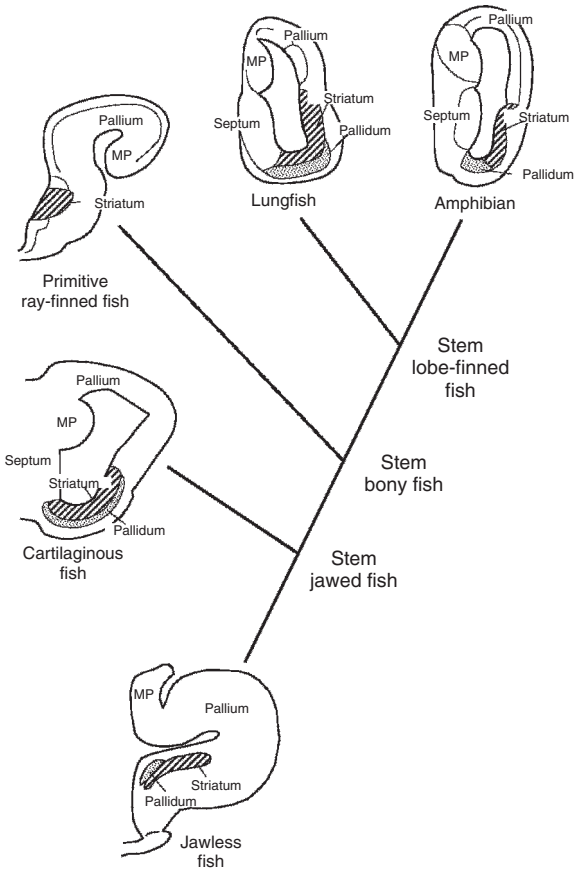

Figure 2 Schematics of frontal sections through the basal ganglia of the right telencephalic hemisphere in representative species from five anamniote groups: a lamprey (jawless fish), a shark (cartilaginous fish), a polypterid (ray-finned bony fish), a lungfish (lobe-finned bony fish), and a frog (amphibian), arranged according to their evolutionary divergences. The basal ganglia in all groups with an evaginated telencephalon (lamprey, shark, lungfish, and frog) consists of a striatum and a pallidum located in the basal telencephalon, beneath the pallial regions. Note that the pallium contains a medial (hippocampal) pallium (MP) in all amniote groups, with the medial pallium located laterally in ray-finned fish due to their telencephalic eversion. A striatum is evident in the ventral uneverted part of the telencephalon in ray-finned fish, but a pallidum is not well defined. Medial is to the left and dorsal to the top in all schematized sections.

topography and cytology less reminiscent of those in cartilaginous fish (Reiner and Northcutt, 1992). For example, in lungfish, the only lobe-finned fish whose basal ganglia has been studied (Reiner and Northcutt, 1987), the ventrolateral sector of the telencephalon contains both a striatum and a globus pallidus, by neurochemical and hodological criteria (Table 1). As in cartilaginous fish, the striatal sector contains SP^+ and ENK^+ neurons, but unlike in cartilaginous fish these are located in a cell-rich periventricular zone of neurons. The SP^+ and ENK^+ striatal neurons give rise to projections to a ventrocaudal neuronal cell group that appears comparable to globus pallidus (Figure 2), both because of this striatal input and because its neurons contain the neuropeptide LANT6. In this pallidal field, the SP^+ and ENK^+ inputs overlap, indicating that GPi- and GPe-type neurons are intermingled. Moreover, the striatum receives a dopaminergic input from the midbrain and these dopaminergic neurons receive a return projection from SP^+ striatal neurons (Reiner and Northcutt, 1987). The major populations of interneurons characterizing the striatum of amniotes may be scarce in lobe-finned fish basal ganglia (Reiner and Northcutt, 1987; Reiner *et al*., 1998). The pallium occupies the dorsal sector of the telencephalon, but it is unknown whether it projects to the striatum.

In ray-finned fish, a striatum containing SP^+ and ENK^+ neurons (Reiner and Northcutt, 1992; Reiner *et al*., 1998), likely to be GABAergic (Martinoli *et al*., 1990; Medina *et al*., 1994), having reciprocal connections with midbrain dopaminergic neurons (Reiner and Northcutt, 1992; Reiner *et al*., 1998; Rink and Wullimann, 2001), and expressing a *Dlx1/2* homologue has been identified (Stock *et al*., 1996; Wullimann and Mueller, 2004). Consistent with a dopaminergic input, the striatum in ray-finned fish is enriched in D1 and D2 dopamine receptors (Kapsimali *et al*., 2000; Vacher *et al*., 2003). A pallidal field may be present in the subpallium, since a ventral part of the subpallium expresses an *Nkx2.1* homologue (Wullimann and Mueller, 2004). The pallium in at least the more advanced ray-finned fish (i.e., teleosts with a large pallium) projects to the striatum and thalamic projections to the striatum appear to be present in all ray-finned fish (Northcutt, 1981b; Rink and Wullimann, 2004). The major populations of interneurons characterizing the striatum of amniotes are scarce in ray-finned fish basal ganglia (Reiner and Northcutt, 1992; Reiner *et al*., 1998). The extent to which the divergent evolution of the ray-finned fish telencephalon has affected the circuitry or function of the basal ganglia is uncertain. Nonetheless, as in mammals, loss of DA^+ input to ray-finned fish striatum results in 'Parkinsonian' symptoms, i.e., slowed movements or bradykinesia (Pollard *et al*., 1992). In any event, since ray-finned fish are not on the evolutionary line to mammals, further consideration of ray-finned fish basal ganglia anatomy is not entirely relevant here.

25.2.4 Amphibians

The telencephalon in amphibians is tubular in shape and its neurons largely occupy a periventricular position. This similarity to lobe-finned fish reflects the

evolutionary origin of amphibians from lobe-finned fish (Figure 3; see Evolution of the Amphibian Nervous System). The major features of basal ganglia organization demonstrated for lobe-finned fish and cartilaginous fish have also been demonstrated for amphibians (Table 1; Marin *et al*., 1998b; Reiner *et al*., 1998). Additionally, considerable hodological and developmental data are available for amphibians that definitively clarify many aspects of amphibian basal ganglia organization vis-à-vis that of amniotes (Table 1). For example, the ventrolateral sector of the telencephalon contains both a striatum and a globus pallidus, by neurochemical, hodological, and molecular developmental criteria. As in cartilaginous and lobe-finned fish, immunolabeling shows that the striatal sector contains SP^+ and ENK^+ neurons (Marin *et al*., 1997, 1998a, 1998b; Reiner *et al*., 1998), with the identity of the striatum further confirmed by the expression of glutamic acid decarboxylase (GAD) and an amphibian homologue of *Dlx1/2* (Papalopulu and Kintner, 1993; Bachy *et al*., 2002; Brox *et al*., 2003). Moreover, the SP^+ and ENK^+ striatal neurons give rise to projections to a ventrocaudal cell group that appears to be comparable to the globus pallidus (Figure 2), because of this striatal input, because it contains large GABAergic neurons, and because it expresses a homologue of *Nkx2.1* (Marin *et al*., 1998a, 1998b; Gonzalez *et al*., 2002; Brox *et al*., 2003). As is true in other anamniote groups, the SP^+ and ENK^+ inputs overlap in this pallidal field, indicating that GPi- and GPe-type neurons are intermingled. The amphibian striatum receives a dopaminergic input from the midbrain and these dopaminergic neurons receive a return projection from SP^+ striatal neurons (Table 1; Gonzalez and Smeets, 1994; Marin *et al*., 1998a, 1998b; Reiner *et al*., 1998). Both striatum and pallidum are, nonetheless, typically much more cell poor in amphibians, and the dopaminergic input more modest, than they are in amniotes. The types of interneurons characterizing the striatum of amniotes also seem to be scarce in amphibian basal ganglia (Reiner and Northcutt, 1992; Marin *et al*., 1997; Gonzalez *et al*., 2002; Reiner *et al*., 1998). The pallium occupies the dorsolateral sector of the telencephalon, but the major excitatory input to the striatum appears to arise from the thalamus rather than the pallium (Kicliter, 1979; Wilczynski and Northcutt, 1983; Marin *et al*., 1998b; Reiner *et al*., 1998). The basal ganglia in amphibians appears to have its major output to motor areas via a projection to the pretectum and a homologue of the substantia nigra pars reticulata, both of which affect head and eye movements by an input to tectal neurons with descending projections (Wilczynski and Northcutt, 1983; Marin *et al*., 1998b; Reiner *et al*., 1998). As in mammals, loss of dopaminergic input to amphibian striatum results in bradykinesia (Barbeau *et al*., 1986).

25.3 Amniote Basal Ganglia Evolution

25.3.1 Reptiles

Whereas the telencephalic hemispheres of reptiles also possess the same tubular evaginated structure as in amphibians during early development, the pallial and subpallial sectors of the telencephalon become much more cell rich than those in amphibians (Figures 3 and 4; Reiner *et al*., 1998). The

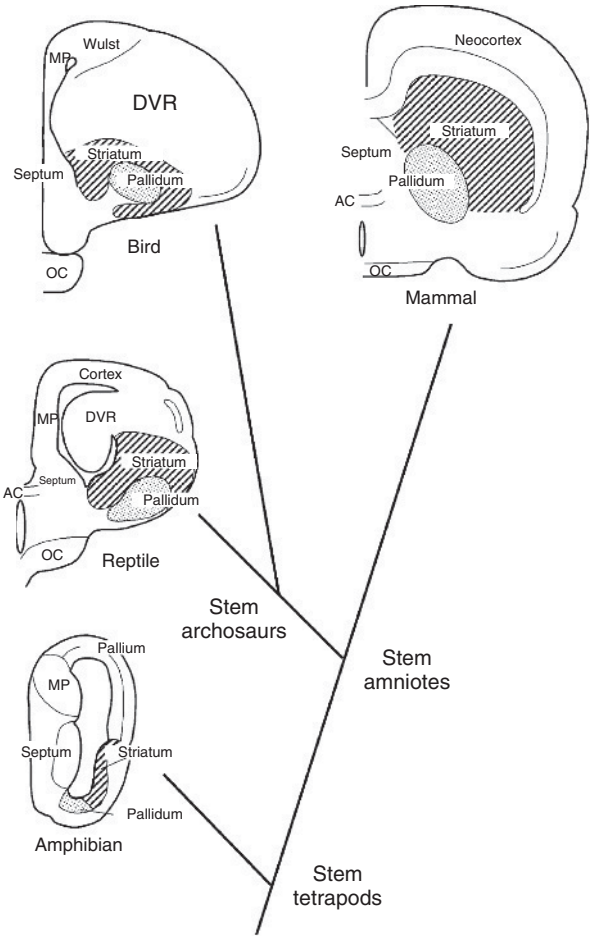

Figure 3 Schematics of frontal sections through the basal ganglia of the right telencephalic hemisphere in representative species from four tetrapod groups: amphibian (a frog), reptile (a turtle), bird (a pigeon), and mammal (a rat), arranged according to their evolutionary divergences. The basal ganglia in all four groups consists of a striatum and a pallidum located in the basal telencephalon, beneath the pallial regions. The pallidum, however, tends to be more laterally located in reptiles and birds than in amphibians and mammals. The phylogenetic distribution of pallidal laterality suggests that this trait arose in the reptilian lineage and was retained in birds. Medial is to the left and dorsal to the top in all schematized sections. AC, anterior commissure; MP, medial pallium; OC, optic chiasm.

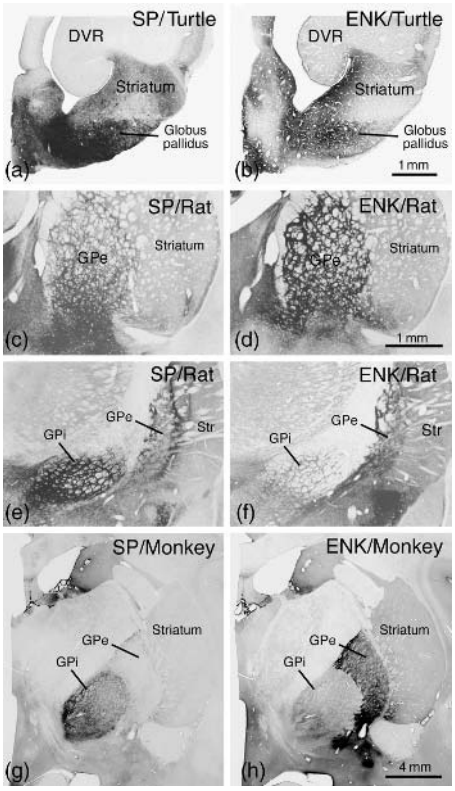

Figure 4 Images of frontal sections through the basal ganglia of one telencephalic hemisphere in a turtle (a), (b), rat (c f), and rhesus monkey (g), (h). Sections were immunohistochemically stained for substance P (SP) (left-hand column) or enkephalin (ENK) (right-hand column). Note the intense SP^+ and ENK^+ immunoreactivity in the ventrolateral wall of the telencephalon that defines the region of the striatum and distinguishes it from the dorsal ventricular ridge (DVR) of the overlying pallium in the case of turtle. As in mammals, the striatum region is rich in SP^+ immunoreactivity due to the presence of numerous SP^+ and ENK^+ neurons and their processes. Note also that the globus pallidus in turtle is rich in both SP^+ and ENK^+ fibers, indicating that in turtles the ENK^+ fiber recipient GPe-type and SP^+ fiber recipient GPi-type pallidal neurons are intermingled. By contrast, in rats and monkeys GPe and GPi pallidal neurons are spatially segregated, more so in rat than in monkey. Medial is to the left and dorsal to the top in all images. GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus; Str, striatum.

basal ganglia of reptiles reflects its inheritance from amphibians, but shows some differences that reflect the elaboration of the thalamus and pallium in reptiles (Table 1; see Evolution of the Nervous System in Reptiles). For example, as in amphibians, the ventrolateral sector of the reptile telencephalon contains both a striatum and a globus pallidus, by neurochemical, hodological, and developmental molecular criteria (Reiner *et al*., 1998; Smith-Fernandez *et al*., 1998). Also as in amphibians, immunolabeling shows that the striatal sector contains SP^+ and ENK^+ neurons (Reiner, 1987b; Russchen *et al*., 1987; Reiner *et al*., 1998), with the identity of the striatum further confirmed by the expression of GAD or GABA (Bennis *et al*., 1991), and a homologue of *Dlx1/2* (Smith-Fernandez *et al*., 1998). Moreover, the SP^+ and ENK^+ striatal neurons give rise to projections to a ventrocaudal cell group that is comparable to globus pallidus, because of this striatal input, because it contains large GABAergic neurons (Bennis *et al*., 1991), and because its neurons contain LANT6 (Reiner and Carraway, 1987; Reiner *et al*., 1998). The globus pallidus is, however, somewhat more laterally migrated than in amphibians (Figure 3). As is true in anamniotes, the SP^+ and ENK^+ inputs overlap in globus pallidus, indicating that GPi- and GPe-type neurons were intermingled in the stem amniotes (Figure 4). The reptile striatum receives a much more substantial dopaminergic input from the midbrain than in amphibians and these dopaminergic neurons in turn receive a substantial return projection from SP^+ striatal neurons (Smeets and Reiner, 1994; Reiner *et al*., 1998). As in mammals, dopaminergic effects on striatum are mediated by D1 and D2 dopamine receptors, with DA agonists inducing hyperkinesia and DA antagonism yielding hypokinesia, indicating a similar role of the dopaminergic system in modulating striatal output as in mammals (Andersen *et al*., 1975; Richfield *et al*., 1987; Reiner *et al*., 1998).

A distinguishing feature of the basal ganglia in reptiles is that it is much larger and more neuron rich than that in amphibians. Concomitant with the basal ganglia enlargement, the pallium in reptiles is also enlarged and is the source of a major excitatory input to the striatum, with the thalamus also providing excitatory input (Gonzalez *et al*., 1990; Butler, 1994a, 1994b; Reiner *et al*., 1998). Moreover, the glutamate receptors employed by specific types of striatal neurons to respond to this excitatory input in reptiles are very similar to those in mammals (Fowler *et al*., 1999). These features of living reptiles indicate that a major telencephalic enlargement and elaboration of corticostriatal circuitry occurred by the evolutionary appearance of stem amniotes (Figure 5; Reiner, 2002). The three major types of striatal interneurons characteristic of the mammalian basal ganglia (cholinergic, $PARV^+$, and somatostatinergic) are also present in the striatum in living reptiles, although they are not as highly abundant as in mammals (Powers and Reiner, 1993; Reiner and Carraway, 1987; Reiner *et al*., 1998). The basal ganglia in reptiles has its major output to motor areas via a projection to the pretectum and to the tegmentum (a substantia nigra pars reticulata (SNr) homologue), which affect head and eye movements

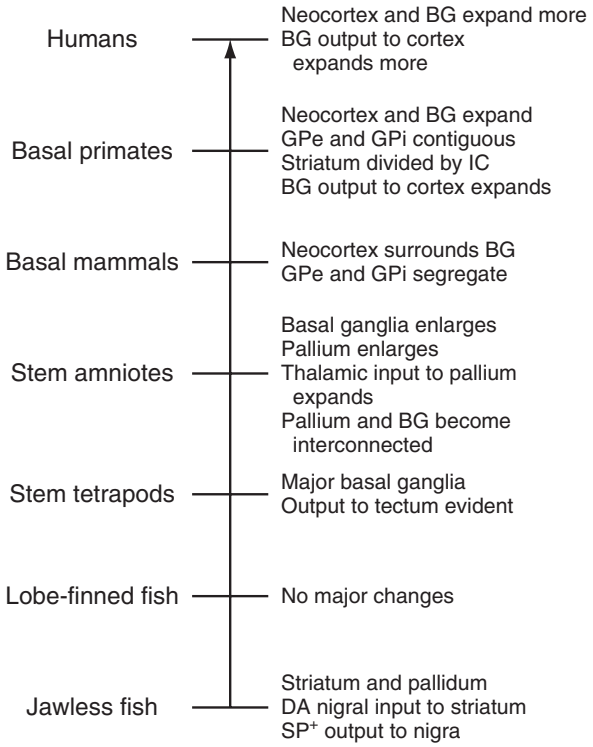

Figure 5 Time line indicating the points in the evolutionary line from jawless fish to humans at which major changes occurred in the basal ganglia.

by input to tectal neurons with descending projections to brainstem premotor cell groups (Reiner *et al*., 1980, 1998; Medina and Smeets, 1991).

25.3.2 Birds

Birds evolved from archosaurian reptiles, of which crocodilians are the only other living group (Chiappe, 1995). Unsurprisingly, therefore, the basal ganglia in birds highly resembles that in reptiles, with the main differences in the available data stemming from the overall telencephalic enlargement in birds and the deeper insights into basal ganglia anatomy and function stemming from the more extensively studied nature of birds (Table 1; Figure 4). For example, as in reptiles, the ventrolateral sector of the avian telencephalon contains both a striatum and a globus pallidus, as defined by neurochemical, hodological, and developmental molecular criteria (Reiner *et al*., 1998). Also as in reptiles, immunolabeling shows that the striatal sector contains SP^+ and ENK^+ neurons (Reiner *et al*., 1998), with the identity of the striatum further confirmed by the expression of GAD or GABA (Veenman and Reiner, 1994), and a homologue of *Dlx1/2* (Smith-Fernandez *et al*., 1998; Puelles *et al*., 2000). Moreover, the SP^+ and ENK^+ striatal neurons give rise to projections to a ventrocaudal cell group that is comparable to globus pallidus, because of this striatal input, because it contains large GABAergic neurons that also contain LANT6, and because its neurons express *Nkx2.1* (Karten and Dubbeldam, 1973; Reiner and Carraway, 1987; Veenman and Reiner, 1994; Reiner *et al*., 1998; Puelles *et al*., 2000). As in reptiles, this cell group is more laterally migrated than in lobe-finned fish, amphibians, or mammals, indicating that the pallidum was more medially located in stem amniotes and that the lateral migration of pallidal neurons evolved in the reptile–bird lineage (Figure 4). The three major types of striatal interneurons characteristic of the mammalian basal ganglia (cholinergic, $PARV^+$, and somatostatinergic) are present in striatum in living birds and as abundant as those in mammals (Medina and Reiner, 1994; Reiner and Carraway, 1987; Reiner *et al*., 1998). The reptilian striatum receives a dopaminergic input from the midbrain and these dopaminergic neurons receive a return projection from SP^+ striatal neurons (Reiner *et al*., 1994, 1998). As in reptiles and mammals, dopaminergic effects on striatum are mediated by D1 and D2 dopamine receptors, with DA agonists inducing hyperkinesia and DA antagonism yielding hypokinesia, indicating a similar role of the dopaminergic system in modulating striatal output as in mammals (Nistico *et al*., 1983; Richfield *et al*., 1987; Dietl and Palacios, 1988; Yanai *et al*., 1995; Reiner *et al*., 1998; Sun and Reiner, 2000; Reiner, 2002).

The pallium occupies the dorsolateral sector of the telencephalon, is much expanded in birds, and is the source of a massive excitatory input to the striatum (Veenman *et al*., 1995; Veenman and Reiner, 1996), with the thalamus also providing excitatory input (Wild, 1987; Reiner *et al*., 1998). The communication between pallium and striatum in birds appears to be mediated by the same two corticostriatal cell types as in mammals (Cowan and Wilson, 1994; Veenman *et al*., 1995; Reiner *et al*., 2001, 2003) and by the same cell type-specific glutamate receptors in striatum as in mammals (Reiner, 2002). The avian basal ganglia has its major output to motor areas via a projection to the pretectum and the tegmentum (an SNr homologue), which affect head and eye movements by input to tectal neurons with descending premotor and motor projections (Reiner *et al*., 1998). Additionally, birds possess a correspondent of the mammalian striato-pallido-thalamic circuit to motor cortex (Medina *et al*., 1997; Medina and Reiner, 2000), which suggests that one may be present in reptiles as well (see Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor

Cortices?). These various striatal output pathways to pretectum, tegmentum, and thalamus are all direct pathway type outputs that appear to facilitate behavior (Reiner *et al*., 1998). Birds have also been shown to possess a subthalamic nucleus and indirect pathway circuitry, as well (Jiao *et al*., 2000). The organization of the avian basal ganglia into direct and indirect striatal output circuits closely resembling those in mammals, and the presence of both SP^+ and ENK^+ striatal outputs in reptiles as well, suggests that the direct–indirect pathway plan of basal ganglia functional organization was already present in stem amniotes (Reiner, 2002).

25.3.3 Mammals

A few major changes in telencephalic morphology appear to have occurred in the evolution of the mammalian basal ganglia from the inferred stem amniote condition. First, from a simple, poorly laminated state, the pallium evolved into the multilayered neocortex that came to wrap around the basal ganglia (Karten, 1969; Puelles *et al*., 2000; Reiner, 2000, 2002). As a consequence, the basal ganglia occupies a more central position in the telencephalon than it does in nonmammals (Figures 2–4). The development of the basal ganglia versus the neocortex, however, reflects its ancestral basal position. The globus pallidus develops from an *Nkx2.1*- and *Dlx1/2*-expressing bulge (called the medial ganglionic eminence, or MGE) at the lower aspect of the lateral telencephalic wall and the striatum develops from *Dlx1/2*-expressing bulge that does not express *Nkx2.1* just above the MGE (called the lateral ganglionic eminence). By a process of extensive lateroventral migration from the pallium, where it meets the subpallium, the neocortex comes to surround the basal ganglia in mammals (Alvarez-Bolado and Swanson, 1996). Unlike in nonmammals, in which GPi and GPe neurons are intermingled, in mammals these pallidal populations occupy separate sectors, in either of two arrangements (Figure 5). In primates, the GPe and GPi are contiguous and distinguishable by their differential neurochemistry, namely, an enrichment in ENK^+ terminals from striatum in GPe and an enrichment in SP^+ terminals from striatum in GPi (Parent, 1986; Reiner *et al*., 1999; Hardman *et al*., 2002). All other mammal groups show a pallidal arrangement that must therefore be the primitive pattern for mammals. In this primitive pallidal pattern, the GPi and GPe are spatially separated, with the GPi enveloped by the internal capsule and seemingly dragged medially to a thalamic proximity. The gap between the two pallidal segments is so large that they have customarily been identified by different names than in primates: the globus pallidus instead of the GPe and the entopeduncular nucleus instead of the GPi. The globus pallidus is, however, clearly homologous to GPe and the entopeduncular nucleus to GPi. As a result, some neuroanatomical atlases for rodents embrace the primate names for the pallidal segments (Paxinos and Watson, 1998). Since it seems generally sound and parsimonious to call homologous structures by the same name (Reiner *et al*., 2004), this policy will be employed here as well.

The primate basal ganglia is also distinguished by being divided by the internal capsule into parts, called the caudate and putamen (Figure 4). Nonprimate mammals vary in the extent to which the striatum is divided by the internal capsule into a caudate and putamen, generally as a function of cortical development (Ariëns-Kappers *et al*., 1936; Parent, 1986). In many mammals with a lissencephalic cerebral cortex, such as some rodents, insectivores, bats, and monotremes, the caudate and putamen are not separated by the internal capsule and rather are pierced by myriad separate thalamocortical and corticothalamic fascicles that coalesce to form the internal capsule ventromedial to the striatum. In other mammalian groups, such as carnivores, ungulates, and some South American rodents, which independently evolved a gyrencephalic cortex, the striatum is divided by an internal capsule, but the point of division varies and differs from that in primates. This variation in internal capsule placement reinforces the notion that it, as well as cortex enlargement and convolution, evolved separately in different mammalian orders (Northcutt and Kaas, 1995). Since mammalian groups that are presumptively closer to the basal mammalian condition, such as monotremes and insectivores, do not show striatal division by the internal capsule, an undivided striatum is likely to be primitive for mammals.

There appear to be no noteworthy differences between mammals and sauropsids in the major types of neurons making up the striatum and pallidum (Table 1). In both amniote groups, the striatum consists of two main neurochemically distinct types of neurons, the SP^+ and the ENK^+ GABAergic neurons, and three types of interneurons, the cholinergic, the $PARV^+$, and the somatostatinergic (Graybiel, 1990; Gerfen, 1992; Reiner *et al*., 1998). In both groups, the projection neurons far outnumber the interneurons and pallidal neurons are large and GABAergic. Moreover, in mammals as in sauropsids, basal ganglia circuitry is organized into the direct–indirect pathway plan (Albin *et al*., 1989; DeLong, 1990; Gerfen, 1992; Reiner *et al*., 1998).

Massive glutamatergic inputs to striatum arise from cortex and thalamus, and a massive dopaminergic input to striatum arises from the substantia nigra pars compacta (Gerfen, 1992; Reiner *et al*., 1998), and the general role of the basal ganglia in motor learning and control seems similar in all amniotes. Mammals do differ from birds and reptiles in that they lack an obvious correspondent of the basal ganglia output to midbrain via pretectum (Table 1; Figure 5; Reiner *et al*., 1998), and their output to motor cortices via the thalamus seems instead more prominently developed, especially in primates (Albin *et al*., 1989; DeLong, 1990; Gerfen, 1992; Reiner *et al*., 1998). Additionally, mammalian striatum is compartmentalized into a network of interlaced zones called striosomes and a much larger sector in which the striosomes are embedded called the striatal matrix (Graybiel, 1990; Gerfen, 1992). These two striatal sectors, which differ in their connectivity with cortex and midbrain, consist of neuronal populations that are more uniformly interspersed in birds and reptiles (Reiner *et al*., 1998). Thus, striosomes are not evident in the striatum of birds or reptiles, but are in all mammals (Künzle, 2005).

Little is known about diversity in basal ganglia organization among mammals, largely because most hodological and neurochemical studies in mammals have focused on only a few groups: rats, mice, cats, and monkeys. Stephan (1979) compared the volume of the striatum in various insectivore, prosimian, and simian species. Although the raw data indicated that striatal volume as a percentage of the telencephalon decreased from approximately 8% to 3% from insectivores to humans, scaling according to body weight revealed an increase in striatal size (relative to body size) from insectivores to prosimians to simians. Along these lines, Stephan (1979) specifically noted that the human striatum would be 14 times larger than that of a basal insectivore of human size. The human cerebral cortex would be larger yet, approximately 30 times the size of that in a basal insectivore. Striatal enlargement, thus, in evolution from basal mammals through primates has contributed to overall telencephalic enlargement, but less so than has expansion and areal diversification of the neocortex (Stephan and Andy, 1969). Neocortical expansion and diversification, in particular, exceed striatal enlargement in the primate radiation from prosimians to simians. Another morphometric study using a slightly different approach also concluded that both neocortex and striatum had expanded progressively in the primate radiation, with the neocortical expansion outpacing the striatal expansion, especially in humans (Clark *et al*., 2001). These findings confirm, as suggested by the connectivity data, that cortex and striatum are functionally linked and that cortex does not take over the role of striatum, as had been presumed in early twentieth century ideas about telencephalic evolution (Ariëns-Kappers *et al*., 1936; Herrick, 1948, 1956).

Hardman *et al*. (2002) performed a quantitative morphological study on the size and neuronal abundance of several additional basal ganglia cell groups or targets in rats and several primate species. Immunolabeling for various cell type-specific markers was used to objectively define the extent of the different cell groups measured, which included the GPe, GPi, STN, substantia nigra pars compacta, and SNr. Corrected for overall brain size, the sizes of the GPe, GPi, and STN in relation to one another were relatively constant across the groups examined, with GPe being larger (and more neuron rich relative to brain neuronal abundance) than GPi and with GPi being larger than STN in each species. The substantia nigra was, however, relatively larger and more neuron rich (relative to brain neuronal abundance) in rodents than in the primates examined. This is likely to reflect a relatively greater importance of striato-SNr circuitry in motor control than striato-GPi circuitry in rat than in primate. Whether this is generally true of nonprimates is uncertain, but it suggests a possibly increased role of basal ganglia outflow to motor cortex in primates than nonprimates (Figure 5).

25.4 Mammalian Basal Ganglia Evolution – Outdated Concepts and Terminology

The preceding overview of basal ganglia evolution in vertebrates reveals that the striatum and pallidum are ancient structures, with both apparently being present in jawless fish ancestral to modern jawed vertebrates (Table 1; Figure 5). Thus, the notion that the pallidum (i.e., the so-called paleostriatum) evolved first and is older than the striatum (i.e., the so-called neostriatum) is incorrect. Because the terms paleostriatum and neostriatum reflect and perpetuate outdated ideas about basal ganglia evolution, we recommend their abandonment. Although the evidence was not reviewed here, the notion that the paleocortex (olfactory or pyriform cortex), archicortex (hippocampus), and neocortex evolved successively in evolution is also flawed, since paleocortex and archicortex seem to be equally ancient parts of the vertebrate telencephalon (Northcutt, 1981a; Northcutt and Kaas, 1995; Rodriguez *et al*., 2002). Because the term neocortex refers to a new, uniquely mammalian structure, this

term is, however, debatably suitable. Similarly, the notion that the basal ganglia are a part of the motor system separate from the descending cortical pyramidal tracts is belied by the extensive interconnections of the cortex and basal ganglia and by the output of the basal ganglia to motor cortices. Thus, the classification of the basal ganglia as part of a motor system called the extrapyramidal system is also suspect. The history of basal ganglia evolution seems to be characterized by an increase in neuron number as the telencephalon expanded during the anamniote–amniote transition, with the elaboration of prominent cortical glutamatergic inputs and midbrain dopaminergic inputs, and by an increased role for telencephalic circuitry in motor control all occurring in stem amniotes (Figure 5). In mammals, especially the primate lineage, this trend has been furthered. Nonetheless, the basic direct–indirect pathway circuit plan by which the basal ganglia regulates movement may have already been in place in early anamniotes.

Acknowledgments

Our work summarized here was supported by NS-19620 (A.R.), NS-28721 (A.R.), EY-05298 (A.R.), the Neuroscience Center for Excellence of the University of Tennessee, Memphis (L.M.), and the Spanish Ministry of Education and Science (L.M.), to whom we are grateful.

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26 The Evolution of the Hippocampus

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Glossary

amnesia	Memory impairment characterized by profound forgetfulness.
consolidation	Process by which memory becomes independent of hippocampal region over time.
declarative memory	Memory for facts and events.
episodic memory	Memory for events.
familiarity	Component of recognition memory characterized by a memory in the absence of recollected details.
hippocampal region	Cornu ammonis fields of the hippocampus proper, dentate gyrus, and subiculum.
macrosomatic	Describes an animal with a well developed olfactory system
medial temporal lobe	Portion of brain, present in larger brained mammals such as primates, that contains the hippocampal and parahippocampal regions.
microsomatic	Describes an animal with a poorly developed olfactory system
parahippocampal region	Entorhinal, perirhinal, and postrhinal (parahippocampal in primates) cortices.
place cell	Neuron whose firing rate correlates strongly with animal's location in an environment.
recognition memory	Capacity to judge an item as having been previously encountered.
recollection	Component of recognition memory characterized by retrieval of specific details relating to the incident in which the item to be remembered was encountered.
retrograde amnesia	Loss of information acquired prior to onset of brain damage.
semantic memory	Memory for facts.

26.1 Introduction

The hippocampus is a brain area that has received considerable attention because of its distinctive anatomy and its important role in memory. A particularly productive approach to studying the hippocampus has been to examine either its form or function across mammalian species (Brown and Aggleton, 2001; Burwell *et al*., 1995; Cohen and Eichenbaum, 1993; Insausti, 1993; Squire, 1992) or to consider whether homologous structures exist in other vertebrates (Aboitiz *et al*., 2002; Bingman *et al*., 2003; Day, 2003; Jacobs, 2003; Salas *et al*., 2003; Sherry and Schacter, 1987). The present article builds on these previous efforts and considers the evolution of the mammalian hippocampus from both anatomical and functional viewpoints. Based on this dual approach, we make two main points.

First, the anatomy of the hippocampal region is largely conserved across mammals. The connectivity and cytoarchitectural features of the hippocampus, dentate gyrus, and subiculum are

remarkably similar for all mammalian species for which information is available. Further, the surrounding cortices in the parahippocampal region also show a substantial degree of conservation across the mammalian taxon. The anatomical details of the hippocampal and parahippocampal regions are not identical from species to species, but these differences are overshadowed by the substantial divergence in the organization of the neocortex. Moreover, structures homologous to the mammalian hippocampus appear in birds and reptiles, yet these structures do not directly parallel the distinct subdivisions of the hippocampal region. Accordingly, the first half of the article considers the anatomical homology of the mammalian hippocampus and details its features along with those of the adjacent parahippocampal region. This half ends by looking to birds and reptiles for evidence of how the hippocampus may have appeared in the earliest mammals.

Second, the hippocampus serves the same fundamental mnemonic function across mammals, and homologous structures in other vertebrates may support a similar capacity. Today, we know that the distinctive anatomy and physiology of the hippocampus anchors only one of several memory systems of the mammalian brain. Together with the parahippocampal region, the human hippocampal region enables a record of our experiences that can be subsequently brought back to mind as facts and events (Manns and Squire, 2002; Poldrack and Gabrieli, 1997; Schacter *et al*., 1998). This notion of memory as conscious recollection is difficult to extend to experimental animals, yet other operating characteristics of hippocampus-dependent memory apply equally well across mammals, including rapid learning, complex associative organization, and flexibility in retrieval (Eichenbaum and Cohen, 2001). The second half of the article tracks the progression of thought regarding the function of the mammalian hippocampus, starting with a point around 50 years ago at which the function of the hippocampus was uncertain and was presumed to be different for humans and experimental animals.

Current research focuses on identifying the fundamental principles that define hippocampus-dependent memory across species. We argue that this effort will be best served by taking an evolutionary approach. The divergence of mammals has provided a natural experiment in which a largely conserved hippocampal system can be explored among neocortical conditions that differ across species. The goal of this approach is to highlight the conserved function of the hippocampus and downplay most species-specific distinctions as a byproduct of differing neocortical inputs.

It is worth pointing out that the reader will find articles Reconstructing the Organization of Neocortex of the First Mammals and Subsequent Modifications, Sex and Species Differences in Hippocampal Volume, Evolution of the Nervous System in Reptiles, The Evolution of Vocal Learning Systems in Birds and Humans, The Hippocampal Formation in Food-Storing Birds, and Evolution of Vertebrate Olfactory Subsystems in the present work as very relevant and informative.

26.2 Anatomical Homology

26.2.1 Anatomy of the Mammalian Hippocampal and Parahippocampal Regions

26.2.1.1 Terminology The hippocampus is highly interconnected with several neighboring and closely interconnected structures, and its anatomy is best understood in combination with these structures (see Amaral and Witter (1995) for a more extensive anatomical review). Indeed, the term hippocampus is often used to refer to not only the cornu ammonis (CA; or hippocampus proper) but also to the dentate gyrus. The hippocampus proper is contiguous with the subiculum, and here these regions together with the dentate gyrus are called the hippocampal region. The adjacent parahippocampal region includes the entorhinal, perirhinal, and postrhinal (parahippocampal in primates) cortices and provides the majority of cortical input to the hippocampal region and is the recipient of its major cortical outputs (Burwell *et al*., 1995).

26.2.1.2 Gross morphology The hippocampus proper and dentate gyrus are both curled sheets of cortex that are rolled together to form a tube-like structure. In many small-brained mammals, the long axis of the tube begins at a medial, dorsal locus just posterior to the septum, and curves to a ventral and caudal apex before bending and heading rostrally and slightly laterally within the temporal region (see Figure 1a for the position of the hippocampal region in the rat brain). In mammals with larger brains, the general shape of the elongated tube has been conserved, but the structure has slid down along the same septotemporal axis described above so that in primates, the hippocampus is contained within the medial aspect of the temporal lobe (Figure 1). The parahippocampal region surrounds the hippocampal region and therefore takes on a different position

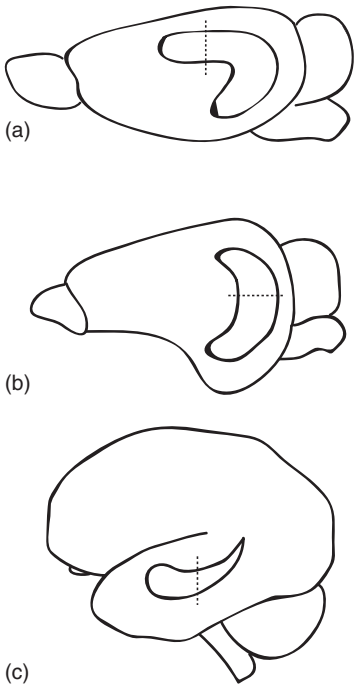

Figure 1 Position of the hippocampus in the brain of a rat (a), tree shrew (b), and human (c). The difference in position between small, medium, and big-brained mammals suggests that the hippocampus has slid through evolution along its long axis to the point that, in humans, the hippocampus is contained entirely within medial portion of the temporal lobe. The dashed lines in each drawing indicate cross sections perpendicular to the long axis of the hippocampus, similar to those depicted in Figure 2.

in the brain according to the position of the hippocampal region (Burwell, 2000).

26.2.1.3 Cytoarchitecture In all mammals, a cross section of the hippocampal region taken perpendicular to the long axis reveals the densely packed cell layers of the hippocampus proper and dentate gyrus, which at most septotemporal levels appear to fit together like interlocking arcs. Figure 2 shows examples from four species. The hippocampus proper consists of pyramidal cells bounded above and below by cell-sparse layers and can be divided into three subfields: CA1, CA2, and CA3. The pyramidal cells in CA1 were originally distinguished from those in CA3 on the basis of size; CA1 pyramidal cells are slightly smaller (Ramon y Cajal, 1911). The CA2 subregion is a small transitional area between CA1 and CA3. The dentate gyrus is also a curved sheet of three-layered cortex, although its principal cells are granule cells. Immediately adjacent to CA1 is the subiculum, another three-layered section of cortex whose principal cells are pyramidal neurons. Although the laminar organization of each subregion represents a 'simple' cortical organization with only one layer of principle cells, the principle cells in each subregion are wrapped in a plexus woven from interneurons.

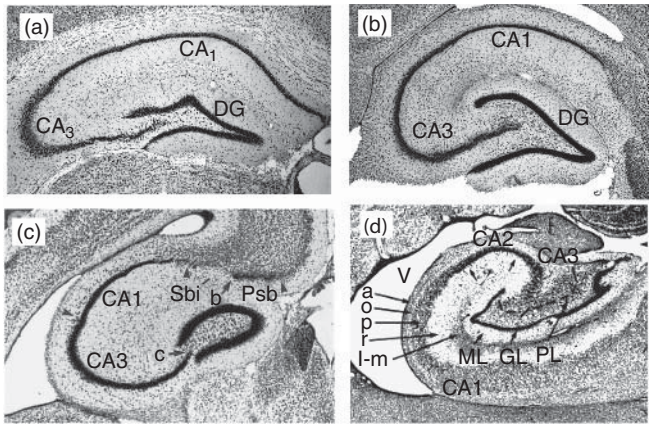

Figure 2 Cross sections taken perpendicular to the long axis of the hippocampal region in a mouse (a), tree shrew (b), tenrec (c), and human (d). The cell fields (CA1, CA3) of the hippocampus proper and the dentate gyrus (DG) appear similar in all four mammals. Note that CA1 appears below CA3 in the human hippocampus due to the evolutionary slide illustrated in Figure 1. See original sources for additional information. a, Reproduced from Van Groen, T., Kadish, I., and Wyss, J. M. 2002. Species differences in the projections from the entorhinal cortex to the hippocampus. *Brain Res. Bull.* 57(3 4), 553 556, with permission from Elsevier. b, Keuker, J. I., Rochford, C. D., Witter, M. P., and Fuchs, E. 2003. A cytoarchitectonic study of the hippocampal formation of the tree shrew (*Tupaia belangeri*). *J. Chem. Neuroanat.* 26(1), 1 15, with permission from Elsevier. c, Kunzle, H. and Radtke-Schuller, S. 2001. Hippocampal fields in the hedgehog tenrec. Their architecture and major intrinsic connections. *Neurosci. Res.* 41(3), 267 291, with permission from Elsevier. d, Reprinted from Amaral, D. G. 1999. Introduction: What is where in the medial temporal lobe? *Hippocampus* 9(1), 1 6.

The adjacent entorhinal, perirhinal, and postrhinal/parahippocampal cortices have a more complicated laminar organization than the three-layered areas in the hippocampal region (for detailed descriptions, see Burwell, 2000; Suzuki and Amaral, 2003). Although the laminar profile of these regions does not directly correspond to the six-layered neocortex, each area of the parahippocampal region is typically partitioned into six layers. The cellular composition of these layers differs between the three regions and, in combination with differing patterns of connectivity, defines their borders. However, the cytoarchitectural details differ even within a region, and no single criterion can be used to define all the borders of any region. Further, the entorhinal, perirhinal, and postrhinal/parahippocampal cortices are typically divided into several subregions based on local differences in cytoarchitecture and connectivity. A full account of the cytoarchitecture in these regions is beyond the scope of the present article.

26.2.1.4 Intrinsic circuitry of the hippocampal region The major intrahippocampal connections are serial and unidirectional (Amaral and Witter, 1995). This intrinsic circuit traces a path from the dentate gyrus to CA3 before continuing to CA1 and finally to the subiculum. In addition, the pyramidal cells in CA3 send a substantial number of axons to other pyramidal cells in CA3. The connections between subregions are organized in different patterns, suggesting that information is being transformed in distinct ways at each step in the serial circuit. This organization is depicted in Figure 3 and is described next.

The projection from the dentate gyrus to CA3 involves a lamellar organization such that, at successive septotemporal levels, dentate cells project to the entire transverse extent of CA3 at about the same level (Gaarskjaer, 1986; Swanson *et al.*, 1978). In contrast to this transverse dispersion of connectivity, the projection from CA3 to CA1 involves an organization in which CA3 cells from a given septotemporal level project to about two-thirds of the septotemporal extent of CA1 (Ishizuka *et al.*, 1990; Li *et al.*, 1994). Moreover, the CA3 projections do extend in the transverse plane, although a gradient applies such that CA3 pyramidal cells close to the CA1 border project to the adjacent CA1 cells and CA3 pyramidal cells closest to the dentate gyrus project to CA1 cells closest to the subiculum. The projection from CA1 to subiculum is perhaps the most structured of the intrahippocampal projections and is organized in at least two dimensions: along the transverse axis and along the septotemporal axis (Amaral *et al.*, 1991). Three nearly discrete transverse columns project to the subiculum such that the third of CA1 cells closest to the subiculum project to the third of the subiculum that is immediately adjacent to CA1. The middle third of CA1 projects to the middle third of the subiculum, and the third of CA1 closest to CA2 (farthest from the subiculum) projects to the third of the subiculum farthest from CA1. Like the CA3 to CA1 projection, the CA1 to subiculum projection is also distributed along the septotemporal axis such that CA1 cells at any septotemporal level project to about one third of the septotemporal extent of the subiculum.

The anatomy of the hippocampal region offers insight into the potential role of its subregions. The

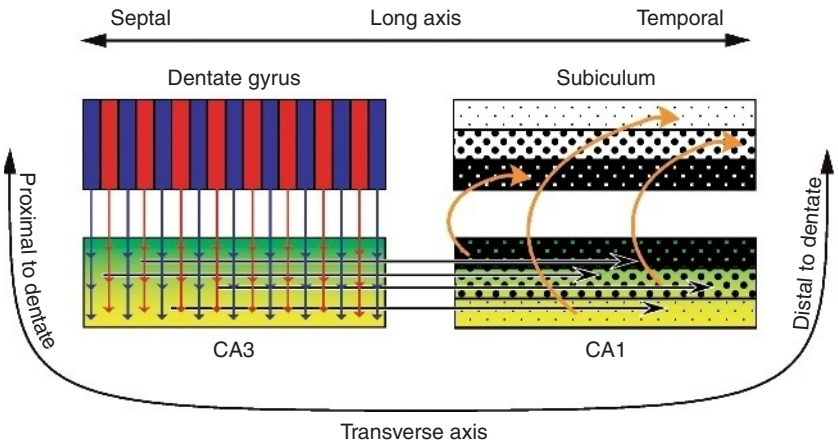

Figure 3 Connections between subregions of mammalian hippocampal region. The dentate gyrus projects to CA3 in a lamellar fashion (red and blue slices), whereas CA3 projections to CA1 are dispersed along the long axis. A gradient also applies to this projection (green to yellow) such that CA3 cells proximal to the dentate gyrus tend to project to CA1 cells distal to the dentate gyrus. The projections from CA1 to subiculum are partitioned into three transverse columns (indicated by dot patterns). Further, projections from CA1 reach about a third of the septotemporal extent of the subiculum. See text for more details.

orthogonal dispersion gradients of dentate gyrus to CA3 projections (transverse) and CA3 to CA1 projections (septotemporal) suggest that these connections might be involved in the final stage of reformatting sensory information in the service of enabling arbitrary associations within and across modalities. The recurrent CA3 to CA3 connections might also participate in this process. In comparison, the more organized CA1 to subiculum projection could represent the first step in repackaging new associations in a format compatible with the topography of the neocortex. In any case, it is clear that the serial circuit through the hippocampal region is not simply a passive relay of information.

26.2.1.5 Connections with the entorhinal cortex In addition to the prominent serial organization of intrahippocampal connectivity, there are parallel direct connections between each of the hippocampal subregions and the entorhinal cortex in all mammals (Figure 4). Thus, although some information traveling through the hippocampal region might proceed serially, there are also 'shortcuts' into and out of each subregion. The projections from the entorhinal cortex are collectively called the perforant path, but the trajectory of this path differs for CA1 and subiculum on one hand and dentate gyrus and CA3 on the other (Witter *et al.*, 2000b). The projections to CA3 and dentate gyrus originate mostly in layer II of the entorhinal cortex, whereas the projections to CA1 and subiculum originate mostly in layer III of the entorhinal cortex. The layer III projection is mirrored by direct return projections from the subiculum and CA1 to the entorhinal cortex.

The projections from entorhinal cortex to the hippocampal subregions can be distinguished further on the basis of the areas within entorhinal cortex from which the connections originate (Witter, 1993). Specifically, there are two parallel pathways, one arising from the lateral entorhinal area (LEA) and the other arising from the medial entorhinal area (MEA). The line bisecting LEA and MEA is not actually perpendicular to the lateral/

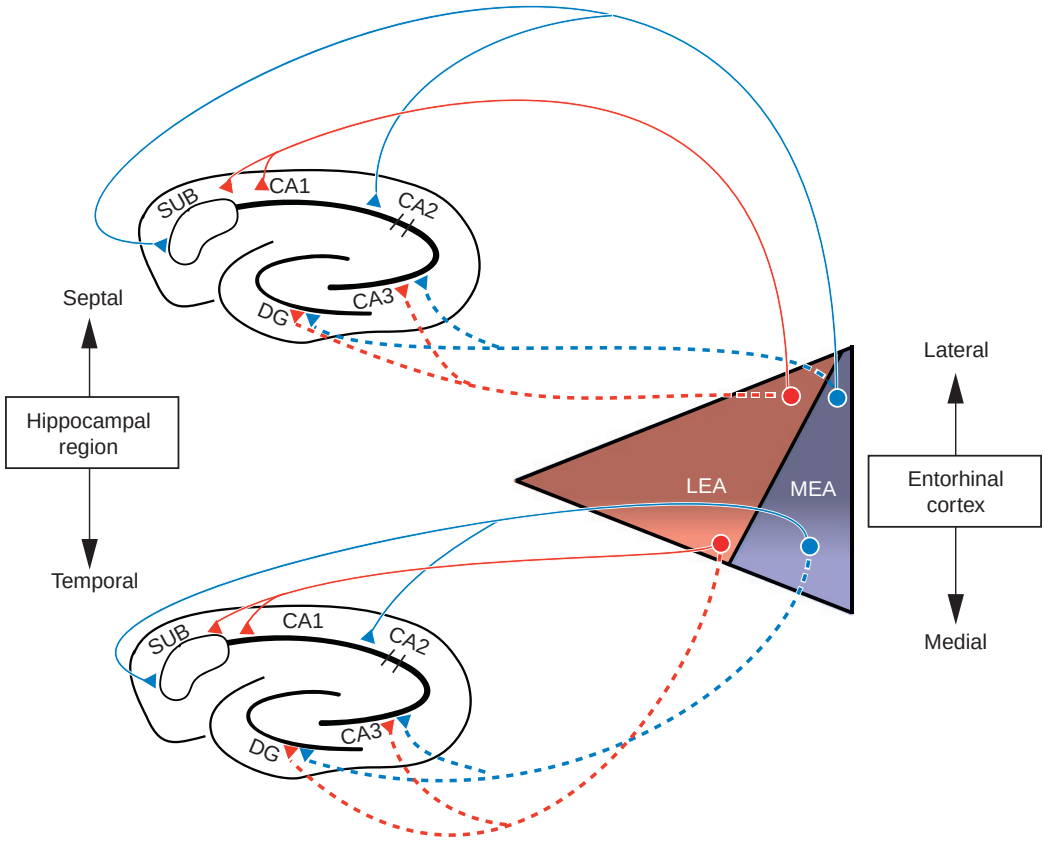

Figure 4 Organization of the mammalian perforant path. Three trends are apparent in the entorhinal projections to the hippocampal region. First, the lateral medial axis of entorhinal cells corresponds to a septotemporal termination gradient in the hippocampal region. Second, separate branches of the perforant path originate in LEA and MEA. These projections are combined in the dentate gyrus and CA3 but are kept separate in the subiculum and CA1. Third, projections to the dentate gyrus and CA3 originate primarily in layer II of the entorhinal cortex (dashed lines), but projections to the subiculum and CA1 originate primarily in layer III (solid lines).

medial cardinal axis in any species, such that LEA and MEA are defined by the origins of the two pathways rather than by their cardinal positions. Both pathways contain projections from layers II and III and project to each of the four hippocampal subregions. However, the pattern in which the fibers from both pathways terminate in hippocampal targets differs between CA3 and dentate gyrus on one hand and CA1 and subiculum on the other (see Figure 4). The projections from LEA and MEA target the same subsets of dentate and CA3 cells. In contrast, the projections from LEA target portions of CA1 and subiculum that differ from the portions targeted by the projections arising in MEA. In particular, projections arising in LEA target CA1 and subiculum cells near the subiculum/CA1 border, whereas projections arising in MEA target CA1 and subiculum cells farthest from the subiculum/CA1 border. Thus, information passing through LEA and MEA appears to be combined in the dentate gyrus and CA3 but kept separate in the subiculum and CA1. Of course, both CA1 and the subiculum also receive intermixed LEA and MEA information via the serial input from dentate gyrus and CA3.

One additional feature characterizes the overall topography of the entorhinal inputs into the hippocampal region. Projections originating in the lateral aspect of the entorhinal cortex (including the lateral aspects of both the LEA and MEA) terminate largely in the septal end of the hippocampal subregions. Conversely, projections originating in the medial aspect of the entorhinal cortex (including the medial aspects of both the LEA and MEA) terminate largely in the temporal end of the hippocampal subregions.

26.2.1.6 Intrinsic circuitry of the parahippocampal region The LEA and MEA can be further distinguished on the basis of their inputs originating in the rest of the parahippocampal region. LEA receives more cortical projections from the perirhinal cortex, whereas MEA receives more cortical projections from the parahippocampal/postrhinal cortex (Witter *et al*., 2000a). This difference is distinguished even further by the fact that perirhinal cortex appears to receive a different subset of olfactory and neocortical inputs as compared to parahippocampal/postrhinal cortex (Burwell and Amaral, 1998a; Suzuki and Amaral, 1994). The postrhinal/parahippocampal cortex receives more inputs from cortical areas important for allocentric spatial information. The perirhinal cortex receives more inputs from olfactory areas and neocortical areas important for nonspatial information. Based on these observations, one view of the connectivity of the parahippocampal region is that information reaching the perirhinal cortex follows a path through the LEA to the hippocampal region that runs parallel to the path taken by information reaching the postrhinal cortex and continuing through the MEA (Witter *et al*., 2000a). These pathways appear to be largely combined in dentate gyrus and CA3 but kept at least somewhat separate in CA1 and the subiculum. However, the notion of parallel, functionally distinct input streams is tempered by the presence of a substantial projection from parahippocampal/postrhinal cortex to perirhinal cortex (and a smaller return projection) in addition to connections between LEA and MEA.

26.2.2 Summary of Anatomy of the Hippocampal and Parahippocampal Regions

The animals of the mammalian taxon represent a great diversity of habitats, means of locomotion, preferred diets, and social structure. They also represent a great diversity of neuroanatomy. For example, tenrecs and hedgehogs are both small-brained insectivores whose neocortex is predominately composed of primary sensory areas (Catania *et al*., 2000; Krubitzer *et al*., 1997). In contrast, the brains of primates contain numerous neocortical areas that are devoted to integrating information across modalities. Further, the amount of tissue devoted to a particular sensory modality also varies substantially between species (Krubitzer and Kaas, 2005). In macrosomatic animals such as rodents, large portions of the brain are involved in processing odors. In other animals, vision (e.g., primates), audition (e.g., bats), or somatosensation (e.g., star-nosed moles) have become disproportionately represented in the brain. Further, the anatomy of the hippocampal and parahippocampal regions includes many complex and highly organized patterns of interconnectivity. This complexity would seem to provide many opportunities for divergence throughout evolution, especially when considering that many other features differ substantially between species. Thus, it is surprising that the predominant trend with respect to the anatomy of the hippocampal and parahippocampal regions is one of conservation rather than divergence. Figure 5 shows an evolutionary tree of some of the animals discussed in this article and is meant illustrate how the diversity of these selected mammals contrast with the conservation of the anatomy of the hippocampal and parahippocampal regions. Nevertheless, there are differences between species with respect to the anatomy of the hippocampal region and to a somewhat greater extent the

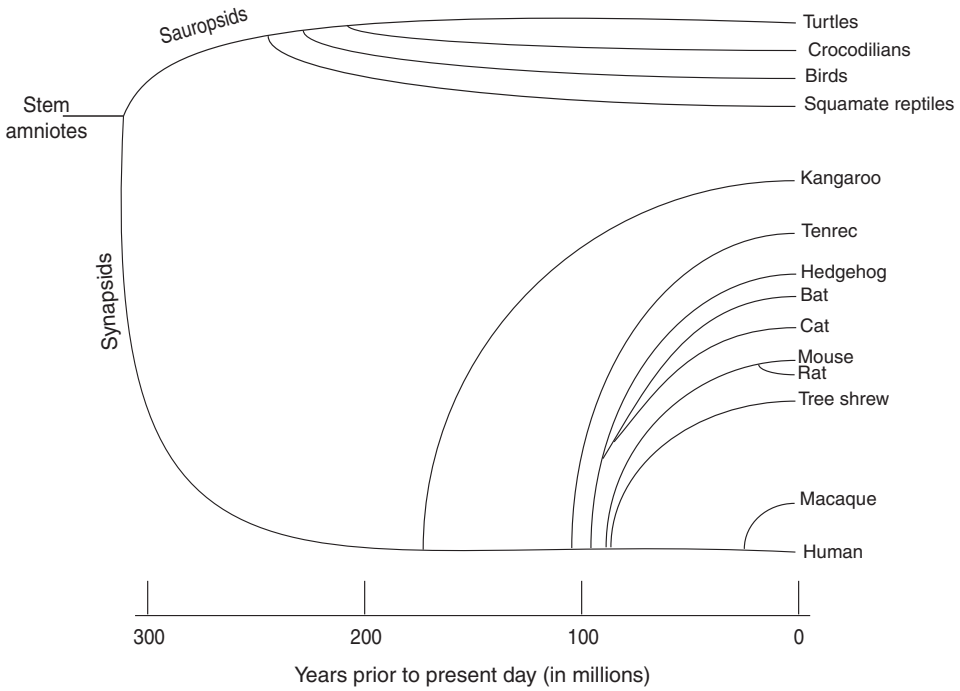

Figure 5 Phylogeny of birds, reptiles, and selected mammals. The dates are estimates based on recent molecular techniques (Arnason *et al.*, 2002; Springer *et al.*, 2003).

parahippocampal region. These differences are considered next.

26.2.3 Anatomical Differences between Species

26.2.3.1 Hippocampal region Species differences in the hippocampal region are best characterized as refinement rather than reorganization. The intermediate subregion CA2 can be clearly distinguished in primates (Bakst and Amaral, 1984; Green and Mesulam, 1988), but its identification is more difficult in smaller-brained mammals such as hedgehogs and tenrecs (Kunzle and Radtke-Schuller, 2001; West *et al.*, 1984). Also, the border that demarcates the transition from CA1 to subiculum is less clearly defined in small-brained mammals (Kunzle and Radtke-Schuller, 2001; West *et al.*, 1984). Furthermore, in hedgehogs, but not in tenrecs or in larger-brained mammals, the mossy fiber projection from dentate gyrus to CA3 invades CA1 to some degree (Kunzle and Radtke-Schuller, 2001; West *et al.*, 1984). Taken together, the overall similarities in cytoarchitectural plan and connectivity described above far outweigh these minor differences, especially when considering the complexity of the region's circuitry. Nevertheless, the trend for the hippocampal region appears to be one of increasing distinction between subregions as a refinement that often comes with evolution.

26.2.3.2 Entorhinal cortex The entorhinal cortex also follows an evolutionary trend of increasing diversification. In most mammals studied, including the hedgehog, LEA is clearly distinguished from MEA (Insausti, 1993; West *et al.*, 1984). One possible exception is the tenrec, in which the entorhinal cortex was poorly differentiated from the piriform cortex and LEA and MEA were not distinguished from one another (Kunzle and Radtke-Schuller, 2001). Nevertheless, in other mammals, the tendency is for entorhinal cortex to increase in diversity with brain size, such that six, seven, and eight subdivisions have been identified in the rat, monkey (macaque), and human, respectively (Insausti, 1993).

Despite the increasing complexity of the entorhinal cortex and refinement of the hippocampal region, the highly structured organization of connectivity between the areas is quite similar across mammals. In particular, the specific topography of entorhinal inputs to the hippocampal formation (Figure 4) is similar in all species for which detailed anatomical information is available, including bats (Buhl and Dann, 1991), mice (Van Groen *et al.*, 2002), rats (Witter, 1993), cats (Witter and Groenewegen, 1984), and monkeys (Witter and Amaral, 1991). However, there are several exceptions that are worth mentioning. In mice, the entorhinal projection to CA3 originates predominately in layer III rather than in layer II as

described in larger-brained mammals, including rats (Van Groen *et al*., 2002). Further, several species differences have been noted in the laterality of projections between regions. For example, projections from the subiculum to entorhinal cortex are solely ipsilateral in rats but are bilateral in cats and monkeys (Amaral and Witter, 1995). It is not always the case that bigger-brained mammals show more anatomical refinement than smaller-brained mammals. For example, LEA and MEA projections to dentate gyrus terminate at the same portions of the granule cell dendrites in monkeys (Witter and Amaral, 1991). In contrast, in the other mammals studied (bats, mice, cats, and rodents), projections from the LEA terminate in the superficial third of the granule cell dendrites, whereas projections from MEA terminate in the middle third of the granule cell dendrites (Buhl and Dann, 1991; Van Groen *et al*., 2002; Witter, 1993; Witter and Groenewegen, 1984). Thus, whereas there is a trend of increasing diversification and refinement over the course of evolution, there does not appear to be a clear shift in the organization of the connections between the hippocampal region and entorhinal cortex. Indeed, in comparison with the dramatic differences in neocortex between mammals (Krubitzer and Kaas, 2005), the hippocampal region and entorhinal cortex are surprisingly similar.

One potentially important distinction between macrosomatic and microsomatic mammals involves the prominence of olfactory input to the entorhinal cortex (Insausti *et al*, 2002). In particular, a direct projection exists in rats from the olfactory bulb to almost the entire extent of the entorhinal cortex (Price, 1973). In contrast, in the macaque, the olfactory bulb projects to only one of the seven subdivisions of the monkey entorhinal cortex, which was estimated to comprise 15% of the region's total area (Witter *et al*., 1989). The corresponding entorhinal subdivision in humans comprises less than 5% of the total human entorhinal cortex (Insausti *et al*., 1995). To the extent that the projection from the olfactory bulb to entorhinal cortex represents the prominence of olfactory processing in the hippocampal region, there is a clear trend toward the reduction of olfactory input to the hippocampus in microsomatic mammals.

26.2.3.3 Perirhinal and postrhinal/parahippocampal cortices The entorhinal cortex appears to have undergone more changes over the course of evolution than the hippocampal region, and the perirhinal and postrhinal/parahippocampal cortices may have undergone even more changes than the entorhinal cortex (Burwell, 2000). However, this observation is limited by the fact that detailed anatomy of the perirhinal and postrhinal/parahippocampal cortices regarding the neocortical afferents, interconnectivity, and projections to the entorhinal cortex and hippocampal region are available for only the rat (Burwell and Amaral, 1998a, 1998b) and the macaque (Lavenex *et al*., 2002, 2004; Suzuki and Amaral, 1994, 2003). Although these animals represent only one branch on the mammalian evolutionary tree (see Figure 5), they differ substantially in that the rat is a nocturnal, macrosomatic, and relatively small-brained mammal, whereas the macaque is a diurnal, microsomatic, and big-brained mammal. Thus, the commonalities in anatomy between the two mammals can highlight fundamental organizational principles of the perirhinal and postrhinal/parahippocampal cortices, and the differences can illustrate one path taken by the parahippocampal region in the evolution of big-brained mammals.

In both the rat and the macaque, the perirhinal and postrhinal/parahippocampal cortices represent major routes of entry into the entorhinal cortex and hippocampal region (Burwell and Amaral, 1998b; Suzuki and Amaral, 1994). Many unimodal and polymodal cortical regions project to the perirhinal cortex or to the postrhinal/parahippocampal cortex. The incoming information is presumably processed and passed on to the entorhinal cortex, where it is likely further processed before being relayed to the hippocampal region. In both the rat and the macaque, the perirhinal cortex tends to project more to the LEA of the entorhinal cortex, and the postrhinal/parahippocampal cortex tends to project more to the MEA (Witter *et al*., 2000a). Also, in both mammals, a strong projection from the postrhinal/parahippocampal cortex to the perirhinal cortex is met with a more modest return projection (Burwell and Amaral, 1998a; Lavenex *et al*., 2004). Thus, the hippocampal and parahippocampal regions can be described as a hierarchy of connectivity in which the perirhinal and postrhinal/parahippocampal cortices are positioned near the top and funnel information into the LEA and MEA, information which is then combined in the hippocampal region (Lavenex and Amaral, 2000; Witter *et al*., 2000a).

However, compared to the macaque, the patterns of connectivity in the rat less clearly conform to the idea of a hierarchy. In the macaque, more than two-thirds of the cortical input to the entorhinal cortex originates in the either the perirhinal or postrhinal/parahippocampal cortices (Suzuki and Amaral, 1994). In comparison, less than one-fourth of cortical afferents of the entorhinal cortex in rats

originate in these regions (Burwell and Amaral, 1998b). Some, but not all, of this difference can be accounted for by considering the prominent olfactory input in the rat that bypasses the perirhinal cortex and projects directly to the entorhinal cortex. Thus, the more rigidly serialized hierarchy in the monkey suggests that the primate entorhinal cortex (and therefore the hippocampal region) receives information that is on average even more highly processed than it is in the rodent. Further, the macaque entorhinal cortex reciprocates its strong perirhinal and postrhinal/parahippocampal input with equivalently strong return projections. In the rat, the projections into the entorhinal cortex are stronger than the return projections (Burwell and Amaral, 1998a).

26.2.3.4 Neocortical input to the parahippocampal region The most notable difference between the parahippocampal regions in rats and monkeys relates to the makeup of cortical information projecting into the perirhinal and postrhinal/parahippocampal cortices. Indeed, there are numerous dissimilarities that relate generally to the differences between a small-brained and a big-brained mammal (Krubitzer and Kaas, 2005). Compared to the rat neocortex, the macaque neocortex is substantially more invaginated and shows a more defined laminar organization. The macaque also has disproportionately enlarged frontal lobes and has more unimodal and polymodal association cortical areas. Further, the macaque displays a much more elaborate visual system. However, olfactory and somatosensory cortex is disproportionately larger in rats and thus may provide more detailed odor and tactile information to the parahippocampal region. Thus, even if the parahippocampal region was identical between rats and monkeys, differences between the species in terms of the kinds of information processed by the perirhinal and postrhinal/parahippocampal cortices would be virtually assured by the substantial neocortical differences. Furthermore, because the inputs to the parahippocampal region largely determine the input to the hippocampal region, one might also expect to observe differences in the content of information that is available to the rat and the monkey hippocampus. Thus, although the rat and macaque parahippocampal regions may share a separation of spatial and nonspatial inputs between postrhinal/parahippocampal cortex and perirhinal cortex, respectively, the details of the spatial and nonspatial information reaching these structures likely differs markedly between the species.

26.2.4 Summary of Anatomical Homology

The organization of the neocortex differs substantially between mammals, and because neocortical organization determines the organization of inputs to the parahippocampal region, the information fed into the parahippocampal and hippocampal regions might differ considerably across the taxon. Nevertheless, the internal anatomical details of the hippocampal and parahippocampal regions are quite similar across mammals. Indeed, the appearance of the hippocampus is so similar – even in mammals such as the tenrec whose brain is thought to resemble those of the earliest mammals – that one might expect to find brain structures resembling the hippocampus in animals who shared a common ancestor with mammals. Accordingly, we next envision the earliest mammals and consider whether birds and reptiles have a brain structure that is homologous to the mammalian hippocampus.

26.3 Ancestral Homologue of the Hippocampal Region

The earliest mammals appeared during the Triassic period, more than 200 Mya. They were likely small, nocturnal animals who relied on their well-developed olfactory abilities to capture their insect meals (Allman, 1999). The ongoing diversification of mammals increased around 65 Mya, coincident with the Cretaceous/Cenozoic boundary that marks the extinction of dinosaurs (Springer *et al.*, 2003). The increased diversification was likely due at least in part to the decrease in competition from dinosaurs, which opened many diurnal habitats, but was probably also due to the increasing separation of land masses (Hedges *et al.*, 1996). In any case, early mammals shared a recent ancestor with sauropsids, a group of animals that today includes birds and reptiles (Kumar and Hedges, 1998; see Figure 5). The brains of modern mammals appear quite different from those of birds and reptiles, but there are also many similarities between the taxa. These commonalities can suggest how the brains of the early mammals might have appeared. Indeed, both reptiles and birds have brain regions that are thought to be homologous to the mammalian hippocampus (Colombo and Broadbent, 2000; Aboitiz *et al.*, 2002).

26.3.1 A Hippocampal Homologue in Birds and Reptiles

26.3.1.1 Anatomical similarities Regions within the medial cortex in reptiles (possibly including

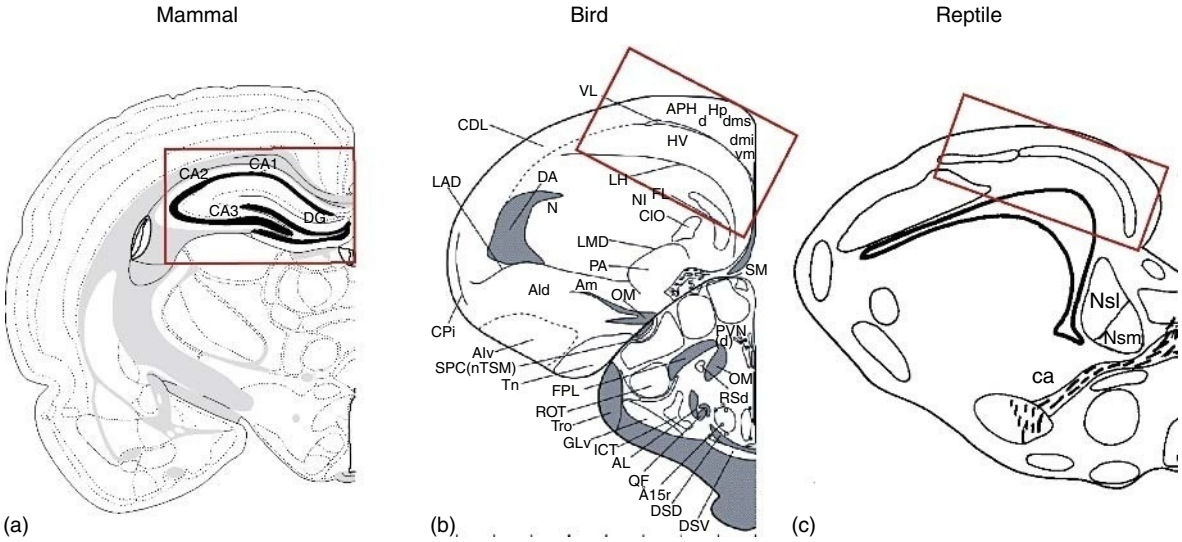

Figure 6 Brain regions thought to be homologous to the mammalian hippocampus in birds and reptiles (indicated by rectangles). One hemisphere from a rat, chicken, and lizard (gecko) is shown. See original sources for additional information. a, Reproduced from Swanson, L. W. 1998. Brain Maps: Structure of the Rat Brain, 2nd edn., with permission from Elsevier. b, Courtesy of Wayne Kuenzle. c, Reproduced from Hoogland, P. V., Martinez-Garcia, F., Geneser, F. A., and Vermeulen-VanderZee, E. 1998. Convergence of thalamic and cholinergic projections in the 'dentate area' of lizards. *Brain Behav. Evol.* 51(2), 113 122, with permission from S. Karger AG, Basel.

dorsomedial cortex) and medial pallium in birds (area hippocampus and area parahippocampalis) share several anatomical features with the mammalian hippocampus. For example, in all three cases the hippocampal homologue is a three-layered section of cortex that develops from the pallial telencephalon and is situated medially in the brain, adjacent to a main ventricle (Figure 6). In all three groups, the hippocampal homologue receives prominent projections from visual and olfactory cortices (Atoji *et al.*, 2002; Hoogland and Vermeulen-Vanderzee, 1993; Lavenex and Amaral, 2000). Also, the hippocampal homologue in all three groups shows physiological evidence of synaptic plasticity, suggesting an ability to associate incoming information (Bliss and Lomo, 1973; Muñoz *et al.*, 1998; Shapiro and Wieraszko, 1996). Thus, as in mammals, the hippocampal homologue in birds and reptiles may serve as a site of integration from already processed information.

26.3.1.2 Anatomical differences At the same time, there are notable dissimilarities between the mammalian hippocampus and the homologous regions in reptiles and birds. Superficially, neither the reptilian medial cortex nor the avian hippocampus have distinguishable subfields equivalent to those of the hippocampus proper, the dentate gyrus, and subiculum. Nor does the architecture of the reptilian or avian hippocampus resemble the characteristic shape of interlocking arcs formed by the densely packed cell layers of the hippocampus and dentate gyrus in mammals (Figure 6). Furthermore, dissimilarities in the distribution of sensory pathways between mammals and sauropsids suggest substantial differences in the kind of information processed by hippocampal homologues. In mammals, the hippocampus enjoys a confluence of highly processed unimodal and polymodal information (Lavenex and Amaral, 2000). Through its connections with the adjacent entorhinal, perirhinal, and parahippocampal/postrhinal cortices, the hippocampus receives input from widespread cortical areas. These connections suggest that the hippocampus serves as a central associative node in the cortical network that supports integrative processing in the mammalian brain. Likewise, in birds and reptiles, the hippocampal homologue also enjoys prominent connections with cortical/pallial regions. However, in comparison with the mammalian forebrain, the sauropsid cortex appears to play a disproportionately smaller role in cognitive functioning. Instead, higher-order functions that are mediated by the neocortex in mammals are supported by the dorsal ventricular region in reptiles and by the archistriatum/arcopallium in birds (Aboitiz *et al.*, 2002). Moreover, the hippocampal homologue in birds and reptiles enjoys few direct or indirect connections with these regions (Atoji *et al.*, 2002; Dubbeldam, 1998; Hoogland and Vermeulen-Vanderzee, 1993; Ten Donkelaar, 1998). Thus, the hippocampal homologue in sauropsids receives a more limited range of connections and may support a more specific subset

of associative abilities as compared to the mammalian hippocampus.

26.3.1.3 Functional similarities A growing number of studies suggest that the reptilian medial cortex and the avian hippocampus share some functional similarities with the mammalian hippocampus (Bingman, 1992; Day, 2003; Jacobs, 2003; Salas *et al.*, 2003). Among the most commonly studied functions of the hippocampus in mammals is spatial memory (reviewed more comprehensively in the second part of this article). Ablation studies have indicated that the hippocampal homologue in reptiles and birds is important for spatial learning functions that are known to rely on hippocampal function in mammals. These experiments show that damage to the putative hippocampal homologue disrupts performance when the animal must learn relationships between distant environmental cues to identify important places in the external world but not when learning can be supported by approaching a specific landmark at the site of a reward (Bingman *et al.*, 2003; Salas *et al.*, 2003). For example, turtles with damage to the medial cortex were impaired at learning the location of an unmarked goal in an open field water maze surrounded by visual cues but were unimpaired when the target was marked by a consistent visual stimulus (Lopez *et al.*, 2003). Similarly, homing pigeons with damage to the hippocampal homologue were able to orient themselves using the sun and familiar local landmarks at the release point but appeared deficient at navigating by using cues they saw along the route home (Gagliardo *et al.*, 1999). Consistent with these findings on hippocampal damage, studies on the firing properties of hippocampal neurons also suggest a possible similarity in spatial representations by the hippocampus. Many studies in rodents and other mammals have identified hippocampal neurons that fire selectively when the animal is in a particular place within its environment (O'Keefe and Nadel, 1978). Recently, cells with the same property have also been identified in the avian hippocampus (Siegel *et al.*, 2005). Thus, one commonality between mammals, reptiles, and birds is that the hippocampal homologue appears to be important for associating spatial relationships between environmental cues and learning places where important events occur.

26.3.1.4 Functional differences? The comparative anatomy of the hippocampus is consistent with the notion of a more limited scope of information processing in sauropsids than in mammals, and the results of several ablation studies are consistent with the idea as well. Mammals with damage to the hippocampal region have been found to be impaired on a variety of nonspatial as well as spatial memory tasks (Eichenbaum *et al.*, 1999; also discussed in more detail below). For example, in one study (Bunsey and Eichenbaum, 1996), rats were trained on a transitive inference task in which they learned a series of overlapping paired odor associations. When presented with odor A, rats were rewarded for selecting odor B, and when presented with odor B, rats were rewarded for selecting odor C. Having learned the A–B and B–C pairs, normal rats also responded to C when presented with A, demonstrating they had linked the two paired associates and could infer the relationship between the indirectly related items A and C. Rats with damage to the hippocampus could gradually acquire the paired associates but did not show the transitive inference for A–C. In contrast to the findings on rats, a similar study found that birds with hippocampal lesions performed just as well as intact birds on the transitive inference task (Strasser, *et al.*, 2004). Similarly, several studies have shown that mammals with damage to the hippocampal region are impaired in learning sensory discrimination reversals (e.g., Murray and Ridley, 1999), whereas turtles with lesions to the medial cortex are unimpaired at discrimination reversal learning (Grisham and Powers, 1990). These findings suggest that the hippocampal homologue in birds and reptiles might be more selectively involved in associations that involve a spatial component.

26.3.2 Summary of the Ancestral Homologue

The earliest hippocampus was likely a medial portion of a simple cortical mantle, and this medial portion was likely connected with dorsal and lateral portions of the mantle that processed visual and olfactory information, respectively. The visual and olfactory connections might have been brought together to form stimulus–stimulus associations to support spatial learning abilities in early mammals. However, it is unclear if this spatial learning ability was the sole ancestral function of the hippocampal homologue. It remains possible that further studies in birds and reptiles will uncover additional nonspatial associative abilities supported by the hippocampal homologue in these animals. Furthermore, even if the sauropsid hippocampal homologue is determined to be involved solely in forming associations that involve a spatial component, it cannot be assumed that this spatial specificity represents the ancestral condition. Indeed, several recent studies in goldfish have

indicated that the presumed hippocampal homologue in these vertebrates is important for nonspatial as well as spatial memory (Broglio *et al.*, 2005). For example, goldfish with lesions to the hippocampal homologue were impaired in classical conditioning of the eye retraction response when a trace interval intervened between the conditioned and unconditioned stimuli but learned at the normal rate when those stimuli were contiguous (Álvarez *et al.*, 2003), a pattern of results similar to those observed in several species of mammals (Clark *et al.*, 2002). Thus, it is possible that the earliest hippocampal homologue supported a general (spatial and nonspatial) associative function and that this ability became specialized in birds and reptiles. Further studies using the classical conditioning paradigm, for example, might provide the critical evidence.

How might the ancestral hippocampus have supported the integration of information derived from its cortical inputs? Assuming that, as in extant mammals and sauropsids, the inputs to the ancestral hippocampus included prominent olfactory and visual afferents, one fascinating possibility concerns the mismatch in neural topography between olfaction and other sensory modalities. Visual stimuli are typically encoded in a retinotopic fashion that organizes information in terms of the spatial locations of external cues in the environment. By contrast, odors contain no inherent directionality, and accordingly there exists no topographic map for odor location in the olfactory cortex. Thus, associations between odors and other types of stimuli require reformatting in at least one modality to provide a common scheme of organization. For a comparison, consider how visual and auditory information is associated by birds. Both types of information are organized by a spatial topography, and an efficient overlapping map of the sight and sound from a noise-emitting object, such as a rustling mouse, has been identified in the barn owl tectum (Knudsen, 1987). It is difficult to imagine how this topographic organization could efficiently include the odor of the mouse. Further, it is important to consider that the olfactory bulb increased in size in early mammals (Aboitiz *et al.*, 2002). Thus, the increasing adaptive value of olfactory abilities in catching prey may have driven the evolution of the hippocampus to support nontopographic associations between reformatted sensory information. Although olfaction may have made the greatest demand for nontopographic associations, the advantage of such a memory scheme would not have been limited to olfactory associations. Indeed, the hippocampus remains a crucial memory structure in microsomatic mammals including humans, so we might properly assume there is adaptive advantage for a nontopographic associative memory over a broad range of materials and modalities. The next half of the article considers whether this associative ability of the hippocampus is conserved across mammals.

26.4 Functional Homology of the Hippocampal and Parahippocampal Regions Across Mammals

26.4.1 Early Evidence on Hippocampal Function

The mammalian hippocampus appeared over 200 Mya, but the study of its function began in earnest only 50 years ago. The earliest and still compelling insights about hippocampal function in memory began with the dramatic characterization of the patient HM (Scoville and Milner, 1957). In an attempt to alleviate debilitating seizures, an experimental surgical procedure was performed in which large portions of both his right and left medial temporal lobes were resected. The ablation included large portions of the hippocampal region, the entorhinal cortex, and the perirhinal cortex (Corkin *et al.*, 1997). The surgery reduced the intensity of the seizures but also had the unexpected and profound effect of leaving HM virtually incapable of acquiring new memories across a broad range of modalities of information. In striking contrast, his perceptual and motor capacities and other cognitive abilities, including language and attention, appeared normal. In addition, HM's capacity to acquire and retain information in mind for a brief period was also intact, although the new information was lost as soon as his attention was directed away from it. Although the majority of his childhood memories survived the surgery, there was a retrograde loss of memories acquired for several years prior to the surgery. The main interpretation of the findings was that the hippocampus was important for the consolidation of short-term memories into lasting long-term memories.

Immediately following the reports on HM, there were several attempts to determine whether the hippocampus was also involved in memory in monkeys and rats. Results from a large number of studies in rats of operant conditioning, sensory discrimination, maze learning, and avoidance learning were mixed and inconclusive (reviewed in Cohen and Eichenbaum, 1993; Eichenbaum and Cohen, 2001; O'Keefe and Nadel, 1978). The results from rats ranged from severe performance deficits to normal performance. Some studies even observed facilitation of learning following damage to the hippocampus. Thus, based on these early results, one possibility was that the hippocampus served

memory in humans but some other nonmemory cognitive function, such as response inhibition, in experimental animals.

The 1970s saw several important ideas emerge regarding the function of the hippocampus in experimental animals. Hirsh (1974) suggested the hippocampus was critical for context-dependent retrieval but not for modifications of behavior "along the performance line." Olton *et al.* (1979) suggested that the hippocampus was critical for what he called working memory (memory for single events) but not for reference memory (learning that can be applied across many events). O'Keefe and Nadel (1978) put forth the idea that the hippocampus is selectively involved in map-like spatial memory but not in learning guided by nonspatial cues. The ideas were far from consensual as to what specific function might be supported by the hippocampus in experimental animals, but all the views shared in common two features. First, all agreed that the hippocampus was involved in some aspect of memory. Second, all agreed that memory was not a single ability but was instead capable of being separated into multiple forms of memory, one that depended on the hippocampus and others that did not.

The spatial memory view of the hippocampus was the most successful of these early ideas. The idea of map-like spatial memory appeared well-suited to rats, for whom a memory of the area's geographical layout would be advantageous in supporting nighttime foraging. Further, the spatial map theory was supported by compelling results from studies in which action potentials of single hippocampal neurons were recorded while rats performed spatial tasks or merely explored an open field. The main finding of these studies was that many of the principal cells recorded from CA1 and CA3, which are noted for their very low baseline activity, increased their firing rate dramatically when the rat was in a particular location within the chamber (Muller *et al.*, 1987; O'Keefe, 1979; O'Keefe and Dostrovsky, 1971). O'Keefe and Nadel's (1978) interpretation of these findings was that the firing of hippocampal neurons signaled occupancy of a particular coordinate locus, a place field, within a cognitive map established in the hippocampus.

At the same time that these ideas emerged from work in experimental animals, the concept of multiple memory systems was being refined in parallel by work in human amnesic patients. Work with patient HM had already demonstrated that the hippocampus was not needed to acquire new motor skills (Milner, 1962). Yet these findings were typically set aside as motor-based exceptions to the general view that all memory depended on the hippocampus. A key finding came when Cohen and Squire (1980) observed intact learning outside the domain of motor skills in amnesic patients. Patients and age-matched volunteers were asked to read mirror-reversed words over the course of several days. Patients and control participants both improved their reading speed with practice, but only the control participants were able to describe subsequently the details of the testing situation. These results helped make the point that the human hippocampus was important for memory in the everyday sense of the word but was not important for other examples of procedural memory, such as the acquisition of cognitive skills, that are expressed through performance rather than recollection.

The observation of hippocampal cells with place fields in rats was compelling, yet for those who worked with amnesic patients, the idea of a special role for the hippocampus in spatial memory appeared to ignore a large body of data on HM and other amnesic patients indicating a critical role for the hippocampus in nonspatial memory, including verbal memory of recently encountered events that were not prominently spatial in nature. This disjuncture in the findings on rodents and humans exacerbated the already widely held view that the hippocampus supported distinct functions in humans and animals.

26.4.2 Convergence of Ideas on Hippocampal Function in Humans and Experimental Animals

A resolution of the contrasting findings from humans and experimental animals became available only through the systematic administration of similar tasks to humans and experimental animals. Points of contact between humans and experimental animals have now been made with numerous tasks, including the transitive inference task described in the first half of the article (Bunsey and Eichenbaum, 1996; Heckers *et al.*, 2004; Nagode and Pardo, 2002; Preston *et al.*, 2004). Here, we focus on three tasks that have been especially informative, either for the volume of data available or for the clarity of the results across species. The first is a task in which multiple pairwise discriminations are learned concurrently. The second is a recognition memory task called delayed nonmatch to sample (DNMS). The third is classical conditioning of the eyeblink response. A fourth point of contact exists, not in the form of a specific task, but as a pattern of results from several tasks related to premorbid memory in amnesic patients and experimental animals with damage to the hippocampus. That is, there is a similar pattern of temporally graded retrograde amnesia across mammals.

26.4.2.1 Concurrent discrimination One task that has been given to both amnesic patients and monkeys with medial temporal lobe damage is a task in which multiple sets of pairwise object discrimination problems must be learned concurrently. Both healthy humans and intact monkeys learn to identify the correct item for each of up to 20 pairs of randomly assigned junk objects over the course of multiple testing sessions. Monkeys with damage limited mostly to the medial temporal lobe perform as well as intact animals (Buffalo *et al*., 1998; Malamut *et al*., 1984; Teng *et al*., 2000). In contrast, amnesic patients typically attain levels of performance much lower that that shown by healthy individuals (Hood *et al*., 1999; Squire *et al*., 1988). Based on these findings, one possibility was that the hippocampal and parahippocampal regions performed a different function in humans and monkeys, one that was important for the concurrent discrimination task and one that was not. Another possibility was that the hippocampal and parahippocampal regions served similar functions in humans and monkeys, both potentially contributing to the concurrent discrimination task. From this viewpoint, monkeys with medial temporal lobe damage performed normally because their memory impairment was masked by a capacity for habit learning that was underdeveloped in humans (Hood *et al*., 1999).

A third possibility is that the line that divides declarative memory and nondeclarative memory is drawn similarly for monkeys, humans, and possibly all mammals (Squire *et al*., 1988). From this viewpoint, the hippocampal and parahippocampal regions serve the same function in humans and experimental animals, and the capacity for hippocampus-independent procedural learning is also similar for all mammals. The difference is that humans enjoy cognitive skills such as verbal labeling and elaborations, which are presumably absent or less well developed in other mammals, leading them to adopt a memorization strategy. When this strategy is unavailable, as in the case of profoundly amnesic patients, humans appear to be able to fall back on habit learning and show a rate of learning on the concurrent discrimination task that is similar to that shown by monkeys (Bayley *et al*., 2005a).

26.4.2.2 Delayed nonmatch-to-sample After HM's profound amnesia was described (Scoville and Milner, 1957), many studies in experimental animals were performed in attempting to duplicate his damage and memory impairment. One early line of studies focused on a test of recognition memory in which animals were first presented with one of two colored sample stimuli, then following a variable delay, were required to chose the sample stimulus over the other stimulus, that is, to match the sample. The same two color pattern stimuli were used on each trial, with either selected randomly to be the sample on that trial. The expectation was that damage to the medial temporal lobe would reproduce the observation of delay-dependent memory impairment in HM. However, monkeys with medial temporal lobe lesions performed surprisingly well on this and other delayed response tests, even at memory delay intervals of several seconds (Correll and Scoville, 1965, 1967; Drachman and Ommaya, 1964). These findings contributed to the early view that hippocampal function differs in animals and humans.

However, a breakthrough occurred when the task was modified to use different three-dimensional objects on each trial (Gaffan, 1974; Mishkin and Delacour, 1975). Also, because monkeys showed a natural preference for manipulating novel objects, experiments began using a nonmatch rule (i.e., the unstudied object was rewarded) for efficiency. Thus, the procedure was changed to become the now widely used trial-unique DNMS task. The results on the DNMS task were very different than those in the early studies. Monkeys with damage similar to that produced in HM could learn the nonmatching rule and performed normally at very brief delays, but rapidly declined in performance as the delay was increased, thus reproducing the pattern of delay-dependent memory impairment in human amnesia (Squire and Zola, 1996). The DNMS task was also soon adapted for use in rats (Mumby *et al*., 1992). Similar to the results from humans and monkeys, damage that included both the hippocampal region and parahippocampal region resulted in a delay-dependent impairment in recognition memory performance (Mumby and Pinel, 1994). Although researchers have more recently debated whether the hippocampal region itself is important for recognition memory, the use of the DNMS task across species helped identify that the mammalian hippocampal and parahippocampal regions enable long-term memory for items encountered only once. Based on these and other observations, it is now appreciated that rapid, single-exposure learning is a hallmark of hippocampal-based learning.

26.4.2.3 Classical conditioning of the eyeblink response Another example of a success in demonstrating similarities in the profile of hippocampus-dependent learning across species shows an impressive distinction generated by a simple procedural parameter. In classical conditioning of the eyeblink response, a tone is repeatedly followed by

a mild puff of air to one's eye, causing a reflexive blink. After several tone–air puff pairings, subjects (across several mammalian species) begin to blink following tone onset and prior to the air puff, demonstrating the conditioned response. In the standard version (called delay eyeblink conditioning), the onset of the tone precedes the onset of the air puff and continues such that the stimuli then overlap and co-terminate. This simple form of associative learning is supported by a carefully described circuit that includes the brainstem and cerebellum (Christian and Thompson, 2003). In a slightly modified version (called trace eyeblink conditioning), the tone ends before the air puff and a brief ($\leq$1s) silent interval separates the two stimuli. This small gap in time necessitates the recruitment of additional brain structures, including the hippocampus, to support the association of the tone and the puff in the cerebellum. The distinction between trace and delay eyeblink classical conditioning is particularly compelling when one considers that the dependence of trace eyeblink conditioning on the integrity of the hippocampus has been established for mice, rats, rabbits, and humans (Clark and Squire, 2000). Thus, the difference between delay and trace eyeblink classical conditioning suggests something fundamental about the function of the hippocampus that has been conserved though evolution.

Classical conditioning was once viewed as a form of procedural learning, typically outside the domain of critical hippocampal involvement. However, the findings of additional experiments in humans have indicated that acquisition of trace, but not delay, eyeblink conditioning correlates with participants' ability to report information about the relationship between the tone and the air puff (Clark *et al.*, 2002). These results suggest two points. First, the close similarity of the experimental parameters for delay and trace eyeblink conditioning illustrate how subtle the change can be that disposes performance to rely on hippocampus-dependent memory available for verbal report. Second, the relationship in humans between trace eyeblink conditioning and awareness of the stimulus contingencies suggests that something similar might be occurring in experimental animals who successfully acquire trace eyeblink conditioning.

26.4.2.4 Retrograde amnesia In addition to cross-species similarities in the cognitive demands dependent on the hippocampus and in the nature of events represented by the hippocampus, there is also considerable evidence indicating conservation of a time-limited role of the hippocampus in memory consolidation. In numerous studies in experimental animals who were given lesions to the hippocampal region at various intervals after acquiring new information, the result emerged that the hippocampus is needed to retrieve information for a finite period of time (for a review, see Squire *et al.*, 2001). The typical finding is that animals in which the hippocampus was ablated immediately after a training session subsequently displayed impaired performance, whereas animals in which the hippocampus was removed one week to one month after a training session subsequently displayed normal performance. The interpretation of these studies was that the hippocampus was needed for acquisition and initial retrieval of the new memory, but that over time the memory eventually became independent of the hippocampus through a process of consolidation (Squire *et al.*, 2001). Although the critical training–lesion interval varied somewhat from study to study, the retrograde amnesia suggested that the process of consolidation lasts from a few days to a month.

These findings are qualitatively very similar to the pattern of temporally graded retrograde amnesia in HM and other amnesic patients. For example, one study assessed amnesic patients' memory for newsworthy incidents that occurred at various years prior to the patients sustaining damage thought to be limited to the hippocampal region (Manns *et al.*, 2003b). These results suggested that the impact of the hippocampal damage extended to memory from 5 to 10 years prior to onset of damage. Thus, although the results suggest that the process of consolidation is substantially longer in humans as compared to experimental animals, the findings indicate that the hippocampus plays a time-limited role in memory across species.

Taken together, the results of these three specific tasks and the common observation of temporally graded retrograde amnesia strongly suggest that the hippocampus serves a similar role in memory in both humans and in experimental animals. However, much debate surrounds the question of how to best characterize this role. For example, still under debate is whether the hippocampus itself is crucial for simple recognition memory or whether structures in the parahippocampal region support this ability (Manns *et al.*, 2003a; Mumby, 2001; Clark *et al.*, 2001). However, this uncertainty applies equally to the findings on humans, monkeys, and rats. In any case, it is clear that the extended hippocampal memory system, including the parahippocampal region, is important for examples of single-trial learning, including recognition memory judgments, for spatial memory, and for forming arbitrary relationships between stimuli, as in the transitive inference task. The example of trace eyeblink conditioning also illustrates that the hippocampus is crucial for learning under

circumstances in which the capabilities of extra-hippocampal structures are unable to support the learning. In these instances, the hippocampal contribution becomes indispensable.

26.4.3 Remaining Points of Disconnect between Humans and Experimental Animals

With the greater understanding of multiple memory systems and the assurance of consistency of function between humans and experimental animals, researchers could explore hippocampal function in a variety of mammalian species and expect with some confidence that the results would be relevant to the entire taxon. This exploration has led to an understanding that the original features of memory that distinguished hippocampal function in humans and animals are more compatible with a cross-species approach than once was believed.

26.4.3.1 Understanding how place cells relate to the human hippocampus A remaining point of potential discontinuity between species is the difficulty in resolving the prominence of spatial correlates in the firing of rodent hippocampal cells with the observation that the human hippocampus is important for all examples of declarative memory, both spatial and nonspatial. The conclusion that hippocampal neurons fire primarily in association with an animal's location in its environment, whereas the human hippocampus is required for and engaged by a broad variety of nonspatial memories would seem to present a major exception to cross-species similarity of hippocampal function.

However, recent parallel studies in both rats and humans have demonstrated a much broader scope of information encoded by hippocampal neurons in both rats and humans. In rats, a direct comparison of spatial and nonspatial coding by hippocampal neurons was investigated by recording from hippocampal cells as rats sampled nonspatial cues at many locations in an environment (Wood *et al.*, 1999). The rats performed a task in which they had to recognize any of nine olfactory cues that were placed in any of nine locations. Because the locations of the odors were varied systematically, cellular activity related to the odors and to memory performance could be dissociated from activity related to the animal's location. The study found that similar proportions of hippocampal cells fired in association with a particular odor, a particular place, or whether the stimulus was recognized. In addition, a large subset of hippocampal neurons fired in association with only a particular combination of the odor, the place where it was sampled, and the match/nonmatch status of the odor. In a remarkably similar study on humans, Ekstrom *et al.* (2003) recorded the activity of hippocampal neurons in human subjects as they played a taxi driver game, searching for passengers to be picked up and dropped off at various locations in a virtual reality town. Similar to the findings with rats, equivalent proportions of the cells fired in association with particular landmarks, views of the environment, or places occupied in the virtual town. Also, many of these cells fired selectively in association with specific combinations of a place and the view of a particular scene or a particular goal.

Other studies have also reported a remarkable similarity of hippocampal neuron firing patterns in monkeys and humans associated with nonspatial stimulus analysis. Hampson *et al.* (2004) trained monkeys on matching-to-sample problems, then probed the nature of the representation of stimuli by recording from hippocampal cells when the animals were shown novel stimuli that shared features with the trained cues. They found many hippocampal neurons that encoded meaningful categories of stimulus features and appeared to employ these representations to recognize the same features across many situations. Kreiman *et al.* (2000a) characterized hippocampal firing patterns in humans during presentations of a variety of visual stimuli. They reported a substantial number of hippocampal neurons that fired when the subject viewed specific categories of material (e.g., faces, famous people, animals, scenes, houses) across many exemplars of each. A subsequent study showed that these neurons are activated when a subject simply imagines its optimal stimulus, supporting a role for hippocampal networks in recollection of specific memories (Kreiman *et al.*, 2000b). This combination of findings across species provides compelling evidence for the notion that some hippocampal cells represent abstract features of nonspatial stimuli that appear in different experiences.

Studies across species also emphasize the presentation of objects in relation to their location in the environment. Hippocampal cells that represent specific salient objects in the context of a particular environment have also been observed in studies of rats engaged in foraging (Gothard *et al.*, 1996; Rivard *et al.*, 2004) and escape behavior (Hollup *et al.*, 2001) in open fields. In addition, two recent studies highlight the associative coding of events and places by hippocampal neurons in rats and monkeys. In one study, rats were trained on an auditory fear conditioning task (Moita *et al.*, 2003). Prior to fear conditioning, few hippocampal

cells were activated by an auditory stimulus. Following pairings of tone presentations and shocks, many cells fired briskly to the tone when the animal was in a particular place where the cell fired above baseline. Another recent study examined the firing properties of hippocampal neurons in monkeys performing a task where they rapidly learned new scene–location associations (Wirth *et al*., 2003). Just as the monkeys acquired a new response to a location in the scene, neurons in the hippocampus changed their firing patterns to become selective to particular scenes. These scene–location associations persist even long after learning is completed (Yanike *et al*., 2004). These findings are entirely consistent with the findings of prevalent hippocampal neuronal activity associated with conjunctions of events and locations in Wood *et al*. (1999) study on rats and Ekstrom *et al*. (2003) study on humans. Collectively, these findings indicate that a prevalent property of hippocampal firing patterns in rats, monkey, and humans involves the representation of unique associations of stimuli, their significance, specific behaviors, and the places where these events occur.

26.4.3.2 Understanding how episodic memory relates to hippocampal function in experimental animals Another point of potential discontinuity involves a form of declarative memory called episodic memory. Episodic memory is characterized as the ability to replay in mind a particular episode in one's life, and this capacity has been closely identified with hippocampal function in humans (Tulving, 2002). Defined in these terms, episodic memory is considered by some to be a uniquely human ability (Tulving, 1983). If so, then the human capacity for episodic memory represents a break in the continuity of hippocampal research between humans and experimental animals. The difficulty in addressing this issue is that experimental animals are unable to report on their subjective experience.

A less mentalistic definition of episodic memory, and one that is experimentally tractable in experimental animals as well as in humans, characterizes episodic memory as including details about the time and place in which an episode occurred. That is, episodic memory includes information about the 'what', 'where', and 'when' of an event (Clayton and Dickenson, 1998). Experimental animals, including rodents and birds, have demonstrated evidence of the ability to remember where and when unique events occurred (Clayton *et al*., 2003; Dere *et al*., 2005; Eacott *et al*., 2005; Morris, 2001). Further, damage to the hippocampus impairs this capacity (Ergorul and Eichenbaum, 2004). By this definition then, it appears that animals other than humans are capable of episodic-like memory and that this ability depends on the hippocampus. However, the definition of episodic memory as the combination of 'what', 'where', and 'when' may be overly strict and may exclude examples of hippocampus-dependent memory. In particular, tasks that require memory for either temporal order (Fortin *et al*., 2002; Kesner *et al*., 2002) or spatial location (Day *et al*., 2003; O'Keefe, 1993) alone depend on the integrity of the hippocampus. Thus, although the hippocampus in humans and experimental animals appears to be crucial for combining temporal and spatial elements of a particular incident, this capacity does not represent the totality of its function.

Another approach to studying episodic memory in humans and experimental animals focuses on the notion that recognition memory can be supported by two processes: an episodic-like recollection of specific details and a feeling of familiarity with a previously experienced item (Atkinson and Juola, 1974; Mandler, 1980; Yonelinas, 1994). Although the status of familiarity-based judgments is currently under debate (Brown and Aggleton, 2001; Squire *et al*., 2004), it is generally accepted that the human hippocampus is important for recognition memory based on recollection. One approach to quantifying the relative contribution of recollection and familiarity to recognition memory judgments has adopted signal detection theory to characterize recognition memory in terms accuracy (proportion of hits to correct rejections) as either a function of confidence levels or tendency to endorse and item as having been repeated. These measures result in a receiver-operating characteristic (ROC) curve that, by one model, contains the signatures of distinct recollection and familiarity contributions to recognition (Yonelinas, 2001). From this viewpoint, a typical ROC curve can be thought of as composite of a familiarity curve that is symmetric to the diagonal and a recollection line that is asymmetric. Mathematical decomposition of these plots can thus provide numerical estimates of recollection and familiarity. One appeal of this technique is that the definition of episodic-like memory, the numerical estimate of recollection, can be used in both humans and experimental animals.

A recent study reported that the ROC curve in human amnesic patients with hippocampal damage is curvilinear and symmetric, indicating a loss of recollection (Yonelinas *et al*., 2002). Consistent with that finding, a recent study of rats found that the ROC curve of normal rats was both asymmetric and curvilinear, similar to the composite recollection and familiarity ROC curve observed in normal

human subjects (Fortin *et al.*, 2004). In contrast, performance of rats with damage to the hippocampus was best fit by a symmetric curve, suggesting a loss of episodic-like recollection. Although the measure of recollection in these studies is necessarily indirect, the closely parallel results suggest that the hippocampus contributes to episodic memory, or something closely resembling episodic memory, in both humans and experimental animals.

However, it should be noted that the view of the hippocampal function based on the distinction between recollection and familiarity is not consensual. Some argue that the shape of ROC curves can be better explained by factors such as differences in the variability in the perceived memory strength between studied and unstudied items (Donaldson, 1996). Others charge that the idea is based on a psychological dichotomy that is incompatible with the anatomical view of the hippocampal and parahippocampal regions as a hierarchical network of interconnectivity (Squire *et al.*, 2004). Further, the notions of recollection and familiarity are based on terminology and concepts tailored to psychological findings in humans. Although contact has been made between humans and rats with respect to the importance of the hippocampus for recollection, one could argue that the distinction is not one that is ideally suited to an evolutionary approach to the study of memory and the hippocampus.

26.4.4 Possible Divergence between Species

Although the bulk of the anatomical data from mammals regarding the hippocampal and parahippocampal regions indicates that the similarities outweigh the differences, several prominent anatomical differences were noted between rodents and primates. Perhaps the most striking difference is the prominence of olfactory input that reaches the entorhinal cortex in the rat. Based on this observation, one possibility is that the rat entorhinal cortex exhibits specialization in olfactory memory that is not exhibited by the primate entorhinal cortex. Indeed, in one study, rats with hippocampal lesions were nevertheless able to identify a novel odor from among 24 recently encountered odors (Dudchenko *et al.*, 2000). Their performance was presumably supported in part by the entorhinal cortex and was as good as that as shown by healthy rats. The basic procedure was adapted and given to humans with damage to the hippocampal region (Levy *et al.*, 2003). In contrast to the findings in rats, humans with damage to the hippocampal region performed poorly and performed worse than healthy individuals. Although it is difficult to rule out other variables such as the extent of the hippocampal damage, these studies hint that the rat entorhinal cortex might be able to support more odor-related memory abilities as compared to the human entorhinal cortex.

Another difference observed between humans and experimental animals concerns the apparent duration of memory consolidation. In experimental animals, the process apparently requires around a month when damage is restricted to the hippocampal region (Squire *et al.*, 2001). In humans, the process can last several years when damage is thought to be limited to the hippocampal region and can last even longer when damage also includes structures in the parahippocampal region (Bayley *et al.*, 2005b; Kapur and Brooks, 1999; Manns *et al.*, 2003b; Reed and Squire, 1998; Rempel-Clower *et al.*, 1996). Several factors likely contribute to the different timescales observed between humans and experimental animals. First, anatomical studies indicate that the organization of the hippocampal and parahippocampal regions in rats is less hierarchical than it is in the monkey. In the rat, the hippocampal region and the entorhinal cortex show more direct connections with olfactory and neocortical areas than are observed in the monkey (Burwell and Amaral, 1998b; Suzuki and Amaral, 1994). If the trend toward a more strict hierarchy was continued in humans, the decrease in direct cortical connections with the hippocampus and entorhinal cortex might suggest why the process of consolidation takes longer in humans. Second, the type of information being assessed in human studies of consolidation is often very different from the type of information being assessed in experimental animals. Studies in humans typically examine factual information about the world. In comparison, studies in experimental animals typically examine presumably simpler associations. For example, one study in rabbits identified that lesions one day after training, but not 30 days after training, impaired subsequent performance on a trace eyeblink conditioning experiment (Kim *et al.*, 1995). One possibility then is that some of the discrepancy between humans and experimental animals could be accounted for by differences in the complexity of the memories assessed.

A related topic concerns whether or not spatial memory holds a special status in terms of memory consolidation in the rat. In humans, it appears that spatial memory eventually becomes independent of the hippocampus, just as other examples of memory that are acquired by the hippocampus are thought to become. In one study, a profoundly amnesic patient who had virtually complete damage to the hippocampal region was nevertheless able to pretend he

was standing in his childhood neighborhood and point accurately to several town landmarks (Teng and Squire, 1999). In rats, the results are somewhat different. Indeed, most studies in rats have found that the hippocampus remains important for spatial memory for as long as the memory remains measurable (Moscovitch *et al*., 2005). In one study, healthy rats were able to demonstrate memory for a spatial location learned more than 14 weeks previously. However, rats with damage to the hippocampus performed at chance even when the training–surgery interval was 14 weeks (Clark *et al*., 2005). That is, there was no evidence for consolidation of spatial memory in these rats, despite the fact that the training–surgery interval was longer than that typically reveals consolidation of nonspatial memory in experimental animals. It is possible that the rat experienced environmental pressures that caused spatial memory to acquire a status in which its persistence became tied to the function of the hippocampus. However, it is also possible that further study will identify a process of consolidation for spatial memory in the rat. Indeed, one recent study in rats found that post-training lesions to the hippocampus spared memory for locations of different rewards, but only when the animals were exposed to the locations of the rewards very early in life (Winocur *et al*., 2005).

26.4.5 A Species-General Mechanistic Account of the Hippocampus

A challenge in memory research has been to provide a mechanistic account that could connect the anatomy of the hippocampal and parahippocampal regions to the examples of memory that depend on these structures. That is, it is now clear that the hippocampal and parahippocampal regions are important for the initial acquisition and temporary maintenance of declarative memory, but it is unclear exactly how these regions support this capacity.

One promising approach has been the development of anatomically plausible computational models of the hippocampal and parahippocampal regions. One particularly influential model built on the characterization for the hippocampal and parahippocampal regions as a hierarchical network and proposed that the hippocampus served as a central node of synaptic change (McClelland *et al*., 1995). The hierarchical network proposed by the model offered the brain several mnemonic advantages. First, incoming information that would be processed by widespread neocortical sites could be condensed through the process of funneling information through the parahippocampal region on its way to the hippocampal region. Accordingly, long-lasting associations between very different types of information could be made very quickly through a limited number of synaptic changes in the hippocampus. Thus, the model described how the anatomy of the hippocampus enabled rapid acquisition of arbitrary associations. Second, the binding of disparate neocortical sites by the hippocampus might allow the neocortical sites to develop more direct interconnectivity over time. That is, the model described how the process of consolidation might occur. This second point was based on the idea that several neocortical nodes would be joined by the hippocampus into a subnetwork whose co-activity could be propelled by repetition, rehearsal, or spontaneous reinstantiation during sleep. An important idea that emerged from the model was that the hippocampus allowed the brain to acquire new information rapidly and then to gradually interleave that information in existing neocortical networks. That is, according to the model, the hippocampus solves the problem of how to acquire new information quickly without disturbing the delicate network of existing knowledge.

The advantage to models like the one described above is that the concepts should apply equally well to any mammal, provided that the anatomical constraints included in the model are not violated by any species-specific anatomical idiosyncrasies. Indeed, additional computational models will be the most useful to an evolutionary approach when they are based on facets of the anatomy that are shared across mammals. In particular, accumulating evidence suggests that the information arriving at the hippocampal region through perirhinal cortex and LEA differs in content as compared to the information arriving at the hippocampal region through postrhinal/parahippocampal cortex and MEA. Further, the anatomy suggests that the dentate gyrus and CA3 may be important for combining this information and incorporating it with as yet uncombined information in CA1 and subiculum. Computational models that take advantage of this pattern of connectivity, which is shared by at least rats and monkeys, could contribute significantly to understanding principles of hippocampal function that apply across species.

26.5 Conclusions

The anatomy of the hippocampal and parahippocampal regions represents an elegant solution to a difficult memory problem. Natural selection shaped a network of structures that could quickly form stable associations between pieces of information

that bore no topographic similarity to one another. Evidently, a rough approximation of an answer had been sketched out early in the evolution of vertebrates, prior to the emergence of mammals. The modern blueprint appeared in the earliest mammalian hippocampus, and the solution was repeated again and again throughout the taxon. The occasional variations in anatomy between mammals with regard to the parahippocampal region might then represent flourishes added to what was already an anatomical masterpiece.

If the anatomy of the mammalian hippocampus is a finished product, then the study of its function might be best described as a work in progress. On one hand, much is already known about the function of the hippocampal and parahippocampal regions. These structures together support only one kind of memory, a type of memory referred to as declarative memory that is unambiguously important for recollecting episodic details, encoding spatial locations, and forming abstract or arbitrary associations. On the other hand, much is left to be discovered. A question at the forefront of memory research is how the individual components of the hippocampal and parahippocampal regions might each contribute to declarative memory. Accordingly, there is debate as to whether the hippocampus itself is important for aspects of declarative memory such as nonspatial memory and judgments of familiarity or if these abilities are supported by areas within the parahippocampal region. Thus, a clear set of principles that account for all examples of hippocampus-dependent memory has not been agreed upon. The hope is that the psychological, anatomical, physiological, and computational approaches will be combined to produce a view of hippocampal function that makes sense at all levels.

As researchers move toward a consensual view regarding the roles of the hippocampal and parahippocampal regions in memory, one challenge will be the struggle to identify ideas that attain a satisfactory level of psychological specificity for humans while maintaining enough contact with the anatomical and mechanistic details to generalize to all mammals. The goal is to identify the unifying principles that apply to all examples of hippocampus-dependent memory, both in humans and experimental animals. To date, many ideas that have been proposed that contain elements of species-specific psychology, such as spatial memory in rats and episodic memory in humans. The difficulty is that often the elements of psychology vary dramatically between species – despite the fact that the anatomical details of the hippocampal and parahippocampal regions are remarkably similar. For example, humans enjoy certain cognitive skills such as chunking and elaboration that may steer them toward memorization strategies. These abilities are presumably less advanced in experimental animals and therefore might lead animals to rely more on trial and error. In turn, rats may have evolved specialized skill sets that allow them in their nighttime foraging to be highly attuned to spatial layout and their position within it. For neither rats nor humans is it likely that these cognitive skills are fundamentally derived from the hippocampus. Instead, these skills are likely supported by areas outside the hippocampal and parahippocampal regions such as prefrontal cortex and posterior parietal cortex. That is, it is possible that any apparent differences in terms of the psychological properties of hippocampus-dependent memory might be more related to the differences in neocortical input to the hippocampal and parahippocampal regions rather than due to any differences between species for these areas themselves.

An evolutionary approach can help us understand the functional machinery of the mammalian hippocampus. At the same time, an enormous asterisk must follow any statement about our current understanding of the hippocampus, for the vast amount of data come from rodents and primates. Most of the mammalian taxon is unexplored, and every mammal presents an opportunity to probe the function of the hippocampal and parahippocampal regions with a unique neocortical instrument. For example, one might explore how auditory information in the bat echolocation system arrives in the hippocampal region. One might also take advantage of the simple cortex of the hedgehog to pare down the inputs to the parahippocampal and hippocampal regions. Thus, an evolutionary perspective can not only reveal the conservation of hippocampal form but can also offer many opportunities for exploring the function of this form.

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http://www.brainmaps.org High resolution images of the macaque brain.
http://www.med.harvard.edu Navigable atlas of human brain.
http://www.avianbrain.org Newly updated terminology for avian neuroanatomy.
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27 The Evolution of the Cerebellum

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Glossary

allometry The approach to relate the size of (sub divisions of) the brain relative to the size of the body (body parts).

cerebellum The small brain. Posterior division of the brain. Connected to the brainstem through the cerebellar peduncles. The mammalian cerebellum is characterized by many transverse fissures of different depths. Vertebrate cerebella share the lat tice character of the cerebellar cortex: its main elements, the Purkinje cells and the granule cells are oriented at right angles. Cerebellar like structures, such as the dor sal cochlear nuclei, contain these elements in a less geometric structure.

clone The cells produced by one progenitor cell.

endocast The cast of the interior of a skull, which may reveal some aspects of the size and shape of the brain, formerly contained therein.

folia The smallest, leaf like units of the cerebel lar surface. A folium is oriented at right angles to the long axis of a folial chain and delimited by two transverse fissures.

folial chain Two deep longitudinal fissures subdivide the mammalian cerebellum in a median region, the vermis and two lateral hemi spheres (Bolk, 1906). Transverse fissures of different depth subdivide both vermis and hemispheres in folia, lobules, and lobes. The cortex between adjacent folia within vermis and hemisphere, always is continuous. The term 'folial chain' accentuates this continuity.

folial pattern The combination of longitudinal and transverse fissures divides the cerebellar surface into its units. Variations in size and internal composition of these units, determine their positioning and, thus, the gross appearance of the cerebellum. The resulting pattern of these units is named after the smallest units, the folia.

function The function of the cerebellum can be studied at different levels. The algorithm performed by the cerebellar cortex is the most basic function, but is still unknown. Recorded activity or connections of cer tain parts of the cerebellum may indicate whether this unknown algorithm is used within the context of a certain functional system. Lesions or diseases affecting the

	cerebellum or its parts cause symptoms and signs that can be interpreted as a consequence of an alleged function of the cerebellum.
lineage	The origin of a particular cell type from a particular progenitor cell.
lineage restriction	The spatial restriction by temporary boundaries of groups of progenitor cells and their offspring.
lobe, lobule	Collections of folia delimited by deep, transverse fissures. The subdivision of the cerebellum in lobes, and of the folial chains in lobules is an arbitrary decision, depending on the importance given to particular transverse fissures of varying depth and their continuity in vermis and hemispheres.
module	Repeating neural unit with a specific structure, composition, and connections.
nomenclature	Set of terms used to indicate a coherent set of structures. Classical nomenclatures use resemblance of structures to every day objects. Comparative anatomical nomenclatures use criteria derived from the variability and the development of a structure. Use of a particular nomencla ture supposes knowledge of these criteria.
zonal pattern	Purkinje cells are distributed in multiple parallel zones that extend perpendicular to the transverse cerebellar fissures. Criteria to distinguish these zones are (1) the projection of the Purkinje cells of a particular zone to a particular cere bellar or vestibular target nucleus, (2) the innervation of each Purkinje cell zone by climbing fibers from a particular subdivi sion of the inferior olive, (3) the chemoarchitecture of the Purkinje cells of a particular zone. The disposition and extent of the longitudinal zones determine the zonal pattern. Zonal pat terns defined by different criteria generally are congruent.

27.1 Introduction

This article aims to deal with the structure and function of the mammalian cerebellum from an evolutionary point of view. This approach can be only tentative since there are few clues from endocasts of fossil skulls; consequently, the fossil record can give only limited evidence on the evolution of soft tissues. In some vertebrate endocasts, for example, there is a possible cerebellar component that is encased within the petrous bone. In those living mammals in which it is present, this cerebellar subdivision is part of the dorsal paraflocculus, although it is sometimes mislabeled as flocculus in the literature. Since there is little data on the relative size of the entire cerebellum or its parts that can be derived from fossil evidence, and there are no guaranteed living primitive forms, we emphasize insights from comparative anatomy and development. As Bolk (1906) pointed out, there is a common plan to the structure of all mammalian cerebella. Moreover, the histology and the microcircuitry of the cerebellar cortex are preserved features among vertebrates. Comparative anatomy reveals the relative size of the cerebellum and its subdivisions and the variations upon the common plan among mammals, and hence may provide clues as to its evolution. Studies on the connectivity of the cerebellum give indications on the use of the stereotyped cerebellar cortical circuit in different functional systems, and may provide clues on adaptations in cerebellar structure during evolution. Cerebellar morphogenesis and its genetic control provide information on the crucial stages where mutations make these adaptations possible. The link between structure and function, so evident for many other parts of the central nervous system, unfortunately is lacking for the cerebellum. This aspect, the missing link in cerebellar evolution, is discussed in the final section of this article.

27.2 Gross Anatomy of the Mammalian Cerebellum

Figure 1 shows drawings of the human cerebellum. The cerebellum is made up of an extensive cerebellar cortex and its associated deep nuclei, which are located within the white matter. Only a small percentage of the cortex is visible on the surface, since most of the cortex is buried on the banks and in the depths of the fissures. The cortex is divided into a medial vermis and lateral hemispheres. Vermis is thus labeled because of its alleged resemblance to a worm. In the human brain, the vermis is overshadowed by the massive cerebellar hemispheres.

The cerebellum is also divisible into three lobes: anterior, posterior, and flocculonodular.

Figure 2 illustrates a midsagittal section through the cerebellum showing the cortex folded into lobes and lobules by deep fissures. Two of these fissures are of particular importance, because they segregate the three functionally important anterior–posterior divisions. The deepest of these is the primary fissure. The cerebellum in front of the primary fissure is the anterior lobe. Behind the primary fissure is the posterior lobe. The smaller posterolateral fissure separates the posterior lobe from the flocculonodular lobe.

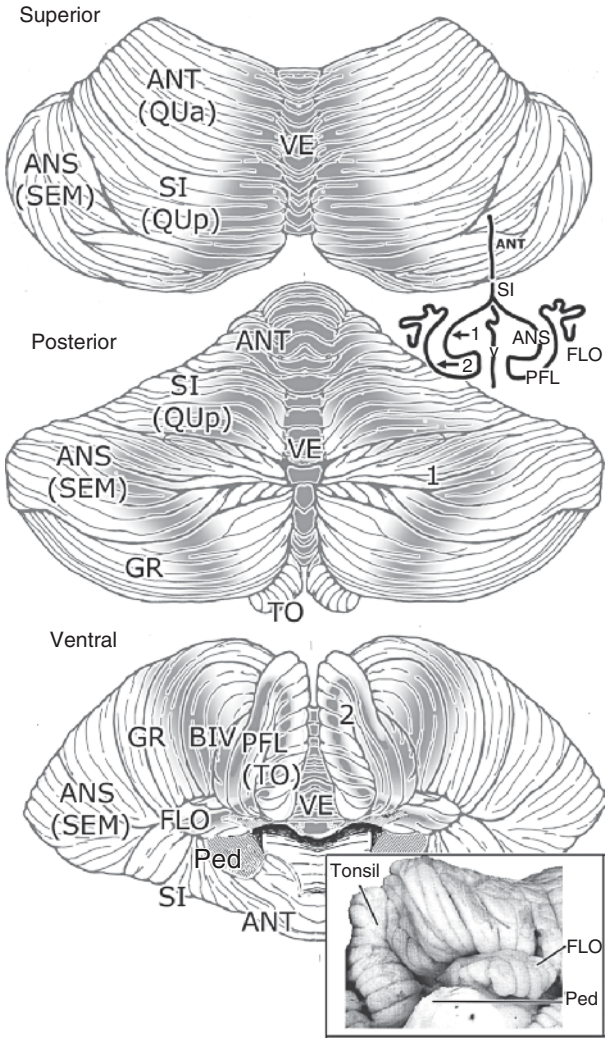

Figure 1 Three diagrams of the human cerebellum. Lobules are indicated with the nomenclature of Bolk (1906), classical names are given in parentheses. The grey band indicates the direction of the folial chains of vermis and hemispheres. The two loops in the folial chain of the hemisphere are indicated as 1 and 2. Insets show Bolk's (1906) wire diagram of the fundamental structure of the mammalian cerebellum and a photograph of the folial loop of the tonsilla. ANS, ansiform lobule; ANT, anterior lobe; BIV, biventral lobule; FLO, flocculus; GR, gracile lobule; Ped, cerebellar peduncles; PFL, paraflocculus; QUa, anterior quadrangular lobule; QUp, posterior quadrangular lobule; SEM, semilunar lobules; SI, lobulus simplex; TO, tonsilla; VE, vermis. Inset Reproduced from Rohen, J. W. and Yokochi, C. 1988. Human Anatomy. Photographic Atlas of Systematic and Regional Anatomy, 2nd edn. Schattauer. Voogd, J. 2003. The human cerebellum. *J. Chem. Neuroanat.* 26, 243 252.

Figure 3 shows a transverse section through the human cerebellum, demonstrating the deeply folded cerebellar cortex, with the cerebellar nuclei embedded within the white matter. The cortex is larger than the nuclei. The pattern of projection from the cortex to the nuclei is orderly. The most medial cortex projects to the middle (fastigial) nucleus and to the lateral vestibular nucleus. More laterally, the cortex projects to the interposed nuclei, called globose and emboliform in the human cerebellum. The cerebellar hemispheres project to the most lateral nucleus, the dentate nucleus. The functional units of the cerebellum are a series of long, parasagittal strips of cortex and their afferent and efferent connections. These will be discussed in detail in a later section.

Over several hundred years, anatomists have been identifying various finer subdivisions of the cerebellum, and there are many and varied systems of nomenclature for these subdivisions (Angevine *et al.*, 1961). Names were assigned long before there was any recognition of whether these subdivisions might be of functional importance. Thus, the most rostral region of the cerebellum was called lingula (originally linguetta) by Malacarne (1776) because it looked to him like a cat's tongue. Most of the traditional nomenclature is based on such superficial resemblance to a tonsil, a ball of wool, or

similarity to a particular geometric shape, such as a pyramid.

27.3 Comparative Anatomy of the Folial Pattern

The cell types and histological structure of the cerebellar cortex are similar among all vertebrates, and they are virtually identical among mammals and lower vertebrates. There is, however, great variability in the relative size and in the morphology of its lobes and lobules. The avian cerebellum, for instance, lacks a clear border between vermis and hemispheres. It consists of a series of simple folia, arranged like the pages of a book. The very small hemispheres are represented by the lateral, unfoliated cortex, and the auricle is the homologue of the mammalian flocculus.

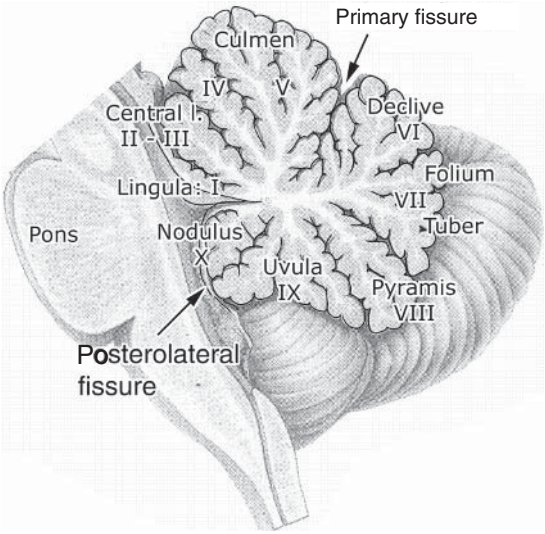

Figure 2 Sagittal section through the human brainstem and cerebellum. Modified from Nieuwenhuys, R., Voogd, J., and van Huijzen, chr. 1988. The Human Central Nervous System. Springer.

Differences in shape and connections of the cerebellum among mammals are obvious (Voogd *et al.*, 1998). The comparative anatomy of the folial pattern of the mammalian cerebellum is based on gross inspection and dissection (Smith, 1903a, 1903b; Riley, 1928, 1929), and on its development (Kuithan, 1894; Stroud, 1898; Bradley, 1904; Bolk, 1906; Larsell, 1937, 1952, 1953, 1970). Larsell and Jansen (1972) combined both approaches. Bolk studied development in order to confirm his ideas on the basic folial pattern of the mammalian cerebellum. For Larsell, the basic pattern is revealed in its development. Inspection and dissection served to confirm this pattern in the adult.

Bolk (1906) emphasized the lack of a distinct border between vermis and hemispheres in the anterior lobe, and in the region immediately behind the primary fissure, known as lobulus simplex, which belongs to the posterior lobe. The transverse fissures in the anterior lobe and simplex run uninterruptedly over the entire width of the cerebellum. Caudal to the lobulus simplex, the cerebellum splits into the median folial chain of the vermis and the two folial chains of the hemispheres. By simple dissection, Bolk revealed the continuity of the folial chain in vermis and hemispheres in all of the 69 mammalian species that he studied (Figures 1, inset, and 4).

The vermis (lobule VII), immediately caudal to the lobulus simplex, is straight in many vertebrates, but it is bent in several carnivores, ungulates, and primates. The caudal lobules of the vermis (VIII, the pyramis; IX, the uvula; and X, the nodulus) show far less variation among species. The folial chain of the hemisphere forms two continuous loops, with a paramedian

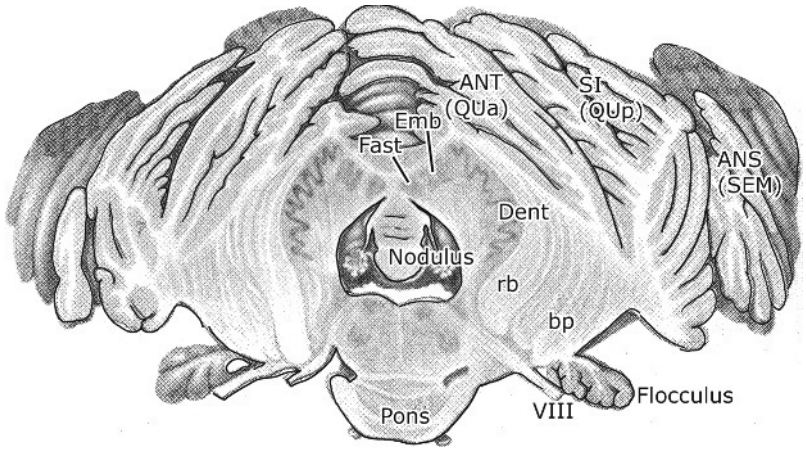

Figure 3 Transverse section through the human brainstem and cerebellum. ANS, ansiform lobule; ANT, anterior lobe; bp, brachium pontis; Dent, dentate nucleus; Emb, emboliform nucleus; Fast, fastigial nucleus; QUa, anterior quadrangular lobule; QUp, posterior quadrangular lobule; rb, restiform body; Sem, semilunar lobule; SI, lobulus simplex. Modified from Nieuwenhuys, R., Voogd, J., and van Huijzen, Chr. 1988. The Human Central Nervous System. Springer.

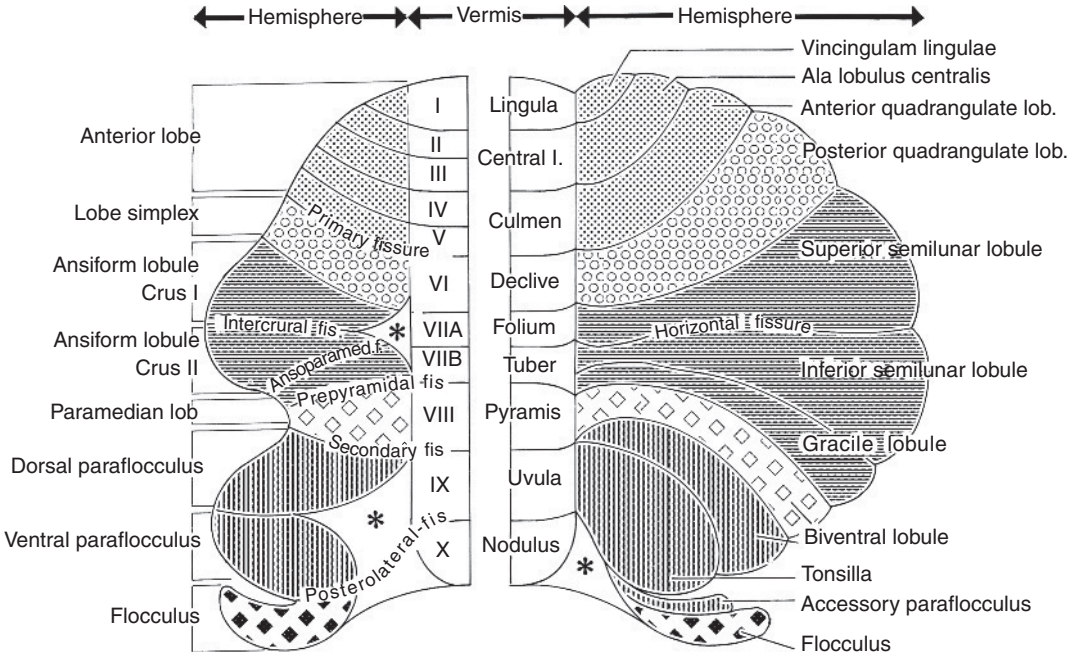

Figure 4 Diagram of the comparative anatomical nomenclature of the cerebellum (left panel) and of the classical nomenclature (right panel). The asterisks indicate superficial medullary areas not covered by cortex. From Voogd, J., Nieuwenhuys, R., van Dongen, P. A. M., ten Donkelaar, H. J. 1998. Mammals. In: The Central Nervous System of Vertebrates (*eds.* R. Nieuwenhuys, H. J. ten Donkelaar, and C. Nicholson), pp.1637 2097. Springer.

segment between the two loops. The rostral loop is known as the ansiform lobule, the caudal loop corresponds to the paraflocculus. The intermediate segment is the paramedian lobule. The caudal-most segment of the folial chain of the hemisphere is turned back upon the paraflocculus as the flocculus, Bolk's uncus terminalis. This basic mammalian folial pattern is present in all mammalian species (Figure 5). In Bolk's view, the anterior lobe and the lobulus simplex form a single growth center. Bolk considered the more caudal lobules of the folial chains of vermis and hemispheres to be mutually independent growth centers. Sultan and Braitenberg (1993) elaborated on Bolk's concept of the folial chains and illustrated this configuration in many mammalian species (Figure 6).

According to Larsell, the posterolateral and primary fissures are the earliest fissures to appear. Together with fissures that form later, they subdivide the cerebellum into 10 subdivisions, from the most rostral lobule I (lingula) to the most caudal lobule X (nodulus). Lobules I–V constitute the anterior lobe; lobules VI–IX are the vermian part of the posterior lobe; lobule X corresponds to the nodulus. Each of the vermian lobules is associated with a lobule in the hemisphere, indicated with the same Roman numeral, with the prefix H. Larsell emphasized the mediolateral continuity of the lobules of vermis and hemisphere. In his words, ". . . it is (also) clear in the adult and in the fetus that the lateral parts, namely lobulus ansiformis, paraflocculus and the lateral continuation of the pyramis are merely lateral extensions of the medial portion" (Larsell, 1937, p. 605).

Bolk attached more importance to the independence of the lobules of the folial chains of vermis and hemispheres. This independence was also emphasized in his studies on the development of the folial pattern of the human cerebellum (Bolk, 1906). He distinguished three rostrocaudal regions. In the rostral cerebellum, comprising the anterior lobe and the lobulus simplex, all of the fissures first appear in the midline and then grow out laterally. In an intermediate region, corresponding to lobule VII and rostral VIII (the pyramis) and the ansiform and paramedian lobules, the interlobular fissures arise medially, but the intralobular fissures arise independently in vermis and hemispheres. In the caudal region of the cerebellum, all of the fissures arise independently in vermis and hemispheres.

There are variations among mammals in the length and width of certain segments of the folial chains. The greatest amount of variability is seen in vermian lobule VII, in the ansiform lobule, and in the paraflocculus. Lobulus simplex, the vermian lobules VIII–X, the paramedian lobule, and the flocculus are less variable, although the width of the folia may vary. In some species, these variations

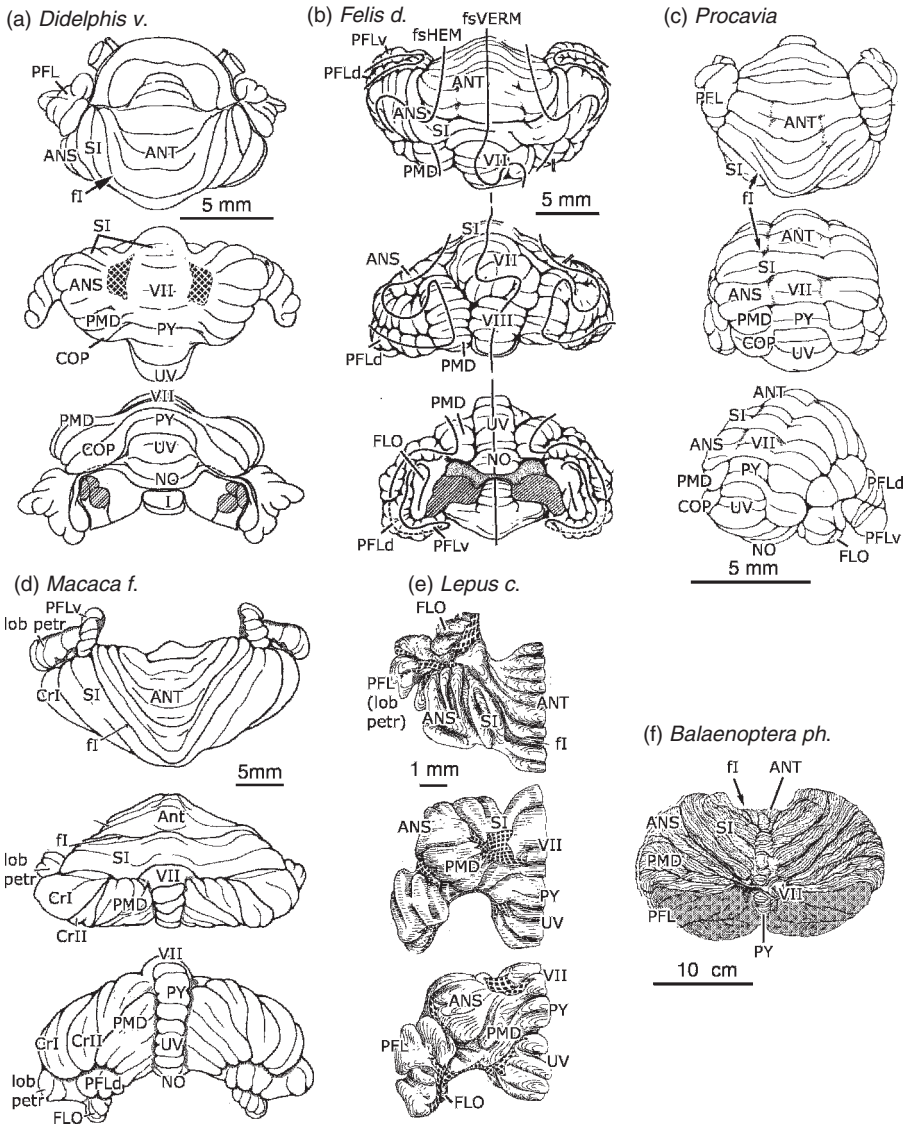

Figure 5 Anterior, dorsal, and caudal (ventral) views of different mammalian cerebella. In (b) the direction of the folial chains of vermis and hemispheres is indicated for the cerebellum of the cat. Medullary areas not covered by cortex are indicated by filled squares in (a) and (e). Note approximately equal width of the folial chains of vermis and hemispheres in opossum (a), cat (b), coney (c), and rabbit (e), and greater width of the hemiphere in monkey (d) and whale (f). A folial loop in the ansiform lobule is lacking in the coney (c). Size and shape of the visual vermis (lobule VII) differs in different mammals: it is large and convoluted in the cat (b), small and straight in the other species. Cetacea are characterized by the large, overall size of the cerebellar hemisphere, especially of the paraflocculus (shaded in f). ANS, ansiform lobule; ANT, anterior lobe; COP, copula pyramidis; CrI (II) crus I (II) of the ansiform lobule; fI, primary fissure; fsHEM, folial chain of the hemisphere; fsVerm, folial chain of the vermis; lob petr, petrosal lobule; NO, nodulus; PFL (dv), paraflocculus (dorsalis, ventralis); PMD, paramedian lobule; PY, pyramis; SI, lobulus simplex; UV, uvula; VII, lobule VII. a, Reproduced from Larsell, O. 1970. The Comparative Anatomy and Histology of the Cerebellum from Monotremes through Primates. University of Minnesota Press. b d, Reproduced from Voogd, J., Nieuwenhuys, R., van Dongen, P. A. M., and ten Donkelaar, H. J. 1998. Mammals. In: The Central Nervous System of Vertebrates (*eds.* R. Nieuwenhuys, H. J. ten Donkelaar, and C. Nicholson), pp.1637 2097. Springer. e, Reproduced from Thunissen, I. 1990. Vestibulocerebellar and Vestibulo-Oculomotor Relations in the Rabbit. Thesis, University of Rotterdam. f, Reproduced from Jansen, J. and Brodal, A. 1954. Aspects of Cerebellar Anatomy. Grundt Tanum.

can be related to the zonal organization of the individual lobules. The details of zonal organization will be discussed in a later section.

Bolk's fundamental plan of the cerebellum was confirmed in Riley's (1928, 1929) studies of the gross anatomy of the mammalian cerebellum and can be recognized in the plots of the number and the width of the folia of several mammalian species of Sultan and Braitenberg (1993), illustrated in Figure 6.

Parallel fibers are the axons of granule cells. They extend within the molecular layer, where they

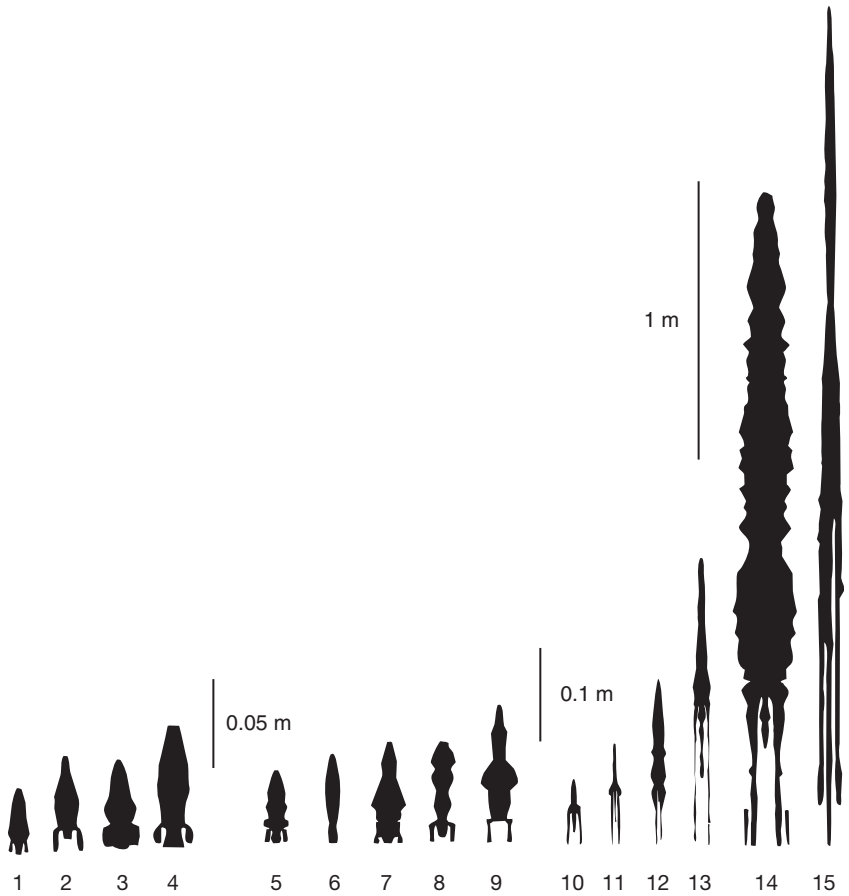

Figure 6 Outlines of the shapes of the cerebellar cortices, obtained by connecting the ends of the most prominent folia. The scale is the same in the laterolateral and anteroposterior direction. Note division of posterior cerebellum in the folial chains of vermis and hemispheres, and the relative width and length of these chains in different species. 1, Mouse; 2, bat; 3, flying fox; 4, guinea pig; 5, rabbit; 6, pigeon; 7, hare; 8, chinchilla; 9, squirrel; 10, dog; 11, cat; 12, macaque; 13, sheep; 14, human; 15, bovine. Magnifications differ for the diagrams 1 4, 5 9, and 10 15. Adapted from Sultan, F. and Braitenberg, V. 1993. Shapes and sizes of different mammalian cerebella. A study in quantitative comparative neuroanatomy *J. Hirnforsch.* 34, 79 92.

contact Purkinje cell dendrites. Mammals vary in the extent to which there is continuity of the parallel fibers between the vermis and hemispheres. This continuity is complete in the anterior lobe, the lobulus simplex, and between lobule VIII (the pyramis) and the paramedian lobule. The cortex is entirely or partially interrupted between lobule VII and the ansiform lobule, and between the caudal vermis (lobules IX, the uvula, and X, the nodulus) and the paraflocculus and the flocculus (Figures 5a and 5e) (Glickstein and Voogd, 1995).

27.4 Allometry and Cerebellar Size

Elephants and mice each have a four-chambered heart that works on the same general principles to pump blood around the body. The brain of an elephant, like its heart, is much larger than that of a mouse. To compare the weight of the brain or any body part between species, it is necessary to consider its relative, not its absolute size. Subdivisions of the brain will also vary with its total size. Allometry is an approach to dealing with such comparisons by plotting the size of each organ against total body size. The same approach can be used to compare the relative size of one or another subdivision of the brain. The general form of the exponential equation that is used is typically in the form $\log Y = k \log X + \log b$, where X and Y are the two structures to be compared and k is the slope of a linear fit to the data. Thus, vast differences can be plotted in the same graph, and an exponential equation becomes linear. If brain weight is plotted against body weight in a log-log plot across a large number of mammalian species, there appears to be a satisfying linearity. But because a whale may weigh over 100 000 times more than a bat, important deviations from the linear fit may not be obvious. The same problem arises in studies in which the volume of a brain subdivision

is plotted against total brain weight (Finlay and Darlington, 1995).

Clark *et al.* (2001) compared the volume of brain subdivisions across several species of insectivores, tree shrews, and primates. They argue that although the telencephalon, and especially the cerebral cortex is relatively large in primates, the cerebellum remains a constant fraction of brain volume. On the basis of this analysis, they grouped mammalian species into several subtypes that they called cerebrotypes. Clark *et al.*'s conclusion about mammalian cerebrotypes was criticized on several fronts. De Winter and Oxnard (2001) used the same data set in a principal component analysis. Their results suggested that a grouping of species by locomotor types is more appropriate than the cerebrotypes postulated by Clark *et al.* Moreover, as Barton (2002) pointed out, Clark *et al.*'s data does, in fact, demonstrate an increase in the relative volume of the cerebellum among the species studied, but the increase proceeds at a slower rate than that of the cerebral cortex. Sultan (2002) has questioned the very basis of Clark *et al.*'s analysis. He argues convincingly that relative volume or weight is not an appropriate measure for the functional importance of a given brain subdivision. A crude volume estimate of subdivisions is inappropriate, since his own work clearly demonstrates that valid comparisons should be based not on volume but on surface extent of the cerebellum.

27.5 Cell Types and Cerebellar Circuitry

27.5.1 Histology of the Cerebellar Cortex

The histological structure of the cerebellar cortex was described and summarized by Ramon y Cajal (1911). Cajal paid attention to the histology of the cortex in lower vertebrates, but his 1911 description is mainly based on the situation in mammals. The following account also describes the cell types and the circuitry of the mammalian cerebellar cortex.

The cerebellum is made up of two fundamental subdivisions: a broad sheet of cells, the cerebellar cortex, and a group of deep cerebellar nuclei that are buried within the white matter. The cerebellar cortex of all vertebrates shares several fundamental features (Nieuwenhuys *et al.*, 1998). Purkinje cells in all species constitute the main output element. The Purkinje cells receive two types of excitatory afferents: the climbing fibers, originating from the contralateral inferior olive, and the parallel fibers, which are the axons of granule cells. Granule cells receive their input from many sources outside of the cerebellum, all of which terminate as mossy fibers. The axons of Purkinje cells terminate in the cerebellar and vestibular nuclei.

Figures 7a and 7b are sketches showing the structure of the cerebellar cortex. Purkinje cell bodies form a single layer throughout the entire extent of the cortex. They have large flask-shaped cell bodies, and their axons constitute the only output from the cortex to the cerebellar nuclei. Purkinje cells are GABAergic and inhibitory. Their dendrites branch extensively in a plane perpendicular to the cerebellar folium. Their axon projects to the nuclei, giving off axonal collaterals within the cortex. By far the most numerous cells in the cerebellum, indeed in the entire mammalian brain, are granule cells. These are small cells, measuring about 7 μm in diameter, packed densely in the granular layer of the cortex. The axon of granule cells ascends to the outer molecular layer of the cortex, where it branches in a characteristic T fashion, and extends as a parallel fiber within the molecular layer. Parallel fibers are labeled as such because they are oriented parallel to the course of the cerebellar folia to which they project. The ascending axon of the granule cell and its parallel fiber branches contact Purkinje cell dendrites and dendrites of interneurons. In addition to Purkinje and granule cells, there are three other types of neurons, all three of which are inhibitory. Golgi cell bodies reside in the granular layer. Their dendrites extend into the molecular layer, where they are contacted by parallel fibers. Their axons ramify within the granular layer, where they terminate on dendrites of granule cells. Golgi cells use glycine as their inhibitory neurotransmitter. Basket and stellate cell bodies are in the molecular layer. Their dendrites and axons extend in a plane perpendicular to the long axis of the folia. Basket and stellate cells use GABA as their neurotransmitter. Basket cell axons give off several branches, each of which surrounds Purkinje cell bodies like a wicker basket. They have powerful inhibitory connections that are concentrated at the axon hillock of the Purkinje cell. Stellate cells inhibit the Purkinje cell dendrites.

Purkinje cells are activated by way of two totally independent systems of afferent fibers. Mossy fibers originate from the spinal cord, multiple centers in the lower brainstem, and the pontine nuclei. Mossy fibers branch extensively and terminate on the dendrites of the granule cells, which in turn connect to the Purkinje cells by way of parallel fibers. Some mossy fibers provide collaterals to the cerebellar nuclei. Each Purkinje cell also receives a completely different type of input, a single climbing fiber, because their terminations ascend along the dendrites of the Purkinje cells, making multiple

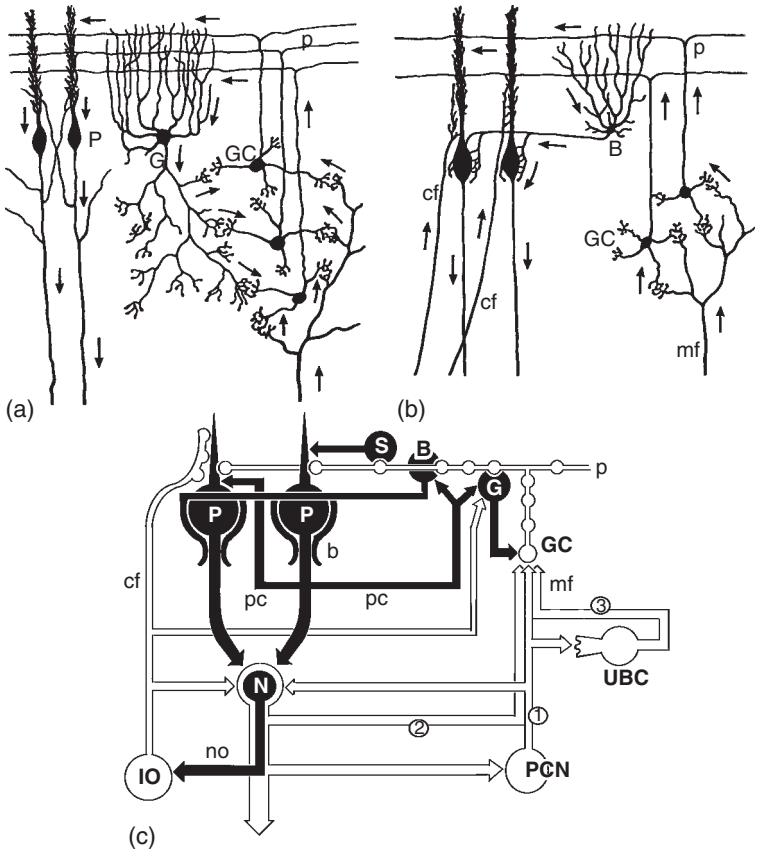

Figure 7 a, Diagram showing the main mossy fiber-granule-cell Purkinje-cell circuit and the innervation of the granule cells by the axonal plexus of the Golgi cell. b, Diagram of the climbing fiber innervation of the Purkinje cells, and the basket cells with their axonal baskets surrounding the Purkinje cell perikarya. c, Diagram of the cerebellar circuitry. Inhibitory neurons are indicated in black. B, basket cell; b, pinceau of basket all axons; cf, climbing fiber; G, Golgi cell; GC, granule cell; IO, inferior olive; mf, mossy fiber; no, nucleo-olivary axons; pc, recurrent Purkinje cell axon collaterals; P, Purkinje cell; p, parallel fibers; PCN, precerebellar nuclei; S, stellate cell; UBC, unipolar brush cell; 1, extracerebellar mossy fiber; 2, nucleo-cortical mossy fiber; 3, mossy fiber collateral of unipolar brush cell. a and b, Redrawn from Ramon y Cajal, S. 1911. Histologie du système nerveux de l'homme et des vertebrés. Maloine.

and direct contacts. Climbing fibers all arise solely from the inferior olivary nucleus on the opposite side of the cerebellum, giving off collateral fibers to the cerebellar nuclei as they ascend to the cortex.

Both inputs to the Purkinje cells, the mossy fiber–parallel fiber system and the climbing fibers, are excitatory, but they terminate on different segments of the Purkinje cell dendritic tree. The climbing fiber terminates on short, stubby spines on the proximal, smooth portion of the dendrites. Parallel fibers contact long-necked spines of the distal spiny branchlets of the Purkinje cell dendritic tree.

The histology of the cerebellar cortex is very similar in mammals and in lower vertebrates. Purkinje cells, granule cells, and Golgi cells have been identified in all vertebrate genera. The main differences concern the lamination of the cortex, the spatial segregation of Purkinje cells and granule cells in certain forms, and the shape of the Purkinje cell dendritic tree. In fish, the proximal smooth dendrites with their climbing fiber afferents are located within the Purkinje cell layer and the spiny branchlets ascend into the molecular layer. In mammals, smooth and spiny branches are found throughout the molecular layer. In birds there is an intermediate arrangement in which the smooth branches are restricted to the lower half of the molecular layer (Nieuwenhuys *et al.*, 1998).

Cerebellar nuclei are not present in all lower vertebrates. In fish, the connections of the cerebellum with other parts of the central nervous system take their origin from cells that are located within the Purkinje cell layer (eurodendritic cells). Unlike the cerebellar nuclei, they are not buried in the white matter, but their axons, like those from nuclear cells in mammals, project to targets outside of the cerebellum. A similar type of cortical neuron with long, extracerebellar connections has been described only

in a fourth cerebellar layer of Pinnipedia (Ogawa, 1934).

The cerebellar nuclei are arranged from medial to lateral, with their major input from the overlying cerebellar cortex. The most medial cortex of the cerebellar vermis projects to the medial nucleus of the cerebellum, also called the fastigial nucleus (Figure 8). The lateral vestibular nucleus, which is in fact a fourth deep nucleus, receives its input from the lateral vermis. The most lateral fibers of the cerebellar hemisphere project to the lateral cerebellar nucleus, also called the dentate nucleus. Between the fastigial and dentate nucleus are the anterior and posterior interposed nuclei, also called globose and emboliform in the human cerebellum. The interposed nuclei receive Purkinje cell axons from the intermediate zone of the cerebellar cortex. Size and structure of the cerebellar nuclei are directly related to the size and the configuration of their Purkinje cell input and to the weight and the construction of the functional motor, sensory, and cognitive systems which serve as their targets.

27.5.2 Purkinje Cell Zones. Morphology, Connections, and Chemical Identity

The output of the cerebellum is organized as a series of independent modules. Each module consists of one or more longitudinal zones of Purkinje cells, oriented perpendicular to the transverse fissures, its cerebellar or vestibular target nucleus, and a climbing fiber system, innervating both the Purkinje cells

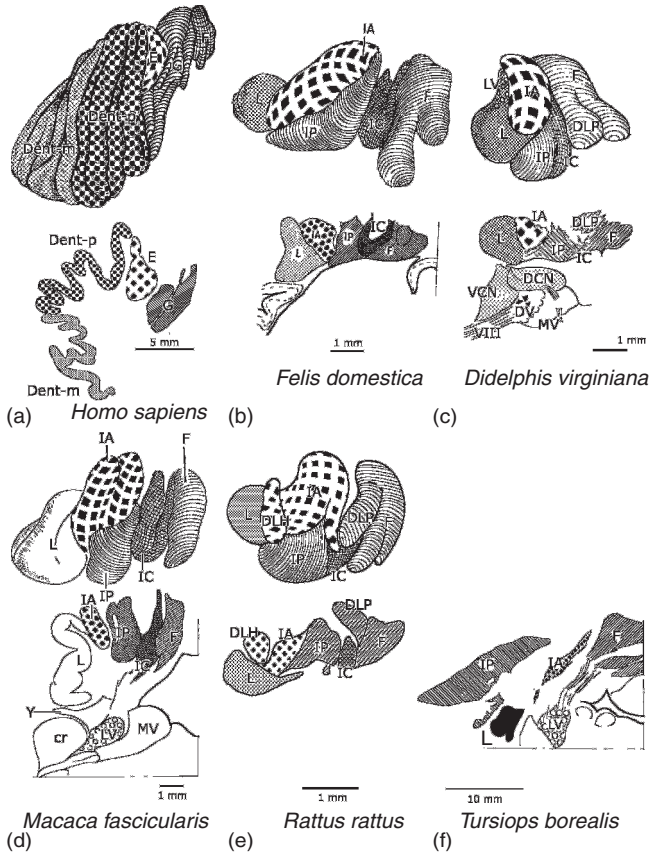

Figure 8 Diagrams of graphical reconstructions of the cerebellar nuclei (upper panels) and of a transverse section through the nuclei (lower panels) in different mammals. The interstitial cell groups (IC) are a nucleus located between the fastigial and interposed nuclei, which serves as the target nucleus of the X and CX zones. The dorsolateral hump (DLH) and the dorsolateral protuberance of the fastigial nucleus (DLP) have only been described in rodents and/or marsupials (c, e). Group Y is particularly well developed in primates (d). It serves as one of the target nuclei of the flocculus. For the dolphin (f; *Tursiops truncatus*), no graphical reconstruction was available. cr, restiform body; DCN, dorsal cochlear nucleus; Dent-m, macrogyric portion of human dentate nucleus; Dent-p, microgyric portion of human dentate nucleus; DLH, dorsolateral hump; DLP, dorsolateral protuberance of fastigial nucleus; DV, spinal vestibular nucleus; E, emboliform nucleus; F, fastigial nucleus; G, globose nucleus; IA, anterior interposed nucleus; IC, interstitial cell groups; IP, posterior interposed nucleus; L, lateral cerebellar nucleus; LV, lateral vestibular nucleus; MV, medial vestibular nucleus; VCN, ventral cochlear nucleus; Y, group Y. a, Reproduced from Voogd, J. 2004a. Cerebellum and precerebellar nuclei. In: The Human Nervous System (*eds.* G. Paxinos and J. K. Mai), pp. 321 392. Elsevier. b f, Reproduced from Voogd, J., Nieuwenhuys, R., van Dongen, P. A. M., and ten Donkelaar, H. J. 1998. Mammals. In: The Central Nervous System of Vertebrates (*eds.* R. Nieuwenhuys, H. J. ten Donkelaar, and C. Nicholson), pp.1637 2097. Springer.

and the target nucleus of the module. Among all mammals studied, there is a similar pattern of longitudinal Purkinje cell zones in the mammalian cerebellum (Voogd, 1967; Buisseret-Delmas and Angaut, 1993; Voogd *et al.*, 1996, 2003; Voogd and Glickstein, 1998; Sugihara and Shinoda, 2004; Voogd and Ruigrok, 2004). Three of these zones occupy the vermis (Figure 9). The medial A zone is present along its entire length and projects to the medial (fastigial) nucleus and restricted portions of the vestibular nuclei. Its climbing fibers originate from the caudal medial accessory olive (MAO). The X zone is the next lateral zone. It projects to the junction of the fastigial and the posterior interposed nucleus (interstitial cell groups) and receives climbing fibers from an intermediate region of the MAO. The most lateral zone of the vermis is the B zone. It is only present in restricted anterior and posterior portions of the cerebellum, projects to the lateral vestibular nucleus of Deiters, and receives a climbing fiber projection from the caudal dorsal accessory olive (DAO). The cerebellar hemispheres are made up of seven or eight zones. The most medial of these, the A2 zone (not illustrated in Figure 9), projects to a dorsolateral protuberance of the fastigial nucleus (Figure 8) and is innervated by climbing fibers originating from the medial subnucleus C of the caudal MAO. Three successively more lateral zones, C1, C3, and Y, all project to the anterior interposed nucleus and all receive their climbing fiber afferents from the rostral DAO. The CX zone occupies a strip, immediately medial to C1. It shares its connections with the X zone. Like the B zone, the X, CX, C1, C3, and Y zones are only present in restricted anterior and posterior segments of the hemisphere (i.e., in the anterior lobe and the lobulus simplex and in the caudal ansiform lobule and the paramedian lobule, see below). The C2 zone, which is located between C1 and C3, is connected with the posterior interposed nucleus and receives climbing fibers from the rostral MAO. Two zones in the lateral hemisphere, D1 and D2, project to rostromedial and caudolateral portions of the lateral cerebellar (dentate) nucleus and are innervated by climbing fibers from the ventral and dorsal lamina of the principal olive, respectively. C2 and the D zones are present over the entire length of the cerebellar hemispheres.

This fundamental pattern, with very little variation, is present in all of the species studied. The main differences concern the relative width and the length of the zones in certain regions of the vermis and hemisphere. There are variations among mammals in the length of the A zone, which is located in the middle regions of the vermis, particularly in lobule

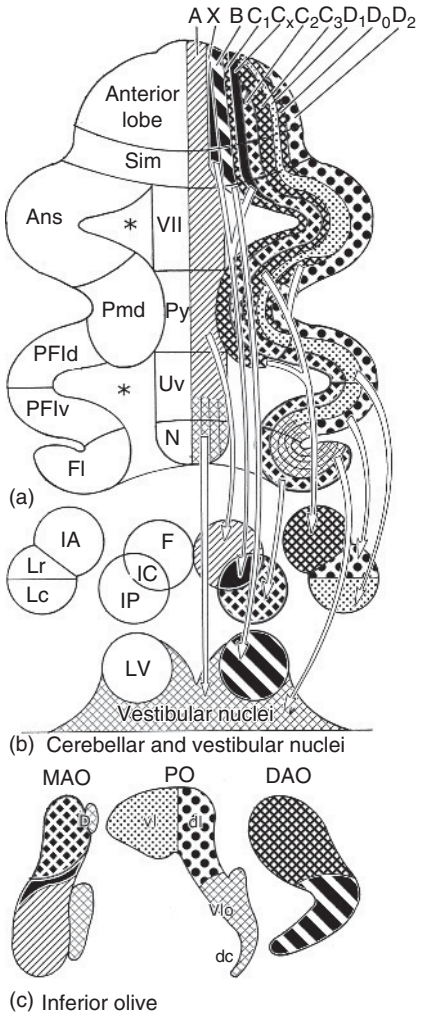

Figure 9 Diagram of the zonal organization in the corticonuclear and olivocerebellar projections. a, Diagram of the flattened cerebellar cortex. b, Diagram of the cerebellar and vestibular nuclei. c, Diagram of a projection of the monkey inferior olive in the horizontal plane. The longitudinal corticonuclear and olivocerebellar projection zones are indicated with capitals (A, X, B, C_X, $C_{1–3}$, Y, $D_{1,2}$). The zones, their target nuclei, and the subnuclei of the inferior olive that project to these zones are indicated with the same shadings. The diagram applies equally to the cerebella of rat, cat, rabbit, and monkey, with the exception of the floccular zones, the most medial one of which is lacking in the monkey. The A_2 zone, only present in the rat, is not indicated. Asterisks, areas without cortex. A, A zone; Ans, ansiform lobule; B, B zone; $C_{1–3–X}$, $C_{1–3–X}$ zones; $D_{1,2}$, $D_{1,2}$ zones; D, dorsomedial cell column; DAO, dorsal accessory olive; dc, dorsal cap; dl, dorsal lamina of the principal olive; F, fastigial nucleus; Fl, flocculus; IA, anterior interposed nucleus; IC, interstitial cell groups; IP, Posterior interposed nucleus; Lc, caudal lateral cerebellar (dentate) nucleus; Lr, rostral lateral cerebellar (dentate) nucleus; LV, lateral vestibular nucleus; MAO, medial accessory olive; N, nodulus; PFlv, ventral paraflocculus; PFld, dorsal paraflocculus; PMD, paramedian lobule; PO, principal nucleus of the inferior olive; PY, pyramis; Sim, lobulus simplex; UV, uvula; vl, ventral leaf of principal olive; vlo, ventrolateral outgrowth; X, X zone. Reproduced from Voogd, J., Nieuwenhuys, R., van Dongen, P. A. M., and ten Donkelaar, H. J. 1998. Mammals. In: The Central Nervous System of Vertebrates (*eds.* R. Nieuwenhuys, H. J. ten Donkelaar, and C. Nicholson), pp.1637 2097. Springer.

VII. This lobule is associated with the control of eye movements. In many carnivore, ungulate, and primate species, this increase in rostrocaudal length leads to the formation of an S-shaped curve in this portion of the vermis (Figure 5b; Voogd and Barmack, 2005). The B, C1, C2, and Y zones are represented only in the rather conserved anterior (anterior lobe and lobulus simplex) and posterior (pyramis, lobule VIII, and paramedian lobule) regions of the cerebellum (Voogd, 2003). They share similar corticonuclear and olivocerebellar connections, which are relatively constant.

Most variations in length and width concern the C2 and the D zones. The C2 zone and its connections with the posterior interposed nucleus and the rostral MAO and the associated brainstem circuitry are hypertrophied in cetaceans, where this zone is associated with the large size of the paraflocculus (Figures 5f, 8, 10, and 11) (Korneliussen, 1967, 1968a, 1968b). The C2 zone in whales was indicated by Korneliussen as the lateral intermediate zone (Figure 10). The D1 and D2 zones, along with the associated regions of the dentate nucleus and the principal olive are greatly enlarged in primates. They are responsible for the great size of the ansiform lobule in nonhuman primates, but also for the width of the folia of the anterior lobe, the lobulus simplex and the paramedian lobule in these animals (Figures 5d and 10). In the human cerebellum, this increase in width of the D zones also affects the homologue of the paraflocculus, the medial belly of the biventral lobule, and the tonsilla (Figure 1). The D1 zone, generally, is the more narrow of the two D zones (Voogd, 2003, 2004a, 2004b). Similarly, in the elephant, it is the dorsal lamina of the principal olive with its projection to the D2 zone that is specifically enlarged (Figure 11) (Verhaart, 1962). There is only one example of a zone, the A2 zone, that exists in some, but not other mammalian species. Judging from the presence of its target nucleus, the dorsolateral protuberance of the fastigial nucleus (Goodman *et al.*, 1993), the A2 zone is present in rodents, lagomorphs, and marsupials, but absent in carnivores and primates (Figures 8c and 8e) (Buisseret-Delmas, 1988a, 1988b).

Purkinje cells are not a homogeneous population; they differ in their biochemical properties. Two populations of Purkinje cells were distinguished by Hawkes and Leclerc (1987) on the basis of their immunoreactivity with an antibody against the zebrin I epitope. Zebrin-positive and Zebrin-negative Purkinje cells are distributed in alternating longitudinal zones (Figure 12). The zebrin pattern is correlated with the distribution of many different substances in Purkinje cells, such as enzymes (5′-nucleotidase and aldolase-C, or zebrin II), certain

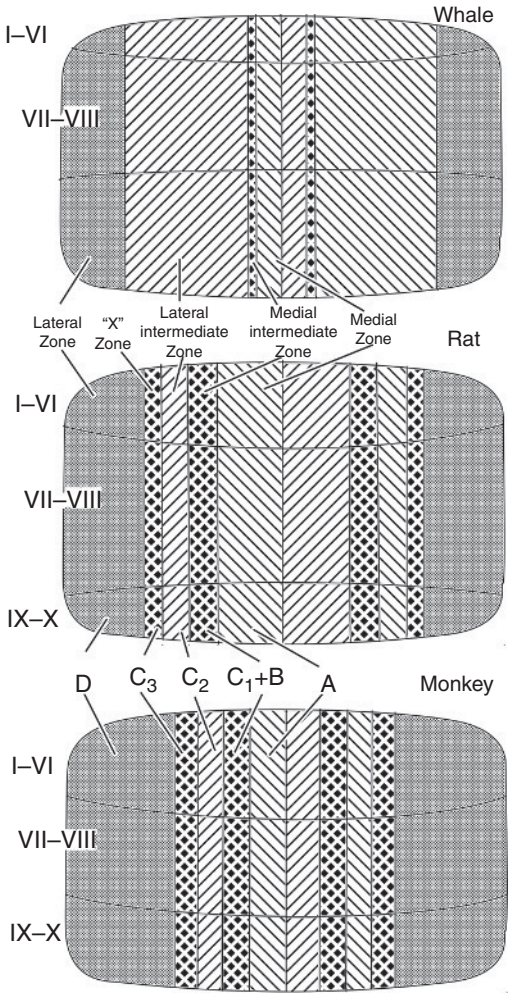

Figure 10 Diagram of the relative width of corticogenetic Purkinje cell zones in different mammals. During early development, the future Purkinje cell zones are present as Purkinje cell clusters at the still unfolded surface of the cerebellar anlage (see also Figure 16). The diagrams depict the relative width of these clusters. Corresponding zones are indicated with the same symbols. The nomenclature of Korneliussen for rat and whale differs from the traditional nomenclature, employed by Kappel. The diagrams of the whale and the rat are based on data from Korneliussen, H. K. 1967. Cerebellar corticogenesis in Cetaca, with special reference to regional variations. *J. Hirnforsch.* 9, 151 185. The data from the monkey are based on Kappel, R. 1981. The Development of the Cerebellum in *Macaca mulatta*. A Study of Regional Differentiation during Corticogenesis. PhD dissertation, University of Leiden.

glutamate transporters, growth factor receptors, etc. There are vast differences in expression of the zebrin II marker across the vertebrate subphylum. For example, basal vertebrates such as sharks and rays reveal uniform expression of the marker. In contrast, all mammalian species show a similar number of zebrin II zones, although the specific patterns are subtly different (Figure 13; Sillitoe *et al.*, 2003, 2005). Recent studies comparing the

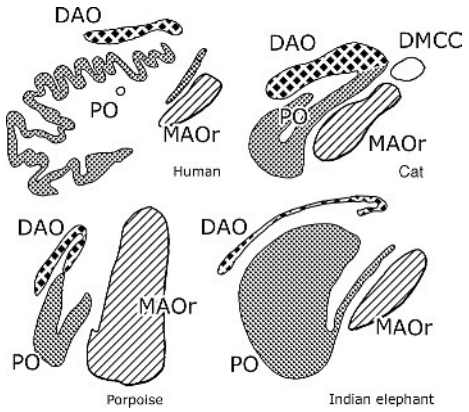

Figure 11 Transverse sections through rostral levels of the inferior olive in different mammalian species. DAO, dorsal accessory olive; DMCC, dorsomedial cell column; MAOr, rostral medial accessory olive; PO, principal olive. The human olive and the olive of the elephant were redrawn from Kooy (1917) . The other diagrams are reproduced from Voogd (2004a, 2004b) and Voogd *et al.* (1998; b f)

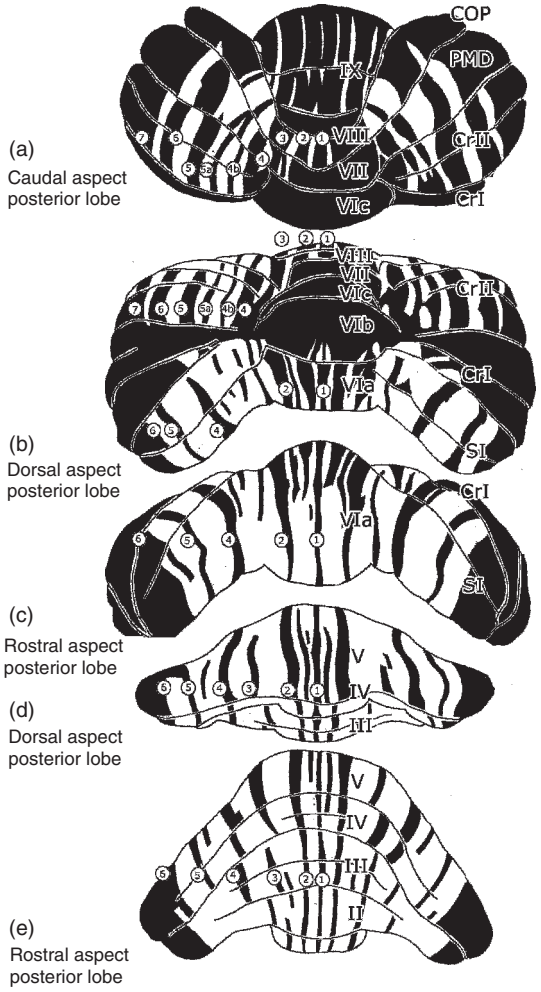

Figure 12 Zebrin-positive and zebrin-negative zones in the cerebellum of the rat. Caudal, dorsal, and rostral aspects of the posterior lobe (a c) and dorsal and rostral aspects of the interior lobe (d, e) are illustrated. Numbers indicate zebrin-positive Purkinje cell zones P1 P7 of Hawkes and Leclerc (1987). COP, copula pyramidis; CrI, CrII, crus 1 and II of the ansiform lobule; PMD, paramedian lobule; S1, simple lobule; I X, lobules I X.

connections and the zebrin-identity of Purkinje cells in the rat showed a close correspondence between the two patterns. Purkinje cells of the B, X, CX , C1, C3, and Y zones are zebrin-negative. Purkinje cells of the C2, D1, and D2 zones are zebrin-positive. The A zone is a composite of zebrin-positive and zebrin-negative areas. Crus I of the ansiform lobule and the paraflocculus and the flocculus, where only the C2 and the D zones are represented, are entirely zebrin-positive (Voogd *et al.*, 2003; Voogd and Ruigrok, 2004; Sugihara and Shinoda, 2004).

Purkinje cell zones are connected by way of the cerebellar and vestibular nuclei with the centers in the brainstem, the thalamus, and the spinal cord (Figure 14; see Voogd, 2003, 2004a, 2004b for reviews). The C1, C3, and Y zones project through the anterior interposed nucleus to the contralateral magnocellular red nucleus and, via the ventrolateral nucleus of the thalamus, to the primary motor cortex. They monitor activity in the rubrospinal and corticospinal tracts. The A, B, X, and CX zones maintain strong connections with the spinal cord, through the cerebellospinal, reticulospinal, and vestibulospinal tracts. Note that these Purkinje cell zones share somatotopically organized somatosensory climbing fiber projections. The connectivity of the C2 and D zones is different. Their target nuclei, the posterior interposed and dentate nuclei, project to centers at the junction of the mesencephalon and diencephalon which, in turn, give rise to strong descending systems to the inferior olive. For the dentate nucleus, the system relays in the parvocellular red nucleus, with the central tegmental tract as its descending system terminating in the principal olive; for the posterior interposed nucleus, the mesodiencephalic nucleus is the nucleus of Darkschewitsch, with the medial tegmental tract, terminating in the rostral MAO, as its descending system. In addition, the posterior interposed and dentate nuclei project to ventral thalamic nuclei with connections to motor, premotor, and prefrontal areas, including the frontal eye fields, and more limited projections to the parietal lobe. The closed cerebello-mesodiencephalic-olivary loops are under strong cortical influence of these same cortical areas (Voogd, 2003, 2004a).

Relative size and connectivity of the Purkinje cell zones and their target nuclei are indicative of adaptations of the cerebellum to changes in the organization of motor, sensory, and cognitive systems of the brain

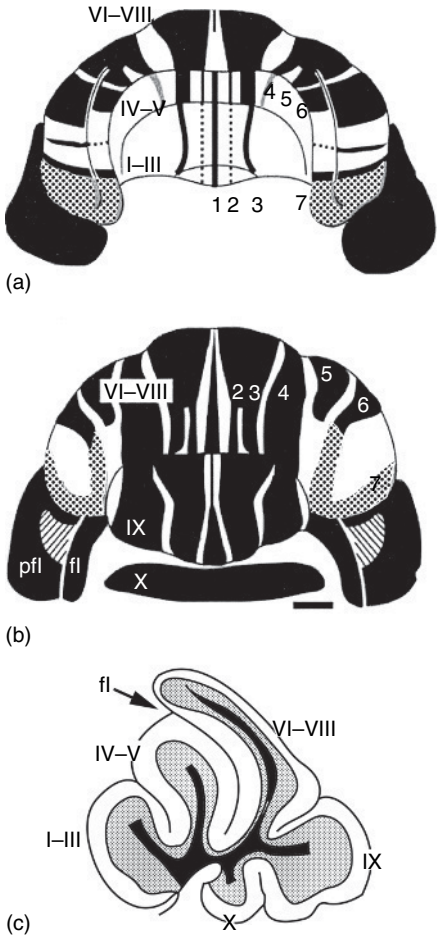

Figure 13 Reconstruction of the location of zebrin II immunoreactive Purkinje cell zones in anterior (a) and posterior (b) views of the cerebellum of the tenrec (*Echinops telfari*). The same zebrin-positive bands 1 7 can be recognized as in the rat (Figure 12). The cerebellum of this basal insectivore can be subdivided into the fused lobules I II and IV V of the anterior lobe, the combined lobules VI VIII with the ansiform and paramedian lobules, the uvula (IX), the nodulus (X), the paraflocculus (pfl) and the flocculus (fl). a and b, Reproduced with permission from Sillitoe, R. V., Künzle, H., and Hawkes, R. 2003. Zebrin II compartmentation of the cerebellum in a basal insectivore, the Madagascar hedgehog, tenrec, *Echinops telfari. J. Anat.* 203, 283 296.

in different species. The prominence of the Purkinje cell zones of the vermis and the C1 and C3 zones in lower mammals, and the large hemispheres and the increase in length and width of the D1 and D2 zones in primates, parallels the shift from a local spinal and brainstem regulation of movement to a situation where movement is largely dependent on the cerebral cortex. Prominent vermal and C zones in lower mammals are associated with prominence of their target nuclei, such as Deiters' lateral vestibular nucleus and the magnocellular red nucleus and their spinal tracts. Prominent hemispheres, extensive D zones, and a large, convoluted and subdivided dentate nucleus are found in primates. Targets of the primate dentate nucleus include extensive and differentiated motor, premotor, and frontal association areas and the large parvocellular red nucleus, which links these cortical areas with the inferior olive and the cerebellum. It has been suggested that the functions of the primate dentate nucleus principally involve cognitive and emotional aspects of behavior (Schmahmann, 1997). However, the great development of the cerebellar hemisphere and the dentate nucleus can also be considered as an adaptation to visual and visuomotor exigencies in primates. Much remains unknown. The functional importance of the C2 zone, which overwhelms the cetacean cerebellum and the presence of additional Purkinje cell zones in rodents remain unexplained.

The caudal portions of the vermis and the hemisphere, i.e., the nodulus and the flocculus, are known as the vestibulocerebellum. The zonal organization of the nodulus and the flocculus represents a modification of the A and D zones, respectively (Figure 9). The afferent and efferent connections of the nodulus are mainly with the vestibular nuclei; the flocculus receives olivocerebellar systems mediating optokinetic information, and projects to vestibulo-ocular and vestibulospinal neurons mediating the optokinetic and the labyrinthine neck reflexes (Voogd and Barmack, 2005).

Both in the flocculus and the nodulus, the Purkinje cells are arranged in a complicated pattern of multiple longitudinal zones. For the flocculus, this is a highly conserved feature. It is present in the flocculus of mammals and birds (Voogd and Wylie, 2004). However, one distinguishing feature is present in the primate flocculus. The folial rosette, which links the flocculus with the paraflocculus (known as the ventral paraflocculus) has increased in size, while retaining its original floccular zonal pattern. This feature has been related to the development of foveal vision and smooth pursuit in primates. The flocculus still subserves the calibration of the vestibulo-ocular reflex, as it does in lower mammals; the ventral paraflocculus mediates smooth pursuit (Voogd *et al.*, 1987; Nagao, 1992; Rambold *et al.*, 2002). It exemplifies how a preserved, anatomical configuration can be used for another purpose.

There are limited data on variations in the zonal pattern between species, and our knowledge on the connectivity of sets of Purkinje cell zones and their target nuclei suggests that the combined A, B, C1, C3, and Y zones show little variation among species. The main differences concern the flocculus and the C2, D1, and D2 zones and their afferent and efferent connections. The functions of these zones and the kind of adaptations provided by their variations in width and length, however, remain largely unknown.

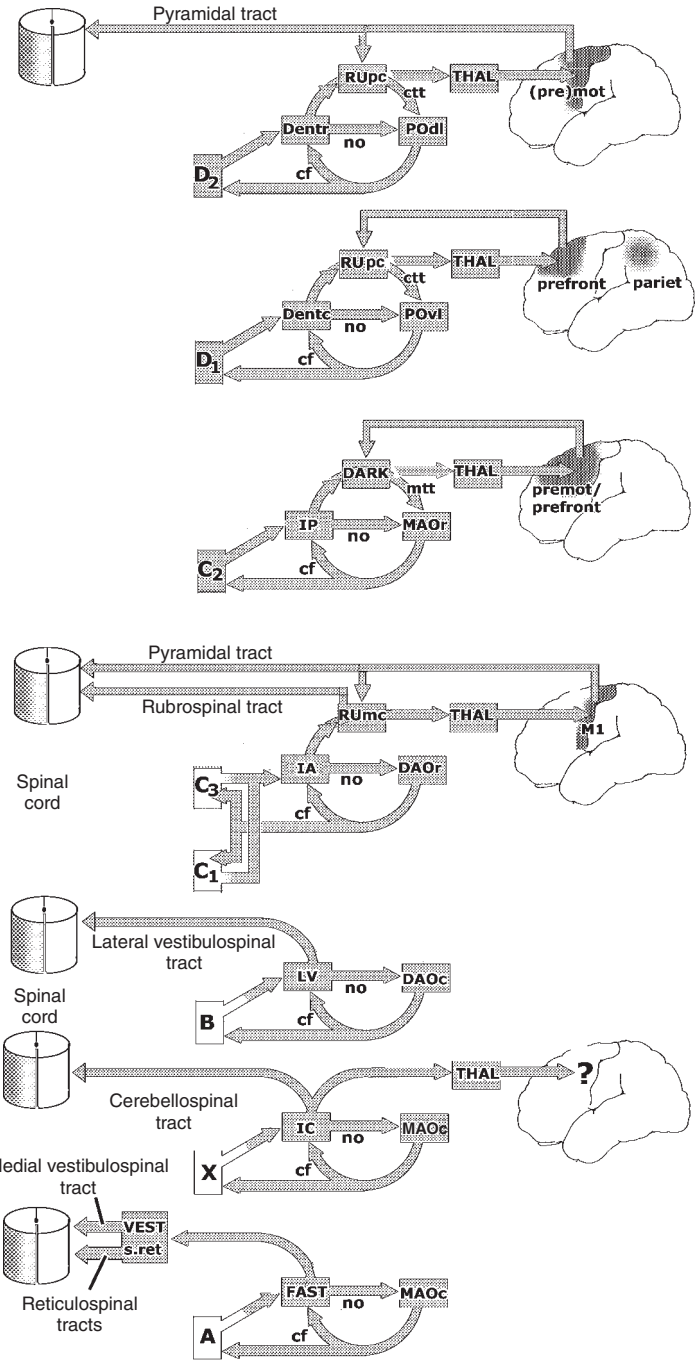

Figure 14 Diagrams of brainstem, thalamic, and cortical connections of the cerebellar Purkinje cell zones. The CX, A2, and Y zones are not included. All zones and their cerebellar and vestibular target nuclei are reciprocally connected with the inferior olive through a GABAergic nucleo-olivary pathway (no) and the climbing fiber projections with their collateral projections to the nuclei (cf). One set of zones (A, X, B, C1, C3, and D2) is connected with the spinal cord, through brainstem-spinal and/or corticospinal pathways. The D1, D2, and C2 zones and their target nuclei give rise to mesencephalo-olivary reciprocal circuits. These circuits are topically organized and include a relay in the parvocellular red nucleus (RUpc) or Darkschewitsch (DARK) nucleus at the mesodiencephalic junction. Similar reciprocal circuits do not exist for the other zones and their target nuclei. The D1, D2, and C2 zones, in addition, project to the cerebral cortex through the thalamus. For the D2 zone, these projections include the motor and premotor cortex; for the D1 zone they include the frontal eye field and prefrontal and parietal areas (see Voogd, 2004a, 2004b). (pre)mot, (pre)motor area; CA X, zones A X; cf, climbing fibers; ctt, central tegmental tract; DAOc/r, caudal/rostral part of the dorsal accessory olive; DARK, Darkschewitsch nucleus; Dentc, caudal dentate nucleus; Dentr, rostral dentate nucleus; FAST, fastigial nucleus; IA, anterior interposed nucleus; IP, posterior interposed nucleus; LV, lateral vestibular nuclues; M1, primary motor cortex; MAOc/r, caudal/rostral part of the medial accessory olive; mtt, medial tegmental tract; no, nucleo-olivary pathway; POdl, dorsal lamina of the principal olive; POvl, ventral lamina of the principal olive; prefront, prefrontal cortex; RUpc, parvocellular red nucleus; RUmc, magnocellular red nucleus; s.ret, reticular formation, THAL, thalamus; VEST, vestibular nuclei.

27.5.3 Mossy Fiber Afferents to the Cerebellar Cortex

The main afferent system of the cerebellum are the mossy fibers. The distribution of the mossy fibers differs from that of the climbing fibers. Mossy fibers generally distribute bilaterally, the mossy parent fibers collateralize into multiple longitudinal aggregates, and mossy fiber systems generally distribute to certain cerebellar lobules only (Wu *et al.*, 1999). Some mossy fiber systems contribute collaterals to the cerebellar nuclei on both sides. The multiple longitudinal aggregates of mossy fiber terminals are topographically related to the longitudinal pattern of Purkinje cell zones and their climbing fiber afferents, but our knowledge on this subject is still far from complete (Voogd, 2004a, 2004b).

Mossy fibers originate both from intrinsic and extrinsic sources. Collaterals of the axons of the cells of the cerebellar nuclei and the axons of a recently discovered cell type of the granular layer, the unipolar brush cell (Dino *et al.*, 2000), terminate as mossy fibers.

By far the largest single extrinsic source of mossy fiber afferents for humans, primates, and many other species is the pontine nuclei. Mossy fibers also originate from sensory relay nuclei, and as collateral systems from interneuronal pools of the spinal cord and the brainstem (the spino- and trigeminocerebellar tracts), certain reticular nuclei (the lateral, paramedian, and reticular tegmental nuclei), the vestibular nuclei, and the adjacent perihypoglossal nucleus (with its subsidiaries). Spino-, vestibulo-, and reticulocerebellar connections are constant among most mammalian species, although variations related to the prevalence of trigeminal over spinal connections in marsupials and rodents, as compared to carnivores, ungulates, and especially primates may also be expressed in the cerebellum (Voogd *et al.*, 1998). These mossy fiber systems terminate preferentially in anterior and posterior regions of the cerebellum, in relation to the A, B, C1, C3, and the Y zones, which, as pointed out above, constitute the stable backbone of the mammalian cerebellum.

On a higher resolution, a fine-grained microzonal topography is present in both the climbing and mossy fiber systems projecting to these zones, which subserve the local interaction of both afferent systems, believed to be at the core of cerebellar functioning (Ekerot and Larson, 1973; Garwicz *et al.*, 1998; Brown and Bower, 2001; Serapide *et al.*, 2001; Voogd *et al.*, 2003).

The pontocerebellar system is the final link in the main cerebrocerebellar pathway, although from a comparative anatomical point of view, it may have arisen as a tectocerebellar connection. In birds, two small medial and lateral pontine nuclei receive their afferents from the tectum and project to middle lobules (corresponding to lobule VII) and the caudal cerebellum (lobule IX, the uvula), regions that also receive an input from the pons in mammals (Freedman *et al.*, 1975). The tectopontine projection is preserved in mammals, but, in addition, the mammalian pontine nuclei now receive profuse projections from the cerebral cortex (Figure 15) (Münzer and Wiener, 1902; Mower *et al.*, 1979; Hartmann-von

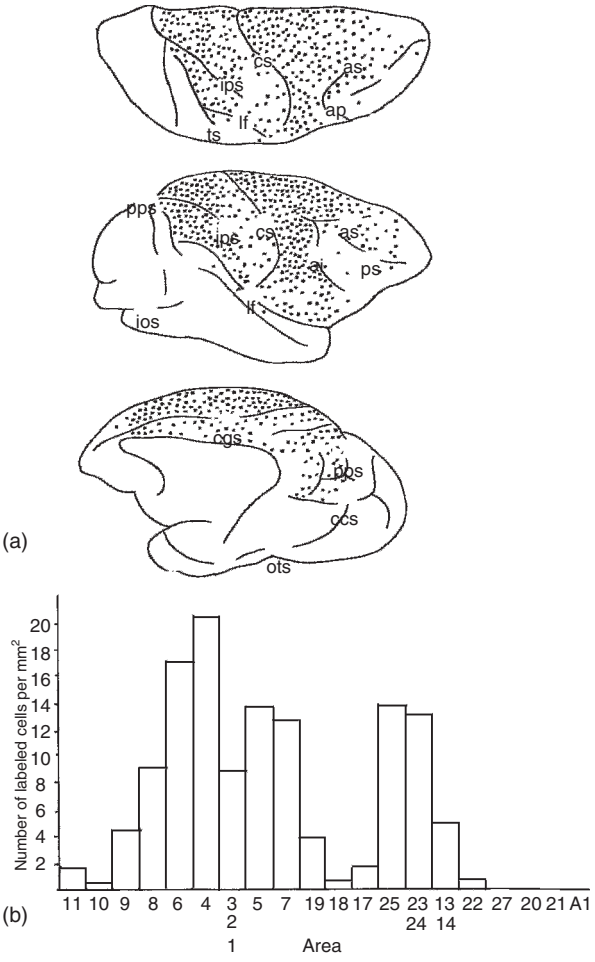

Figure 15 Distribution of corticopontine neurons in macaque monkeys. a, Distribution of retrogradely labeled neurons in the left cerebral hemisphere from a complete wheat germ agglutinin-coupled horseradish peroxidase filling of the pontine nuclei. b, Bar graphs illustrating the numbers of retrogradely labeled neurons in different cortical areas. ai, arcuate sulcus, inferior branch; as, arcuate sulcus, superior limb; ap, arcuate sulcus, inferior limb; ccs, calcarine fissure; cgs, cingulate sulcus cs, central sulcus; ios, inferior occipital sulcus; ips, intraparietal sulcus; lf, lateral (Sylvian) fissure; ots, occipitotemporal sulcus; pps, postparietal sulcus; ps, principal sulcus; ts, superior temporal sulcus. From Glickstein, M., May, J., and Mercier, B. 1985. Corticopontine projection in the macaque: The distribution of labelled cortical cells after large infection of horseradish peroxidase in the pontine nuclei. *J. Comp. Neurol.* 349, 51 72.

Monakow *et al.*, 1981; Glickstein *et al.*, 1980, 1985). These projections fall into two groups. One group, originating from the primary motor, the premotor, and the primary sensory cortices, contains collateral projection from the pyramidal tract (Ugolini and Kuypers, 1986). The second, originating from prestriate, posterior parietal, and prefrontal areas, may be considered in part as a collateral projection from the corticotectal system (Baker *et al.*, 1983; Keizer *et al.*, 1987). The largest evolutionary changes in mossy fiber connections occur in the second corticopontine cerebellar system. The pyramidal collateral system may subserve the coordination of skilled movements. The corticotectal collateral system probably is involved in the execution of sensory guided movements. The prominence of visual association and visuomotor areas of the cerebral cortex in the primate corticopontine projection indicates the importance of vision in the evolution of the primate cerebellum.

Pontocerebellar mossy fibers probably distribute to all cerebellar lobules, with the exception of the nodulus and the flocculus (Voogd, 1967; Kawamura and Hashikawa, 1981; Gerrits and Voogd, 1982, 1986; Glickstein *et al.*, 1994). A longitudinal zonal pattern in their termination was recently described by Serapide *et al.* (2001). In the anterior lobe and the lobulus simplex, they project to the apical and lateral portions of the folia, covering the spino-, reticulo-, and vestibulocerebellar mossy fibers, which terminate in more basal regions of the granular layer. A similar pattern may be present in the pyramis (lobule VIII) and the adjacent paramedian lobule. The apical coverage of pontocerebellar mossy fibers is much thicker in primates, where spino- and reticulocerebellar mossy fibers may never reach the cerebellar surface. Pontocerebellar mossy fibers terminate in lobule VII of the vermis and the uvula (lobule IX), in the ansiform lobule, and densely in the paraflocculus, the regions that display most variations in their folial pattern.

27.6 Embryological Origin of the Cerebellum

27.6.1 Determination and Origin of the Cerebellar Primordium

The past decade has seen rapid growth in our understanding of the molecular and cellular mechanisms underlying the embryological origins of the cerebellum. This has been fueled mainly by a great increase in knowledge on the mechanisms of action of key developmental control genes that sculpt the body plan in a large spectrum of animals ranging from insects to mammals.

In spite of the enormous species diversity with respect to size, foliation, and mediolateral patterning of the cerebellum, the following basic embryological scheme likely applies to all vertebrates. The cerebellum is derived from an embryonic territory at the junction of the developing midbrain and hindbrain, the so-called mid–hindbrain boundary, or MHB. Chick/quail transplantation experiments have revealed an organizing influence of a band of cells at the MHB called the isthmus (Marin and Puelles, 1994; Crossley *et al.*, 1996; Hidalgo-Sanchez *et al.*, 1999; reviewed in Joyner *et al.*, 2000). This region directs the formation of the midbrain, cerebellum, and anterior hindbrain and can induce midbrain and cerebellum tissue when placed ectopically. Genetic analysis in mice has shown that the position of the isthmic organizer is fixed at the boundary between the domains of expression of two homeodomain transcription factors, Otx2 and Gbx2 (Millet *et al.*, 1999; Broccoli *et al.*, 1999; reviewed in Joyner *et al.*, 2000). Otx2 is typically expressed throughout the embryonic forebrain and midbrain with a caudal border at the MHB; Gbx2 is expressed in the anterior hindbrain with a rostral limit at the MHB (Figure 16). Each of these factors mutually excludes the other from its territory. Using transgenic methods to artificially expand either expression territory results in malpositioning of the MHB and a variety of effects on development of the midbrain and cerebellum.

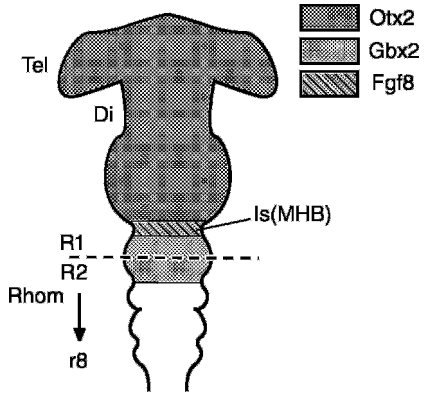

Figure 16 Position of the mid hindbrain boundary (MHB) is genetically determined. Mutually exclusive domains of expression of Otx2 and Gbx2 define the position of the MHB and isthmus region of the developing vertebrate embryo. The organizer activity of the isthmus leads to the specification of cell fates in the midbrain, cerebellum, and rostral hindbrain. The major brain vesicles are indicated. Tel, telencephalon; Di, diencephalon; Mes, mesencephalon; Rhom, rhombencephalon; Is, isthmus. Shadings correspond to expression domains of developmental control genes. Redrawn and adapted from Joyner, A. L., Liu, A., and Millet, S. 2000. Otx2, Gbx2, and Fgf8 interact to position and maintain a mid hindbrain organizer. *Curr. Opin. Cell Biol.* 12, 736 741.

All vertebrates, from fish to birds to mammals, have a similar embryonic pattern of brain vesicles that include the mesencephalon (midbrain) and rhombencephalon, the latter of which is made up of two secondary brain vesicles classically called metencephalon (cerebellum) and myelencephalon (hindbrain). The embryonic hindbrain is subdivided into eight transient swellings called rhombomeres, labeled R1 (the most rostral; really equivalent to the metencephalon) through R8 (Keynes and Lumsden, 1990; Lumsden and Krumlauf, 1996) (or R0–R7 in zebra fish; see Moens and Prince, 2002) (Figure 16). The cerebellum develops from the rostral rhombic lip, which corresponds with the part of the rhombic lip in R1. The rhombic lip is the dorsal rim of the alar plate, which gives attachment to the thin roof plate of the fourth ventricle. The border between the rostral and caudal rhombic lip is located at the greatest width of the fourth ventricle, between R1 and R2 (Figure 17). While expression boundaries of Hox and other control genes coincide with transient morphological constrictions between rhombomeres in many vertebrates, and genetic analysis indicates an intrinsic program controlling the segmental appearance of the hindbrain, there is no evidence of rhombomeric boundaries in the mature hindbrain. Thus, the major brain vesicles, the isthmic organizer, and rhombomeres are transient lineage restriction compartments for the generation of cellular diversity required for mature brain function (Fraser *et al.*, 1990; Keynes and Lumsden, 1990; Zervas *et al.*, 2004).

Several studies have examined the embryological origins of the cerebellum and its major afferent motor nuclei. For example, the inferior olive is generated from R8 and rostral spinal cord (Cambronero and Puelles, 2000), and the pontine nuclei are generated from R2–R8 (Marin and Puelles, 1994, 1995; Rodriguez and Dymecki, 2000). Although several studies using chick–quail chimera analysis indicate a contribution to the cerebellum from both the mesencephalon and metencephalon (or R1) (Martinez and Alvarado-Mallart, 1989; Hallonet and LeDouarin, 1993), this may be dependent upon the precise definition of the mes-/metencephalic boundaries. For example, another study in chick, using the marker Otx2 to define the caudal limit of the mesencephalon, indicated that the cerebellum is derived wholly from the metencephalon (or R1) (Millet *et al.*, 1996). A more recent study using an inducible fate-mapping technique in mice supports this view (Zervas *et al.*, 2004). This study suggests that lineage restriction boundaries restrict the intermingling of mes and met progenitor cells, and that the isthmus region (organizer), separating the mes and met, acts to further restrict any mixing of cells in these two neuromeres, in addition to influencing their distinct fates. Whether the cerebellum is derived wholly from R1 or not, the patterns of expression of key developmental control genes (En, Wnt, Otx, Gbx, Pax, Fgf8, etc.), which determine the positions of the isthmus and subsequent development of the midbrain and cerebellum, are highly conserved from fish to mammals. Thus, this highly conserved developmental program, activated in all vertebrates, guarantees the formation of the same basic brain substructures but allows for the expansion of features within these substructures required for species diversification.

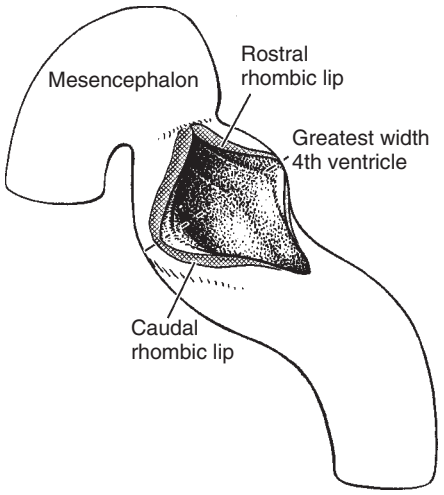

Figure 17 Diagram illustrating the position of the rostral and caudal rhombic lip.

Many of the genes that control specification of the cerebellar primordium are vertebrate orthologues of genes that control formation of the fruit fly body plan, such as Engrailed (En1, En2), Wingless (Wnt-1), Orthodenticle (Otx1, Otx2), etc. While no brain structure exists in the fruit fly that might remotely be considered a cerebellum, it is possible to trace the evolutionary origins of cerebellar development based on expression of these same genes in primitive chordates. In Amphioxus and Ascidians, for example, there is a single Otx gene expressed in the head region with a sharp posterior boundary of expression reminiscent of that in vertebrates (Williams and Holland, 1998). It is possible that the Otx territory in these primitive chordates is functionally homologous to the forebrain and midbrain in vertebrates. As in vertebrates, the rostral-most boundary of Hox gene expression in these species is posterior to the caudal boundary of Otx expression (Figure 18). While not yet identified in Amphioxus, Pax gene

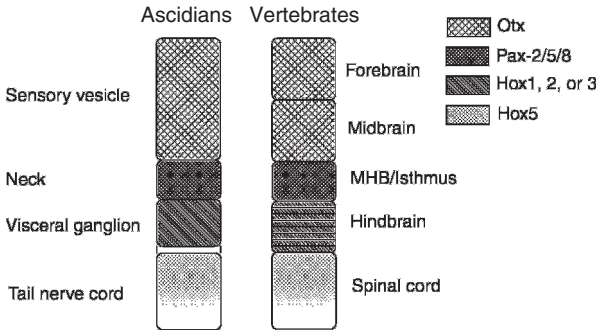

Figure 18 Comparison of the ancestral chordate neural tube structure to that of vertebrates. Homologous functional domains in the neural tubes of ascidians and vertebrates are hypothesized based on the observed expression domains of orthologous developmental control genes. The MHB of ascidians does not give rise to a cerebellum, and therefore new functions are acquired by this domain in vertebrates. Similarly, modification of these functions and the acquisition of new ones are likely to explain evolutionary changes within the vertebrate cerebellum. The subdivision of the rostral neural tube into forebrain and midbrain is hypothesized to be a novelty of vertebrates due to novel rostral expression domains of developmental control genes such as Dmbx 1 that emerge in the vertebrates (not shown). Di, diencephalon; Is, isthmus; Mes, mesencephalon; MHB, midbrain hindbrain boundary; r1/8, rhombomere 1/8; Rhom, rhombencephalon; Tel, telencephalon. Redrawn and excerpted from Takahashi and Holland (2004).

expression in Ascidian embryos is wedged in between the Otx and Hox domains. Therefore, it is possible, but by no means proven, that this region could be homologous to the MHB of vertebrates, but without the capacity to generate a cerebellum.

This is analogous to the observation that these primitive chordates, which do not have a neural crest, nonetheless show expression of crest regulatory genes in the lateral plate (e.g., Snail; Langeland *et al.*, 1998; Erives *et al.*, 1998; for review, Shimeld and Holland, 2000). Thus, the Pax-positive MHB cells and the Snail-positive lateral plate cells of primitive chordates may be the evolutionary progenitors of cerebellum and neural crest, respectively.

Primitive basal vertebrates such as lampreys have a neural crest and a primordial cerebellum with primitive Purkinje cells (Larsell, 1967). So far, in the limited number of cases where it has been examined, expression of known Purkinje cell markers such as Zebrin II cannot be detected in these primitive cells (Lannoo and Hawkes, 1997). The failure to detect these cell markers is most likely due to species divergence of the specific biochemistry of Purkinje cells or of marker protein structure, rendering them undetected by antisera generated to vertebrate proteins. Nevertheless, all other vertebrate species that have been examined, from fish to humans, express most of the classic Purkinje cell markers, many of which deal with Ca^{2+} metabolism (calbindin, parvalbumin, IP3 receptor type 1, etc.).

27.6.2 The Genetics of Cerebellar Morphogenesis and Zone Formation

As described above, there is a great deal of evolutionary conservation of the cerebellar zonal pattern among vertebrate species. Studies from multiple avenues have converged to suggest that the division of the cerebellum into zones occurs by mechanisms that are intrinsic to that tissue and genetically encoded. If this is the case, then it is likely that functional differences among the species could be added by slight modifications of the genetic program underlying this pattern, resulting in expansions or contractions of zonal dimensions and/or cell numbers as the need arises. So far, however, the precise mechanisms defining zonal boundaries within the cerebellum remain elusive. Nevertheless, based on what is known, development of zones in the cerebellum can be viewed as a special case of the general process whereby developmental fields are parcelled into progressively narrower functional domains.

27.6.3 Fate Mapping and Clonal Analysis

Early in its development, the rostral rhombic lip changes in position from a mainly rostrocaudal orientation to a mediolateral one (Hochstetter, 1929). This change in position may be caused by mechanical factors and is related to the development of the pontine flexure and/or differential growth within the cerebellar primordium. More recently Mathis *et al.* (1997) and Sgaier *et al.* (2005) drew attention to this change in position in their interpretations of their fate maps of the cerebellar anlage and suggested that rostral rhombic lip ultimately corresponds to the medial cerebellum and posterior rhombic lip to the lateral cerebellum (Figure 19).

Fate maps of the matrix of the rhombic lip have a spatial and a temporal aspect. Studies of the development of the cerebellum have shown that its neurons are derived from the ventricular matrix of the rhombic lip in a definite sequence (Altmann and Bayer, 1978, 1985). The earliest are the nuclear cells, followed by the Purkinje cells and the Golgi cells. Interneurons of the molecular layer are generated by cells derived from the ventricular matrix, which divide in the white matter during late stages of cerebellar development (Zhang and Goldman, 1996). The cells of the external granular layer (EGL), the secondary matrix that produces the granule cells, are derived from the dorsal margin of the rhombic lip (from the URL, or upper rhombic lip or

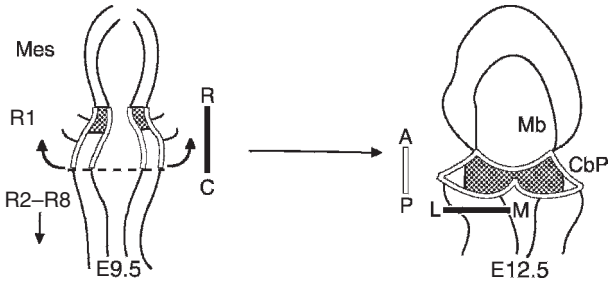

Figure 19 Orthogonal rotation of the A P axis of R1 gives rise to the mediolateral orientation of cerebellar zones. Growth of the brain and cerebellar primordium in mouse between E9.5 and E12.5 results in a 90° rotation of the longitudinal axis of the rhombic lip. The darkly shaded region indicates a zone of cerebellar cell clones marked at E7.5 using an En2-promoter-based genetic fate mapping strategy. The open contour line indicates the rhombic lip and the cerebellar primordium. Note that the boundaries of the marked region change from a primarily anterior posterior (AP) orientation to a mediolateral (ML) one. Mes, mesencephalon; Mb, midbrain; CbP, cerebellar primordium; R and C are rostral and caudal, respectively; A and P refer to anteroposterior axis; M and L refer to mediolateral axis. Adapted from Sgaier, S. K., Millet, S., Villanueva, M. P., Berenshteyn, F., Song, C., and Joyner, A. L. 2005. Morphogenetic and cellular movements that shape the mouse cerebellum; insights from genetic fate mapping. *Neuron* 45, 27 40, with minor modifications.

the germinal trigone) and subsequently migrate over the outer surface of the cerebellum.

Clonal analysis of cerebellar cells using a variety of techniques reveals a number of interesting relationships between cerebellar progenitors and both the determination of specific cerebellar cell types as well as their spatial organization. All of these studies have concluded that granule cells constitute a distinct lineage from all other cerebellar cell types, which is consistent with their initial origin from a spatially separate germinative neuroepithelium. In fact, mutation of the gene *Math1*, a molecular determinant of this unique lineage, results in a complete loss of this germinal region and thus granule cells, with no effect on the specification of Purkinje cells and deep nuclear neurons (Ben-Arie *et al.*, 1997). In contrast, in a study using replication defective retroviruses in chick, it was found that Purkinje cells and Bergman glia frequently occupied the same clone (Lin and Cepko, 1999). This study could find no strong evidence for a clonal relationship among the ventricular matrix-derived neurons. In another study designed only to detect relationships between neurons, however, Purkinje cells, deep nuclear neurons, Golgi neurons, and molecular layer interneurons in mice were often found to occupy the same clones (Mathis *et al.*, 1997). Thus, a picture emerges in which a common self-renewing progenitor in the ventricular matrix gives rise to most cerebellar cells except granule cells, and it does so asymmetrically and sequentially according to the known birth dates of these neurons. However, this view is now in need of some updating due to recent transgenic fate mapping studies. It is currently thought that large glutamatergic deep nuclear neurons, in addition to granule cells, are generated from the Math1-positive upper rhombic lip. Purkinje cells, inhibitory interneurons, and small inhibitory deep nuclear neurons are generated from a Ptf1a-positive ventricular zone immediately subjacent to the Math1 domain (Hoshino *et al.*, 2005; Machold and Fishell, 2005; Wang *et al.*, 2005).

One other feature of these clonal analyses is that clones were typically found to spread nearly the entire rostralcaudal dimension of the cerebellum, but were restricted in their spread along the mediolateral axis. No clones in any study were ever found to cross the midline, even in cases where clones spread rostrally into the midbrain or caudally into the hindbrain. In these cases, such large clones never occupied an entire hemicerebellum. Rather, these early labeled clones completely filled either a median territory (extending from the midline to roughly the edge of the vermis) or a lateral territory, but never both. Both territories extended the full rostralcaudal dimension of the cerebellum (Mathis *et al.*, 1997). The size of the median and lateral territories occupied by an intermediate-size clone varied with the actual clone size, and no fixed boundaries were observed that were respected by all or even several clones, except for the midline. Smaller clones cover less total space, but are similar in that they are oriented like zones, with distinct mediolateral boundaries but fanned out rostrally and caudally. When examined, no obvious relationship has been found to exist between these mediolateral clonal boundaries and boundaries defined by zonal markers, such as Engrailed, Eph's, and Gli, leading to the conclusion that individual cerebellar zones are not lineage restriction compartments (Lin and Cepko, 1999). Nevertheless, clones are always very limited in their spread along the mediolateral direction.

The finding of two precursor pools, a median one and a lateral one in each hemicerebellum, and the relative restriction of clone expansion in the mediolateral direction, may be consistent with what was observed using an inducible fate mapping technique in mouse (Sgaier *et al.*, 2005). This technique made use of the endogenous Engrailed promoters to inducibly and permanently label distinct territories of cerebellar precursors starting from embryonic day 7.5(E7.5) or later. Rather broad territories could be labeled (with lacZ) by this method, extending from the midline to variable lateral positions that depended upon the time of induction and the

promoter used. When the fate of cells that were labeled as early as E9.5 was determined by examination of lacZ expression in mature cerebellum, the mediolateral positions of cells at the time of their labeling were found to be unchanged in the adult. This is consistent with other studies that have observed gene expression in cerebellar cell clusters at E15 that are remarkably akin to the zonal pattern of expression of the same gene in adults (Oberdick *et al.*, 1993; Ozol *et al.*, 1999). From these studies, it seems likely that the rhombic lip is primarily divided by broad patterns of developmental gene expression rather than by lineage restriction, and the overlapping patterns of many such genes may result in embryonic zones with distinct cell fates whose positions are roughly maintained until adulthood. Time of birth of the cells may also play a role in the development of zonal patterns in the distribution of cortical neurons (see below).

It is formally possible that a transient lineage restriction boundary exists between the two clone pools within the rhombic lip, generating a medial and a lateral set of precursor cells, and that zone refinement occurs subsequent to this by overlapping patterns of expression of developmental control genes. At any rate, the rotation of a mostly rostrocaudally oriented pattern in the rhombic lip to one oriented mediolaterally during the period from E9.5 to E11.5 in mouse is consistent with many studies, as indicated above.

27.6.4 Late Embryonic Patterning and Cerebellar Morphogenesis

When Purkinje cells migrate toward the surface of the cerebellar anlage, they collect in a number of mediolaterally arranged clusters (Figures 10 and 20) (Korneliussen, 1967, 1968a, 1986b; Kappel, 1981; Feirabend *et al.*, 1985; Feirabend, 1990). Similar observations on the clustering of the Purkinje cells were made in studies using Purkinje cell-specific markers (Wassef and Sotelo, 1984; Smeyne *et al.*, 1991). Cell strands and/or fiber streams associate Purkinje cells of different clusters with different cerebellar nuclei. These connections of Purkinje cells are obvious even at these early stages, before synaptic connections have developed. The proliferation of enormous numbers of granule cells by the EGL leads to a great increase in the external surface area of the cerebellum and its folding into lobules and folia, most prominently in the rostrocaudal direction. This increase in the surface area causes the spreading out of the Purkinje cells of the clusters into a monolayer. The Purkinje cell clusters have been considered as the primordia of the adult Purkinje cell zones, and this is supported by studying their temporal morphogenesis through the use of zonal markers, as described above (Oberdick *et al.*, 1993; Ozol *et al.*, 1999).

Timing of Purkinje cell production by the ventricular matrix can be further subdivided into several successive stages, each of which gives rise to distinct clusters of Purkinje cells. Both in birds (Feirabend *et al.*, 1985; Feirabend, 1990; Karam *et al.*, 2000) and mice (Hashimoto and Mikoshiba, 2004), Purkinje cell clusters that are born at different dates typically form interdigitating patterns. In mice, early- and late-born clusters are located both in the vermis and in the hemisphere (Figure 21).

Purkinje cell clusters differ in their expression of different polarity genes such as En-2 (Millen *et al.*, 1995; Lin and Cepko, 1998). In the avian cerebellum, moreover, Purkinje cell clusters that are born

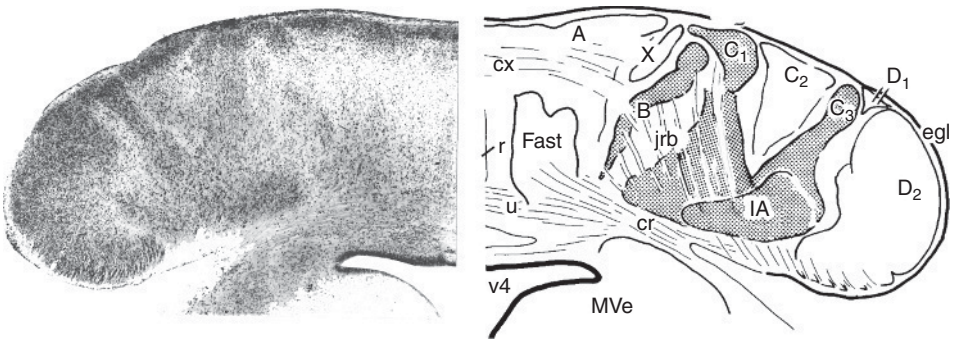

Figure 20 Coronal section through the cerebellum of a 55-day rhesus monkey fetus. Purkinje cell clusters are located at the surface of the cerebellum or are still located in a subcortical position. The cluster that will give rise to the future A zone is related to the fastigial nucleus. Cell strands connect the clusters C_1 and C_2 with their future target nucleus, the anterior interposed nucleus. The posterior interposed and dentate nuclei, the respective target nuclei of the C2 and the D zones are located at a different level. A D, Purkinje cell clusters A D; cr, restiform body; cx, cerebellar commissure; egl, external granular layer; Fast, fastigial nucleus; IA, anterior interposed nucleus; jrb, juxtarestiform body; r, midline recess; u, decussation of the uncinate tract; v4, fourth ventricle. From Kappel, R. 1981. The Development of the Cerebellum in *Macaca mulatta*. A Study of Regional Differentiation during Corticogenesis. PhD dissertation University of Leiden.

on different dates express different cell-adhesion molecules, such as the cadherins (Arndt *et al.*, 1998) and BEN (Chédotal, 1996, 1997), and repulsion molecules such as the ephrins and their receptors (Figure 22) (Karam *et al.*, 2000). The cadherins have been held responsible for the clustering of the Purkinje cells and the setting up of their corticonuclear projections (Arndt *et al.*, 1998; Luo *et al.*, 2004). The ephrins and BEN have been shown to be involved in the patterning of the olivocerebellar projection (Chédotal, 1996, 1997). The Engrailed gene products have been shown to be regulators of ephrin expression in other systems (Logan *et al.*, 1996). A sequence of events seems to emerge that determines the mediolateral zonal patterns in the distribution of the Purkinje cells and their connections. The fate maps of the rhombic lip, discussed in the previous section, and the temporal sequence in the production of the Purkinje cells of the different clusters form key features in their development. Further studies are needed to reconcile the spatial and temporal determinants of these zonal patterns.

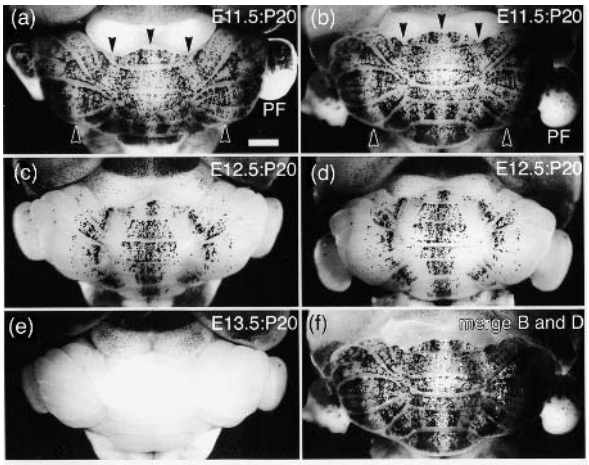

Figure 21 Comparison of the zonal localization of Purkinje cells with different birth dates. Purkinje cells are labeled with birth date-specific gene transfer of the adenoviral vector AdexCAG-NI-lacZ injected into the midbrain ventricles of embryos at E11.5 (a, b), E12.5 (c, d), and E13.5 (e). At P20, each manipulated brain was stained by whole-mount for beta-gal. Purkinje cells born at E11.5 and at 12.5 are distributed in complementary zonal patterns. In (f) panels b and d are superimposed. The arrowheads in a and b indicate beta-gal-negative clusters. PF, paraflocculus. Scale bar: 1 mm. Reproduced with permission from Hashimoto, M. and Mikoshiba, K. 2004. Mediolateral compartmentalization of the cerebellum is determined on the 'birth date' of Purkinje cells. *J. Neurosci.* 23, 11342 11351.

It seems likely that some of the genes that control the very formation of the cerebellar primordium also play a role in the establishment and late embryonic refinement of cerebellar zones. En1, En2, Wnt1, Pax5, etc., are all known to be expressed in a zonal pattern during late embryogenesis of the mouse cerebellum (Millen *et al.*, 1995). That En and Wg (the fly orthologue of vertebrate Wnt genes) participate in establishing boundaries between fruit fly body segments and En/Wnt signaling is conserved in mice (Danielsen and McMahon, 1996) add fuel to the notion that these genes play a role in zone formation. So far, however, proof of a direct role has not been forthcoming. Null mutation of most of these genes results in deletion of the entire cerebellar primordium due to their very early action (McMahon and Bradley, 1990; Wurst *et al.*, 1994), and therefore effects on zones cannot be studied. However, ectopic overexpression of En-2 in mouse cerebellum starting from E15 has a mixed effect. It has no effect on the adult pattern of zones revealed by L7BG3 expression, a lacZ reporter gene driven by a truncated version of the Pcp-2(L7) promoter, but

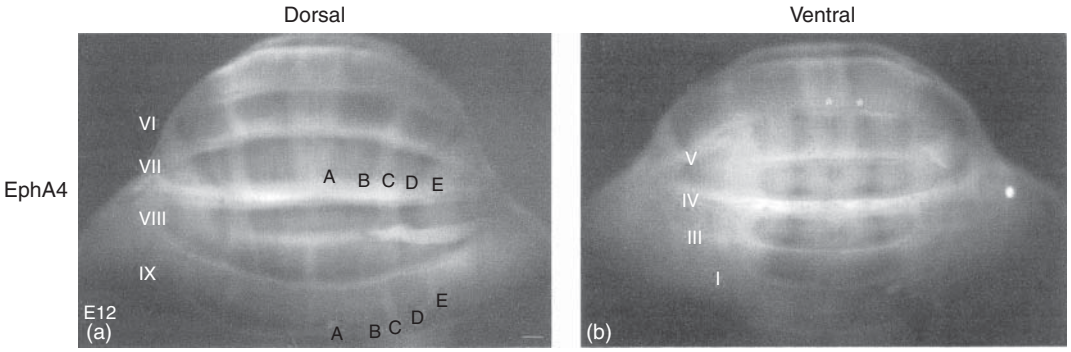

Figure 22 Dorsal and ventral views of EphA4 receptor localization (a, b) in whole-mount immunostaining on chick cerebella taken between stages 36 (E1O) and 38 (E12). Roman numerals refer to cerebellar lobules. EphA4-positive Purkinje cells in bands B and D are born early, EphA4-negative Purkinje cells in bands C and F are born late. The localization of Cadherin 6B (Arndt *et al.*, 1998) corresponds with the localization of EphA4. Reproduced with permission from Karam, S. D., Burrows, R. C., Logan, C., Koblar, S., Pasquale, E. B., and Bothwell, M. 2000. Eph receptors and ephrins in the developing chick cerebellum: Relationship to sagittal patterning and granule cell migration. *J. Neurosci.* 17, 6488 6500.

it has a severe effect on the pattern of stripes revealed by Zebrin II expression (Baader *et al.*, 1999). In addition, it results in an effect on the zonal organization of mossy fiber afferents, which could be explained by disruption of the expression of guidance molecules such as ephrin.

The development of the folial pattern of the cerebellum is closely related to the proliferation and migration of the granule cells in the EGL. An important aspect of transversely migrating EGL cells to the foliation of the cerebellum of the mouse was noticed by Sgaier *et al.* (2005). As previously revealed in chick, the transverse migration is mostly lateral to medial (Ryder and Cepko, 1994). However, one significant difference seems to be that in mouse there is a much greater flow of EGL cells from lateral regions in the posterior vermis (Sgaier *et al.*, 2005). This novel migratory trajectory was hypothesized to correlate with the expansion of the hemispheres in mammals.

The development and the patterning of the afferent climbing and mossy fiber connections were recently reviewed by Sotelo (2004). The inferior olive, the single source of the climbing fibers, and the lateral reticular, the external cuneate, the pontine tegmental reticular, and the pontine nuclei, all of which give rise to mossy fibers, are derived from the region of the caudal rhombic lip (R2–R8, as described above). Temporal and spatial sequences exist in their development from this region (Altman and Bayer, 1987a, 1987b, 1987c, 1987d). Neurons of the inferior olive and certain mossy fiber systems become specified as future mossy and climbing fibers at their birth. The molecular cues for pathfinding and translocation of the cell bodies to their definite positions have been extensively studied. Both mossy and climbing fibers enter the cerebellar anlage very early. Their transverse and longitudinal patterning occurs early, before synaptic connections are established, and, for the olivocerebellar climbing fiber projection at least, is related to the patterning of the Purkinje cells.

27.6.5 Conclusions on the Genetic Control of Cerebellar Development

1. Mutations in genes determining the production and differentiation of cells in the rostral and caudal rhombic lip, which are responsible for setting up the temporal and spatial gradients in the production of different cell types, expressing specific recognition and repulsion molecules, which would subserve the future patterning in their connections, are a possible substrate for natural selection.
2. The variations in the morphology of the mammalian cerebellum are mainly related to variations in length and the width of the C2, D1, and D3 Purkinje cell zones and their afferent climbing and efferent cerebellar nuclear connections in certain lobules of the cerebellum. Such variations are not observed in the zonal composition of the vestibulocerebellum, and the system of the A, C1, C3, and Y zones, which subserves the classical motor functions of the cerebellum. However, there are indications that these zones and their target nuclei can be integrated in newly developed systems, i.e., the development of the ventral paraflocculus in species with foveal vision. Generally, Purkinje cell zonation of the mammalian cerebellum is a highly conserved feature.
3. Mossy fiber systems, namely the corticopontocerebellar system, are subject to great variations among mammals. When the cerebellum is used for new or extended functions, adaptations in mossy fiber afferent systems may occur, which use the conserved, modular output system of the cortex for a new purpose.

27.7 The Functions of the Cerebellum

27.7.1 Historical Aspects

The earliest clues about functions of the cerebellum came from animal experiments. Rolando (1804) made lesions in the cerebellum of mammals and birds and found that the lesions impaired the animals' ability to move. Flourens (1824) agreed, but unlike damage to the spinal cord, the lesions do not abolish movement. Flourens agreed that the cerebellum is involved in the control of movement, but he argued that rather it is the 'coordination' of movement that is lost. Over the course of the nineteenth century, surgical technique improved, allowing more precise control of lesions and longer postoperative survival times. Luciani (1891) studied the long-term effects of cerebellar lesions in mammals. He believed that the observed lack of coordination caused by the lesion is best interpreted on the basis of more elemental deficits in muscle control. Luciani identified these deficits as asthenia, or muscular weakness, atonia, or loss of muscle tone, and astasia, the inability to fuse successive contractions, leading to a characteristic tremor.

Animal experiments strongly influenced the interpretation of the effects of cerebellar lesions in humans. Holmes (1917, 1939) studied the effects of cerebellar lesions on soldiers wounded in the First World War. Citing Luciani, Holmes interpreted most of the deficits in his patients as due to loss of the elementary functions of muscle tone, muscle strength, and loss of the continuity of movements. Babinski (1902) cited the experiments of

Flourens as the basis for his interpretations of the deficits caused by cerebellar lesions in humans. In addition to loss of coordination, Babinski added a characteristic symptom of cerebellar disease: the inability to execute rapid alternating movements, which he labeled adiadochokinesis.

All experimenters and clinicians agree that lesions of the cerebellum cause an impairment of movement. There is, however, no agreement on the underlying cause. Rodolfo Llinas and his colleagues (Llinas and Welsh, 1993; Welsh and Llinas, 1997) argue for a critical role for the inferior olivary nucleus and its efferent climbing fibers in the initiation and timing of voluntary movement. Bower (2002) and his colleagues (Parsons *et al.*, 1997) believe that the cerebellum is entirely a 'sensory' structure. According to this interpretation, the cerebellum receives sensory information that is used to predict the sensory consequences of a movement. Paulin (1993), who shares this view of the sensory role of the cerebellum, pointed out that the motor effects of lesions would be analogous to the effect on an automobile if its windshield were to be shattered. The car seems to perform poorly. Even though its motor, drive shaft, and wheels are intact, it is hard to steer. Thom Thach and his colleagues (Thach *et al.*, 1992) take a middle ground, arguing that the cerebellum serves as a sensory to motor coordinator. The parallel fibers are seen as allowing the coordination of movements among disparate body parts.

27.7.2 The Cerebellum and Plasticity

Humans and other mammals are capable of exquisitely precise control of movement. The nature of this control can best be studied in quantitative detail in eye movements. Saccades are rapid shifts of gaze that are characteristic of foveate animals. It has been estimated that humans execute as many saccades in a lifetime as they do heartbeats. In experiments with humans (McLaughlin, 1967) and monkeys (Straube *et al.*, 1997), it is clear that the saccades are highly accurate in finding a target. How is this accuracy maintained over a lifetime? If a human or monkey looks at a central fixation target and then makes a saccade to a target 15 degrees to the right or left, the saccade is typically made with great precision. If, when the eyes begin to move, the target is displaced by five degrees, the saccade is first made to the original position, and then a catch-up saccade brings the eyes to the new target position. Within a single session, the eyes now make a successively larger saccade. Saccadic adaptation requires the cerebellum (Barash *et al.*, 1999). After lesions restricted to lobule VII and caudal VI of the vermis, monkeys are completely unable to adapt to the altered target position. On average, saccades are made with some reduced accuracy to the presented target, but adaptation to the displaced target is no longer possible.

The failure of saccade adaptation reflects an important underlying function of the cerebellum. Each time a saccade is made, a measure of its accuracy is fed to lobule VII. Small errors due to perturbations such as fatigue are compensated and accuracy is restored.

Similar results show that the cerebellum is involved in other forms of motor calibration. The vestibulo-ocular reflex (VOR) is a mechanism whereby the stability of gaze can be maintained in the presence of head movements. As Melville-Jones and his colleagues have shown (Gonshor and Melvill-Jones, 1973; Melvill-Jones and Davies, 1976), as have Miles and his colleagues (Miles *et al.* 1980; Miles and Lisberger, 1981), the reflex can be modified by changing the direction or size of the image on the retina. The flocculus is an essential link in the long-term adaptation of the VOR. Paired Purkinje cell zones are able to adapt eye movements in the plane of the horizontal or the anterior semicircular canals, via the oculomotor neurons in the superior and medial vestibular nuclei (van der Steen *et al.*, 1994). The floccular zones also are a highly conserved system, present in mammals and birds alike (Voogd and Wylie, 2004). The circuit for smooth pursuit in monkeys includes the primary visual cortex, the middle temporal visual area (MT), and the frontal pursuit area in the arcuate cortex and converges upon the flocculus/ventral paraflocculus. Area MT extracts information about direction and speed of the target. The frontal pursuit area is concerned with the modulation of the visuomotor transmission for pursuit, but is dependent on feedback of the eye velocity command from the cerebellum or the brainstem for this task (Rambold *et al.*, 2002; Tanaka and Lisberger, 2002a, 2002b; Priebe *et al.*, 2003; Osborne *et al.*, 2004). The connections of the frontal pursuit area with the flocculus/ventral paraflocculus are not known, but may use the pontine nuclei (Leichnetz *et al.*, 1984; Glickstein *et al.*, 1985; Fries, 1990). The primate corticopontine system differs from mammals with nonfoveate vision in the presence of strong projections from parastriate and parietal areas belonging to the dorsal visual stream, including area MT. These visual corticopontine projections involve the rostral and lateral pontine nuclei, which project to the ventral and the adjacent dorsal paraflocculus. The primate ventral paraflocculus is an extension of the flocculus, using the same, conserved, zonally organized output system

(Voogd *et al.*, 1987). In lower mammals, it is represented by a single lobule (Gerrits and Voogd, 1982; Voogd and Barmack, 2005). The Purkinje cell zonation of the dorsal paraflocculus is quite different, and consists of the C2, D1, and D2 zones with an output through the posterior interposed and dentate nuclei. Visual corticopontine projections to the dorsal paraflocculus are already present in nonfoveate mammals (Burne *et al.*, 1981), and the output system of this lobule through the C2, D1, and D2 zones is the same in foveate and nonfoveate species. However, both the input and the output of the dorsal paraflocculus have differentiated and now include extensive areas of the parietotemporal and frontal association cortex, both as a source for the corticopontine mossy fiber input and as a target for the visual portions of the posterior interposed and dentate nuclei and, 'inter alia', as the origin of the corticomesodiencephalic principal olive climbing fiber paths to the C2, D1, and D2 zones (Voogd, 2003, 2004a). This differentiation in primates may provide the reciprocal pathways connecting the frontal pursuit area with the effective output through the flocculus/ventral paraflocculus.

27.7.3 Theories of Cerebellar Function

Some authors interpret the role of the cerebellum in the control of movement as being analogous to a problem of industrial control. Miall *et al.* (1993) propose that the cerebellum acts as a Smith predictor, taking their example from the field of industrial chemistry. In a typical arrangement, such as a petrochemical plant, there is a flow of material into a processor, which then acts on that material to produce an output. The output of the plant is monitored, but since online adjustments are too slow to correct for errors, the Smith predictor serves to compensate for the inherent delays. Rather than directly affecting the processor, there is a computer-based model of the processing system which controls production. The output of the plant is continuously monitored. Errors are fed into the model and corrections made. According to this view, the cerebellum is thought to contain an internal model of the motor system. Any deviations from an intended movement are fed into the cerebellum, which continuously compensates for errors in movement.

In addition to its obvious role in the control of movement, some have argued that the cerebellum, particularly the cerebellar hemispheres, plays a critical role in more complex functions, such as cognition, language, and emotion. Peter Strick and his colleagues (Dum and Strick, 2002; Strick, 2003) find the cerebellar hemispheres are preferentially connected by way of the lateral nuclei to the prefrontal cortex. This connection is interpreted as a closed loop; with the cerebral cortex accessing the cerebellar hemispheres and the cerebellar hemispheres projecting back to the same region of cerebral cortex by way of the lateral nuclei. They suggest that this loop plays a critical role in cognition and planning.

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28 Olfactory Cortex: Comparative Anatomy

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28.1 Introduction

The vertebrate olfactory system faces an enormous challenge. When one or a mixture of a seemingly limitless set of odorous molecules is encountered, the olfactory system must identify it, discriminate it from similar odors, recall information that has already been learned about the odor's significance, and incorporate any new information that might accompany the current presentation. Understanding the transfer of chemical information and the circuitry involved at the initial stages of processing yields some clues about how the olfactory system performs such tasks, but it is at the level of the olfactory cortex – complex, often multimodal sensory and association areas characterized by a high degree of convergence and interconnectedness – that odor information is integrated with other sensory cues, learned associations, and internal motivational states to generate a meaningful response to an odor.

This comparative review of the vertebrate olfactory cortical areas first examines what is known about the olfactory cortical areas in rodents, which have been the most well studied and widely used vertebrate experimental subjects in olfactory research. The structure and organization of the two major olfactory cortical areas, the piriform cortex and the anterior olfactory nucleus, are examined in detail, and the afferent, reciprocal, and efferent connections that these two areas have with other cortical structures are considered. Next, the structure and organization of the rodent olfactory cortex are discussed in relation to other mammalian and nonmammalian vertebrate species, and the article concludes with remarks about the potential function of the cortical areas in the vertebrate olfactory system.

28.2 What Is Olfactory Cortex?

In the broadest sense, olfactory cortex is defined as any cortical region that receives information from the olfactory system. The first structure that could be designated in such a way is the main olfactory bulb, which is the target for incoming information from the olfactory sensory neurons in the olfactory epithelium. Although the olfactory bulb is not usually regarded as a cortical structure, it has the features considered by neuroanatomists to characterize cortex: it is divisible into more than two tangential layers, the most superficial layer is a plexiform layer of incoming afferent fibers; it is connected by a network of neuronal processes that give rise to columnar functional units, and these functional units can interact with each other both within and across laminae. Regardless of whether it can be termed olfactory 'cortex,' the olfactory bulb is the first stage of olfactory information processing in the vertebrate central nervous system, and a great deal of synthesis and refinement of the olfactory signal occurs at this stage.

The two classes of principal cells in the olfactory bulb, the mitral and the tufted cells, project to a number of cortical structures, and these third-order

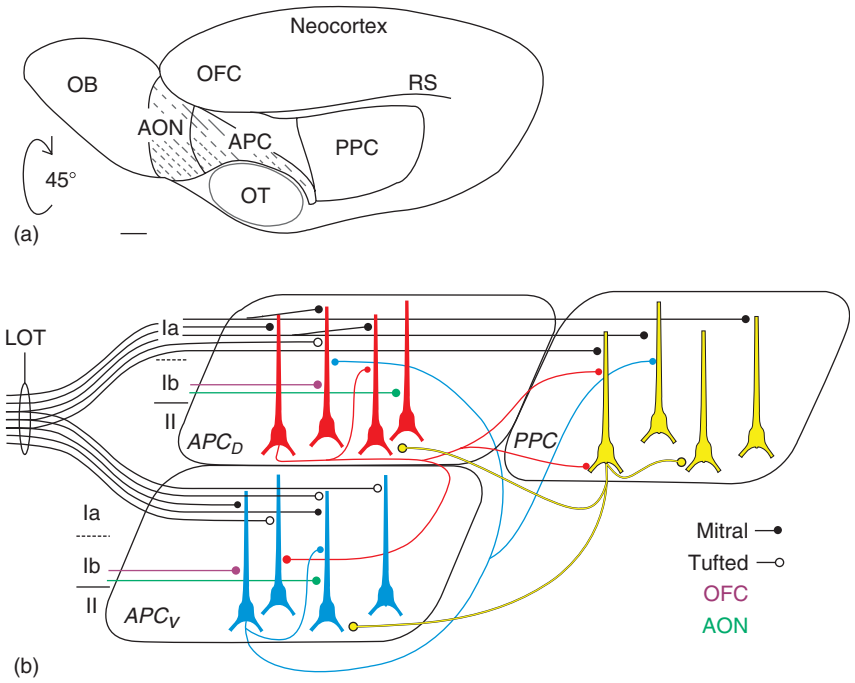

Figure 1 Schematic diagram showing the relative location and connectivity of olfactory cortical areas in the rodent. (a) Ventrolateral view of the rat brain, with the dorsal surface of the brain rotated 45° medially, showing the locations of the olfactory bulb (OB), anterior olfactory nucleus (AON), and the anterior (APC) and posterior (PPC) piriform cortices. The lateral olfactory tract (LOT) overlies the AON and a portion of the APC and is indicated by cross-hatching. Also shown are the orbitofrontal cortex (OFC) and olfactory tubercle (OT). The dorsal portion of the APC forms the ventromedial wall of the rhinal sulcus (RS). Scale bar 1 mm. (b) Circuitry of the piriform cortex. Axon collaterals from the mitral and tufted cells exit the LOT to synapse on the distalmost portion of pyramidal cell apical dendrites in layer Ia of the piriform cortex. Although a light input from tufted cells is shown in dorsal APC (APC_D), it has been suggested that ventral APC (APC_V) may be the only region of piriform cortex that receives input from tufted cells. Tufted cell axons do not project to PPC. Axons from the OFC and from the AON project proximal to the input from the OB in layer Ib of APC. The remainder of input to pyramidal cell dendrites in layer Ib is from associative projections from piriform cortex, often originating in the same subregion as the target cell (so-called 'autoassociative' input). The projection from PPC onto other piriform pyramidal cells is primarily to basal dendrites in layer III. Other inputs to layer III, for example from the amygdala and OFC, are not shown.

structures are traditionally designated olfactory cortex (**Figure 1**). The largest of these is the piriform cortex, which is often referred to as 'primary olfactory cortex,' although this designation has more to do with its size than its functional similarities to other primary sensory cortices. The piriform cortex is also designated the pyriform or prepiriform cortex. The second-largest cortical recipient of mitral and tufted cell fibers is the anterior olfactory nucleus (AON), a large structure (cortical in nature, despite its name) situated within the olfactory peduncle between the olfactory bulb and the piriform cortex. Although the AON has received relatively little experimental attention, it likely plays a central role in processing olfactory information. These two cortical structures will be the focus of this review.

There are other cortical areas that receive direct input from the olfactory bulb, including the olfactory tubercle, lateral entorhinal cortex, prefrontal cortex, and cortical areas associated with the amygdala, although the direct olfactory bulb input to these areas often is nominal. Many of these areas also have reciprocal projections with the piriform cortex and the AON and can thus be designated, along with perirhinal, perihippocampal, and orbitofrontal cortices, as members of higher-order olfactory or multimodal cortical circuits.

28.3 Rodent Piriform Cortex

28.3.1 Organization

The piriform cortex is the largest cortical recipient of direct olfactory bulb projections, and it is also a very prominent part of the rodent brain, accounting for up to 10% of cortical volume in some species. Although it is generally agreed that the area is involved in higher-order processing of olfactory information, its function appears to be complex, and is not altogether well understood. Unlike 'primary' cortical structures in other sensory systems that are devoted to processing a single type of sensory information, the piriform cortex has a highly autoassociative architecture, with an extensive

network of interconnections within and among its various subregions. Moreover, direct afferent fibers from the olfactory bulb make up only a small fraction of the input that neurons in piriform cortex receive on their dendritic tree; all other input to piriform cortex is from other neurons within piriform cortex, from higher-order areas such as orbitofrontal cortex or from structures that are most often associated with other systems or sensory modalities, such as entorhinal cortex and the amygdala.

A second feature of the rodent piriform cortex is its discrete laminar organization perpendicular to the cortical surface (**Figures 1** and **2**). Piriform cortex is three-layered paleocortex. The most superficial layer (layer I) is a highly ordered plexiform layer consisting of two parts: layer Ia, a superficial sublayer that includes axons from the mitral and tufted cells in the olfactory bulb, and a deeper layer, Ib, which consists of associative axons from other neurons in piriform cortex, from other ipsilateral cortical structures such as the AON, and from commissural axons from contralateral olfactory cortical neurons (**Figure 1**). The next deepest layer (layer II) is a compact cell body layer that houses the majority of the principal cells within piriform cortex. layer IIa is a superficial sublamina that houses a specialized population of pyramidal-type cells that lack basal dendrites, termed semilunar cells. The deeper layer, IIb, contains the cell bodies of superficial pyramidal cells and of small multipolar cells (**Figure 1**). Deep to layer II is layer III, which contains cell bodies of deep pyramidal cells, two populations of multipolar cells, and a large number of associational fibers, both from within piriform cortex and from other brain structures.

Deep to layer III is the endopiriform nucleus, which has also been termed layer IV of piriform cortex. This structure is dominated by multipolar neurons, and although it is interconnected with overlying piriform cortex, it does not receive direct input from the olfactory bulb, and its role in olfactory information processing has not been investigated directly.

As in most other cortical structures, the principal neurons are glutamatergic pyramidal cells, and γ-aminobutyric acid (GABA)ergic interneurons are distributed throughout the cortex. Further, the piriform cortex is the target of a variety of neuromodulators, including acetylcholine from the horizontal limb of the diagonal band of Broca, norepinephrine from the locus coeruleus, and serotonin from the raphe nucleus. These neuromodulatory inputs heavily terminate in layers Ib, II, and III, targeting both dendritic and axonal elements.

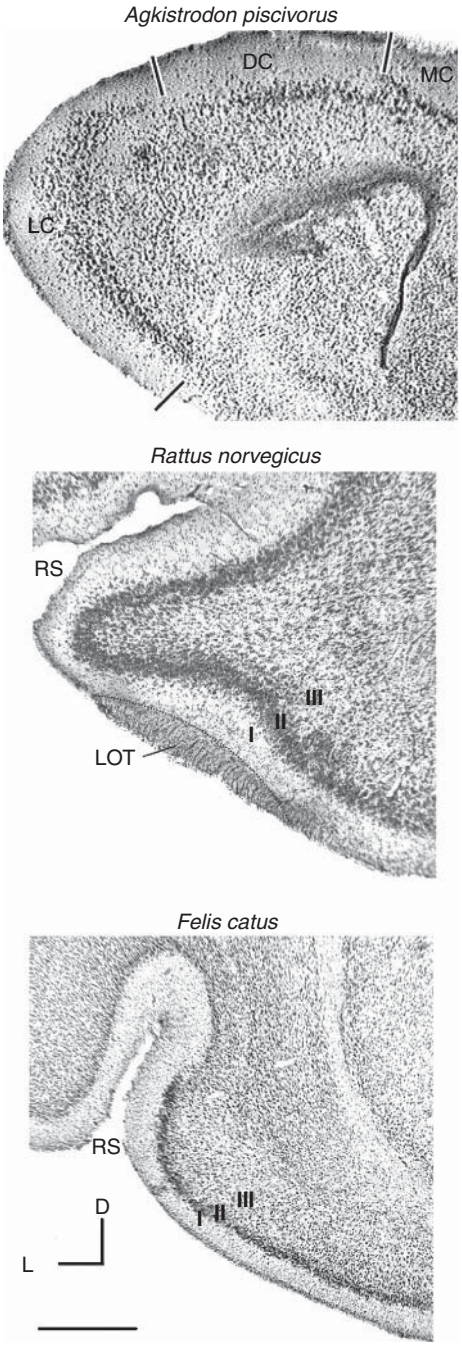

Figure 2 The appearance of olfactory cortex in mammalian and nonmammalian vertebrates. Nissl-stained coronal sections through one hemisphere of one nonmammalian species, the cottonmouth snake (*Agkistrodon piscivorus*) and of two mammalian species, the Norway rat (*Rattus norvegicus*), and the domestic cat (*Felis catus*). In *A. piscivorous*, the medial (MC), dorsal (DC), and lateral (LC) cortices are labeled. The anterior region of LC is the primary olfactory cortex, while more posterior regions of lateral cortex receive convergent olfactory and vomeronasal input. In the mammals, the piriform cortex is shown ventromedial to the rhinal sulcus (RS), with its characteristic three-layered appearance visible in these sections (I, II, and III). LOT, lateral olfactory tract. Scale bar 500 μm.

Piriform cortex is often referred to as a single structure, but it can be divided into five or more subregions on the basis of chemo- and cytoarchitectural features. This review will focus on three main subdivisions: anterior piriform cortex (APC), ventral APC (APC_V), and posterior piriform cortex (PPC; see **Figure 1**). These areas appear similar to one another, but there is good evidence that they receive different inputs, have distinct intrinsic architecture, and provide unique patterns of output. For example, APC_V may be the only portion of piriform cortex that receives input from tufted cells, and it receives a smaller complement of associative fibers originating in piriform cortex than do other subregions. In the rat, this region of the cortex also lacks characteristic GABAergic 'cartridges,' inhibitory features found elsewhere in APC that dampen neuronal activity and control recurrent excitation levels, which may account for the observation that this subregion is among the most epileptogenic sites within the rodent brain. The output from APC_V is almost exclusively to the dorsal APC and to orbitofrontal cortex, which sends a projection back to APC_V.

The PPC receives a much lighter input from the olfactory bulb, and this input is exclusively from mitral cells; tufted cell axons do not reach into the region of the PPC. Layer Ia is distinctly smaller in PPC than in APC, and PPC has a much thicker layer Ib than APC does, reflecting a much heavier associative input from the more anterior subdivisions of piriform cortex.

There is good evidence in the literature that the divisions made according to anatomical features reflect functional differences among subregions of piriform cortex. For example, responses to odors recorded from awake, behaving animals seem to be more stimulus specific in APC whereas responses in PPC appear to incorporate more associative information, perhaps reflecting the relative differences in afferent and associative input seen in each area.

28.3.2 Output

Piriform cortex projects to a widespread and diverse set of forebrain structures. These structures include the olfactory bulb, AON, lateral amygdala nucleus, entorhinal cortex, orbitofrontal cortex, and mediodorsal nucleus of the thalamus. The projections to the olfactory bulb terminate on GABAergic granule cell interneurons and thus allow a cortical feedback control of olfactory bulb excitability. It is not known how these back projections are organized within the olfactory bulb relative to the patterns of olfactory receptor afferent input. The projection to the amygdala derives primarily from the PPC. Both the APC and the PPC receive input from the basolateral amygdala nuclei, though this projection is most pronounced to the PPC. Thus, the PPC has a unique reciprocal relationship with the amygdala, which may be important in the incorporation of hedonic or associative meaning with the representation of odors. The relationship between the piriform cortex and the orbitofrontal cortex is similarly complex, with both direct and indirect (via the dorsomedial thalamic nucleus) projections.

28.3.3 The Piriform Cortex in Other Mammalian Species

In general structure, the piriform cortex in mammalian species outside the class Rodentia is recognizable by its characteristic lamination (**Figure 2**). Experimental study has been made of the piriform cortex of the opossum, cat, dog, and primates. Its relative size within the brain diminishes greatly with the enlargement of the frontal lobes seen in these mammals. In rodents and other small nonprimates such as possums and cats, the piriform cortex is located along the ventral or ventrolateral edge of the forebrain, with the myelinated lateral olfactory tract (LOT) overlying the anterior regions. In primates, the piriform cortex has moved more medially as neocortex has expanded and resides along the dorsal medial edge of the temporal lobe. Both gross anatomy and, as far as is known, local circuitry are generally conserved across these species.

28.4 Rodent AON

The AON is a large structure that lies within the olfactory peduncle, caudal to the olfactory bulb and rostral to the piriform cortex, with which it merges at its caudal end. As noted above, the AON is a cortical structure that was named by early investigators who recognized the outside-in pattern of development of the AON as seen in other brain nuclei, as opposed to the inside-out pattern observed in cortex. Nevertheless, the concept of the AON as a cortical structure has been argued for at least 80 years. The argument is based on structural features – a rigid laminar structure, the presence of pyramidal-shaped principal cells, and its continuity with the piriform cortex caudally – and on its functional role in the olfactory system. Its continued designation as the AON persists as a matter of historical consistency, although it has been proposed that functional groupings within the AON, when identified, should be renamed in a manner consistent with its cortical nature.

28.4.1 Organization

The AON exhibits a laminar structure similar to that of piriform cortex; it receives a direct projection from the mitral and tufted cells within the superficial plexiform layer (layer I), and these synapse on the apical dendritic tree of glutamatergic pyramidal cells that make up the principal cell population of the AON. Pyramidal cell bodies lie in layer II, and deep to layer II is an amorphous, poorly differentiated layer of neuromodulatory and corticofugal inputs to the AON. GABAergic cells and terminals are found distributed throughout the AON, suggesting internal processing of olfactory information within the AON, but the proportion of GABAergic cells that are intrinsic to the AON relative to those which project elsewhere is currently unknown.

One question that has surrounded the internal organization of the AON is whether it can be subdivided into functionally or anatomically discrete units. Traditionally, the AON has been divided into five subregions, termed *pars* (Latin for 'part'), that have been designated according to their physical location: pars medialis, pars dorsalis, pars lateralis, pars ventralis (or ventroposterioralis), and pars externa. Unfortunately, with the exception of pars medialis and pars externa, these designations have been made without a cytoarchitectural basis, and there are no anatomically or functionally defined features to discriminate among the remaining subregions. Nevertheless, ongoing research suggests that the AON is not homogeneous and is likely a collection of functionally discrete subregions that perform separate roles in processing olfactory information.

28.4.2 Output

Three structures are the primary targets for pyramidal cells in the AON: the olfactory bulb, the contralateral AON, and the piriform cortex. The output to the olfactory bulb is widespread, with terminations found within each layer, suggesting that the AON plays an important role in the initial stages of odor processing. It is interesting that there are substantial regional differences in the patterns of projections to the olfactory bulb; for example, projections from pars medialis terminate in the deep granule cell layer of the ipsilateral bulb, while projections from pars externa predominately innervate the inner plexiform layer in the contralateral bulb. The remaining subregions have heterogeneous, bilateral projections to broader regions of the bulb.

The projection to the contralateral AON is substantial and widespread, and the AON is the first structure in the olfactory pathways to exhibit such interhemispheric connections. Because the subregions of the AON that receive input from the contralateral hemisphere also project back to the ipsilateral olfactory bulb, the AON may be an important component in a network that allows interbulbar feedback regulation of activity. Further, because the AON is the first structure to receive input from both olfactory bulbs, it is in a position to compare the incoming signals, perhaps to facilitate localizing olfactory cues in the environment.

The output from the AON to the piriform cortex is heaviest to the central and ventromedial portions of APC, deep to the LOT and extending from the border with the olfactory tubercle laterally to just beyond the border of the LOT. This projection arises predominantly from pars lateralis, with lesser involvement from adjacent regions of pars dorsalis and pars ventroposterior. The projection from the AON to the piriform cortex is bilateral, but evidence from tract-tracing experiments suggests that pars dorsalis may project more heavily to the ipsilateral piriform cortex, while the pars ventroposterior projects more heavily to the contralateral hemisphere.

Perhaps the most striking feature of the projection from the AON to the piriform cortex is that these fibers terminate on pyramidal cells at the most proximal portion of the apical dendritic tree in deep layer Ib. This suggests that the AON is an important source of input for driving activity in piriform cortex, and it indicates that higher-order cortical processing of olfactory information involves complex reciprocal connections between these two cortical structures.

28.4.3 AON: Other Mammalian Species

The AON in mammalian species outside the class Rodentia is recognizable by its location and laminar appearance but has remained poorly studied. In most primates, the AON loses its circular structure as the forebrain expands, and it appears instead as extended groupings or islands of cells at the end of the olfactory stalk. In the macaque monkey, the AON may be only 12 pyramidal cells wide in some coronal sections. This diminished size, and the consequent difficulty in reaching the area with experimental techniques, may contribute the current lack of scientific study of the AON in other mammals.

28.5 Comparative Anatomy of Olfactory Cortex: Nonmammalian Vertebrates

Nonmammalian terrestrial vertebrates lack neocortex, but many have three-layered paleocortex that receives olfactory projections (see **Figure 2**). Output from the olfactory bulb in many of these species can be divided into at least two main projections, a medial

olfactory tract and a LOT, often with the presence of a third, intermediate tract. Mammals have medial projections from the olfactory bulb, for example to the tenia tecta, but do not have a fully separate medial olfactory tract distinct from the LOT. In reptiles, the LOT projects bilaterally to the lateral cortex and to portions of the amygdala. The medial (and intermediate) olfactory tract projects to more medial structures, again bilaterally, including structures along the medial wall of the forebrain.

Similarly, the medial and lateral olfactory pathways in birds project to three (or more) distinct cortical areas; medially, olfactory bulb afferents innervate the regio retrobulbaris and the 'piriform' or 'prepiriform' area, while lateral projections innervate the regio periamygdalaris. Each of these areas receives heavy input from the olfactory bulb, and each exhibits a characteristic three-layered structure. While it has been postulated that the regio retrobulbaris in birds corresponds to the mammalian AON, and the piriform area corresponds to the mammalian piriform cortex, the degree to which any of these areas is similar to mammalian olfactory cortex is not known.

In amphibians and fish, with no true cortex, the olfactory bulb projects to the lateral pallium via the LOT and to the medial pallium via the medial olfactory tract.

Does the olfactory cortex in nonmammalian animals serve a functional role similar to that postulated for mammals? Some evidence suggests that it does. In birds, a portion of the lateral cortex (a region that is analogous to mammalian piriform cortex) is critical for the use of olfactory information. Lesioning this region in homing pigeons does not affect homing behavior when birds are released from familiar locations, but it drastically impairs performance on homing from unfamiliar locations, where olfactory cues are necessary. Further, recent physiological recordings in catfish suggest that the projection to the olfactory forebrain may be spatially organized such that food odors (e.g., amino acids) may be processed in regions distinct from social odors (e.g., bile salts), mirroring a similar functional segregation in the fish olfactory bulb. These findings suggest that the olfactory forebrain in fish and the lateral (olfactory) cortex in birds may play a role in associating chemosensory cues with behavioral outcomes, a role that is analogous to that attributed to piriform cortex in the mammalian brain.

28.6 Summary

The last decade has seen an expansion of cellular and molecular biological techniques applied to sensory and systems neuroscience. Arguably, there is no area of study that has benefited more from this influence than has the study of olfaction, where this work has led directly to an understanding of the structure and function of olfactory receptor neurons and the organization of their projections to the olfactory bulb.

Nevertheless, critical questions regarding the processing of olfactory information remain unanswered, and the answers are likely to come from a study of the olfactory cortical regions. For instance, rodents have little difficulty making discriminations between complex odor mixtures that share components, even though these mixtures evoke activity in overlapping populations of olfactory receptors and mitral and tufted cells. How are such discriminations carried out? How is the pattern of cells activated in the olfactory bulb deciphered by the cortex? Do cortical areas play an active role in shaping the responses of cells in the bulb, as might be suggested by the heavy cortico–bulbar projections?

This review has outlined the structure of the two most prominent olfactory cortical areas in the vertebrate, the AON and the piriform cortex. By virtue of their connections and their position in the olfactory pathways, these structures are likely to play significant roles in olfactory information processing. Although some aspects of organization and circuitry are species specific, each of these cortical structures is characterized by heavy reciprocal relationships with the olfactory bulb, and with higher-order cortical areas, in all vertebrate species that have been studied. Consequently, a complete understanding of olfaction will be achieved only after the roles of these cortical structures have been identified.

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29 Vestibular System

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Glossary

1°	Primary afferent (vestibular nerve).
2°	Second order vestibular neuron (vestibular nucleus).
AC	Anterior semicircular canal.
AIN	Abducens internuclear neuron.
ampulla	A bulb like hollow structure housing a sensory organ.
analogous	Similar in function, but without phyletic continuity (e.g., human hands and the tongue of a cha meleon used for prey catching: they both do the same thing at one time, but their origins are completely different).
ATD	Ascending tract of Deiters.
C_2	Second cervical vertebra.
C_7	Seventh cervical vertebra.
cilia	Sensory processes of hair cells.
common crus	Common leg (Latin), a portion of the semicircular canal system shared by two canals.
cupula	Receptor system of the labyrinth in the semicircular canal ampullae detecting angular accelerations (rotations).
ectoderm	One of the three germ layers formed during the gastrula stage in embryogenesis and giving rise to, among other organs, the ner vous system. The other two germ layers are the mesoderm and the entoderm.
FP	Frankfurt plane.
hair cell	Sensory cells of the inner ear and some other sense organs whose name derived from their mechani cally sensitive cilia, the so called kinocilia and the stereocilium. During a stimulus, e.g., mechani cal or auditory, these cilia undergo a bending and via a specific ion conductance perform a mechanoe lectrical transduction.
halteres	Fast moving (rotating) clublike righting organs of flies, working much like gyroscopes.
HC	Horizontal semicircular canal.
hemilabyrinthectomy	Global extirpation of the labyr inth of one side, resulting in distinctive lesion symptoms.
homologous	Inherited from a common ances tor (with phyletic continuity), but not necessarily similar in function (e.g., the limbs of horses and sea lions, used for walking in one case, and swimming in the other case; another example are the wings of birds and bats which

	are both homologous and analo gous, since they were both derived from forelimbs and are used for flying).
Hor	Plane of horizontal semicircular canals.
HorC	Horizontal canal.
III	Oculomotor nucleus.
IN	Internuclear neuron.
invertebrates	Animals without a back bone (spinal column), e.g., insects, worms, spiders, crabs.
IO	Inferior oblique.
IR	Inferior rectus.
IV	Trochlear nucleus.
kinematics	Muscle actions upon a movable body part, e.g., a limb, an eye.
lateral line	A sensory system present in many aquatic vertebrates of mechanor eceptive (water current) or electroreceptive nature (electric fields).
LR	Lateral rectus.
MN	Motoneuron.
MR	Medial rectus.
neural crest	Inductive tissue to form sensory and neuronal elements appearing between the neural tube and the surface ectoderm.
neuromast cell	Hair cell of the lateral line system.
optokinetic reflex	Eye (or head) movements elicited by large moving visual scenes, e.g., when observing the passing landscape in a moving train.
otic placode	Thickening of the ectoderm and precursor of the otocyst.
otoconia	Ear stones consisting of calcium carbonate crystals embedded in the otolith membrane.
otocyst	Invagination of the otic placode forming a cyst at first that later subdivides and gives rise to the complex adult three dimensional structure of the labyrinth.
otolith	Receptor system of the labyrinth, so called graviceptor, detecting lin ear accelerations (translations).
Otx	Member of a gene family (orthodenticle).
PC	Posterior semicircular canal.
rhombomere	Elements of segmentation of the rhombencephalon formed during embryology and thought of as an expression of developmental organization.
semicircular canal	Tubelike structure of the labyr inth filled with endolymph to detect angular accelerations.
SO	Superior oblique.
SR	Superior rectus.
statocyst	The balance organ of an invertebrate.
VAPC	Vertical anterior parasagittal canal.
vertebrates	Animals with a spinal column (back bone), i.e., fish, amphi bians, reptiles, birds, mammals.
vestibulo ocular reflex	Eye movements elicited by stimu lation of the labyrinth.
VI	Abducens nucleus.
VPPC	Vertical posterior parasagittal canal.
VTC	Vertical transverse canal.

29.1 Introduction

The sense of balance, the vestibular system, is our unknown sense. We recognize its existence only under pathological conditions, such as seasickness, dizziness, vertigo, etc. Among the classical five senses, i.e., vision, taste, smell, touch, and hearing, our sense of balance is not mentioned. Quite often, the sense of balance is just considered as an appendix of the auditory sense due to the anatomical unity of cochlea and vestibular apparatus, the so-called inner ear (Figures 1a and 1b). The inner ear is really a fabulous example of the engineering capabilities of nature and evolution, as it is one of the most complex anatomical structures in the vertebrate history: in humans, we find two hypersensitive hyperprecise sensory organs housed within the space equivalent to that of an M&M ball – the auditory sense and the sense of balance. Moreover, under normal life conditions, we are not even aware of the latter's existence. The sense of balance could thus be considered our sixth sense, and its functions are manifold. At least four different and vital functions should be mentioned:

1. postural control and postural stabilization;
2. reflex movements;
3. perception of self-movement; and
4. autonomous control.

29.2 Spatial Coordinates

A large portion of our daily activity requires moving between different positions. To that end, we use different means of locomotion or transport, e.g., individual locomotion (walking, running, swimming), or with the aid of mechanical devices (bicycle, car, train, escalator, etc). During all these dynamic events, we have a sensation of self-motion, which we will largely attribute to visual inputs. The

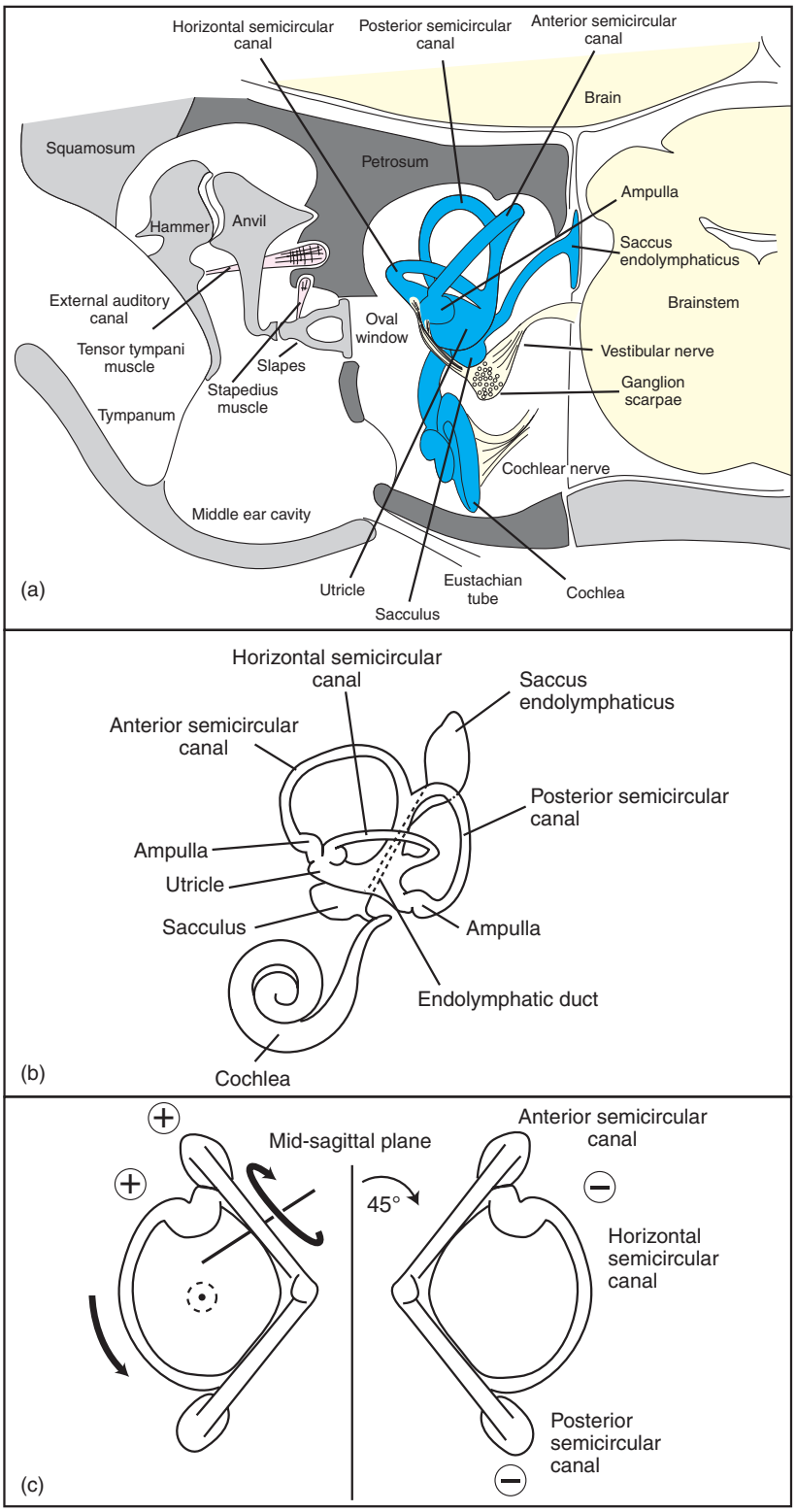

Figure 1 Anatomy of the organ of the sense of balance. a, Position of the labyrinth in the cranium. Cross section of the right Os petrosum with a frontal view of the right labyrinth and its topographical neighborhood. b, Lateral view of the left human labyrinth showing the portions of the sense of balance (semicircular canals and otoliths) and of the sense of hearing (cochlea). c, Spatial orientation of an idealized semicircular canal system (top view). Anterior and posterior canals are oriented vertically, horizontal canals are oriented horizontally. The vertical canals are oriented 45° off the midsagittal axis (diagonal orientation). Note bilateral symmetry, mutual orthogonality between canals, and the push pull operational mode illustrated for the right posterior and the left anterior canals, and the right and left horizontal canals. When one canal becomes excited (+), its coplanar counterpart becomes inhibited ( ). Canal on-directions are indicated by the directions of the arrows about the canal rotation axes. The combined excitatory and inhibitory responses of all canals during head movements produces a meaningful activity pattern in the afferent nerves and recipient brain nuclei to represent a movement vector in physical space (Werner, 1960).

role of the sense of balance in this function is often not realized, although fast reflex movements, such as certain eye movements or postural control adjustments are mediated by this sensory system. Triggering a fast eye movement via the sense of balance (the vestibulo-ocular reflex, VOR) requires only 16 ms, while eliciting the corresponding reflex via the visual system (optokinetic reflex) takes 80–150 ms.

Humans live in a three-dimensional environment; however, they rarely use the third dimension for every day transport in comparison to many bird and fish species, or even nonhuman primates. Nevertheless, our sense of balance uses a sensory organ to detect self-movements in three-dimensional space. Two fundamentally different movement categories have to be distinguished: rotations and translations. Each one of these has three degrees of freedom. Classically, these movements are described in a Cartesian coordinate system anchored to the head, which includes one vertical axis (along the gravity vector), and two earth horizontal axes, one naso-occipital (sagittal) axis, and one interaural (transverse) axis. All three axes intersect at one point in the middle of the head. It has to be mentioned, however, that the Cartesian coordinate system, as its name implies, is man-made (i.e., by the French philosopher and natural scientist René Descartes, 1596–1650) and bears no significance for the way biological systems developed movement detection systems during the course of evolution.

29.3 Receptors of Movement Input: The Labyrinth

29.3.1 Anatomy

The inner ear is a bilateral organ. It is located inside the petrosal part of the temporal bone of the cranium (Figure 1a). The balance organ is part of the inner ear and consists of the semicircular canals and the otoliths (Figure 1b). At first sight, the twisted and three-dimensional structure of the inner ear looks quite complicated and has earned the balance organ the name 'labyrinth'.

Semicircular canals and otoliths are sense organs, which detect accelerations. The semicircular canals detect angular accelerations (rotations), the otoliths linear accelerations. An example for a ubiquitous and permanent linear acceleration is earth gravity (gravity vector). Under normal living conditions, we rarely spend a thought about gravity, but when gravity becomes absent, the effects can be dramatic, as during space flight under microgravity conditions with resulting space motion sickness.

29.3.1.1 The semicircular canals The operational mode of the semicircular canals is independent of gravity. The canals are filled with a fluid, the so-called endolymph, which, during a given head movement, causes a so-called endolymph current, which displaces receptor cells inside a specialized area of the canal lumen, the so-called ampulla (Wilson and Melvill Jones, 1979; Graf, 2003). An important characteristic of the macroscopic anatomy of the semicircular canals is their three-dimensional orientation. The ensemble of the six canals, three on each side, forms a physical coordinate system to detect angular accelerations in three-dimensional space. The semicircular canal system on each side of the head consists of a horizontal (lateral) canal, and two vertical canals (one anterior and one posterior canal) (Figure 1c). The horizontal canal is lightly tipped upward (about 30° in humans) at normal head resting posture (Figure 2a; see also Figure 2b). The vertical canals are oriented about 45° off the midsagittal plane of the head (Figure 1c).

The orientation of the semicircular canals in the head follows three interdependent functional principles:

1. Bilateral symmetry: both labyrinths are mirror symmetric.
2. Reciprocal operational mode: during head rotations receptors in a given canal will be excited, while the receptors in the contralateral coplanar canal will be inhibited; the so-called push-pull system.
3. Mutual orthogonality of canals: the functional planes of the canals enclose angles of 90°, or close to that value (Figure 1c).

The semicircular canal system thus constitutes an intrinsic sensory reference frame system, which provides a blueprint for the spatial coordination for a number of reflex functions and sensory interactions (Cohen *et al.*, 1965; Schaefer *et al.*, 1975; Simpson and Graf, 1981, 1985; Simpson *et al.*, 1981; Graf, 1988; Graf *et al.*, 1988; Leonard *et al.*, 1988).

29.3.1.2 The otoliths By contrast to the semicircular canals, the otoliths are receptors, which depend on the presence of gravity (graviceptors). They detect linear accelerations and do not function in microgravity. Most vertebrates, including humans, posses two otoliths on each side: the horizontal utriculus and the vertical sacculus. At normal resting posture of the head, the utriculus seems to be oriented earth horizontally (Figure 2b). The receptor cells of the otoliths are embedded in the so-called otolith membrane, which contains the

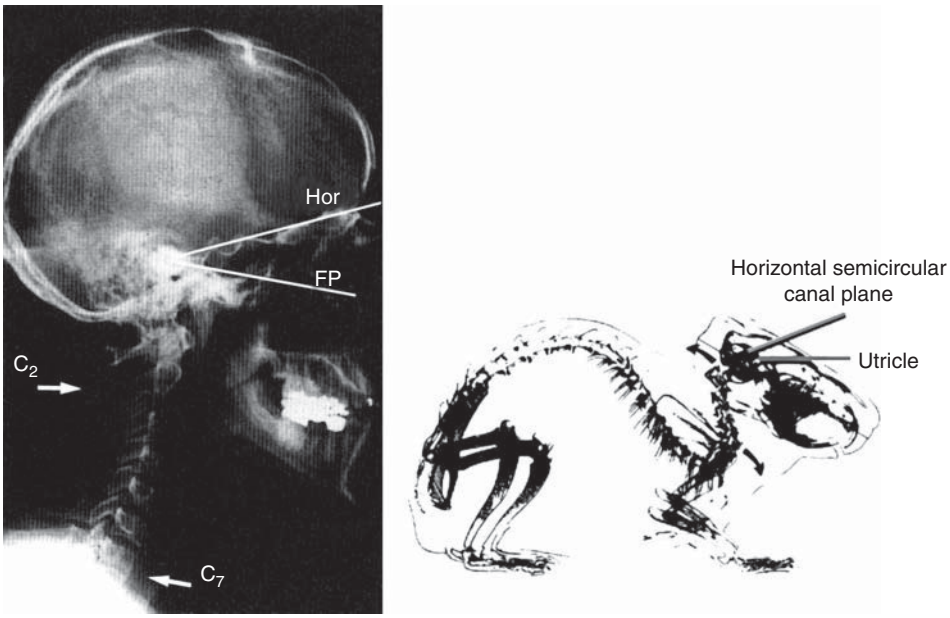

Figure 2 Lateral views of head and labyrinth orientation during normal head posture at rest in a biped and a quadruped. a, Radiograph of a human skull. C_2, C_7, second and seventh cervical vertebras; FP, Frankfurt plane; Hor, plane of horizontal semicircular canals. b, Artist's rendering of a lateral radiograph of an awake and unrestrained guinea pig. Note vertical orientation of the cervical vertebral column. Arrows indicate the direction of possible movement, i.e., at the upper cervical columns, only extension movements are possible, since the head is held in the extreme flexed position at the atlanto-occipital articulation, and at the cervicothoracic junction only flexion movements are possible, since here the vertebras are held at extreme extension. In both cases, the cervical vertebral columns are held vertically with the horizontal canals kept tipped upward by approximately 20° 30°. At this position, the utricles would be positioned about earth horizontally.

otoconia. During a displacement of the head from the normal upright position, the otoconia will slide across the otolith membrane and produce a shear force upon the receptor cells.

29.3.2 Evolutionary History of the Labyrinth

The phylogenetic origins of the vertebrate labyrinth are not known. The only living protochordate, *Amphioxus*, possesses sense organs, namely, a median eye, and bilateral balance organs (Lacalli, 2001; Lacalli *et al.*, 1994, 1999; Figure 3), but no functional–physiological data are available. Furthermore, only fragmentary fossil records exist that testify to the beginning of vertebrate life in Cambrian times, more than 500 Mya. However, there now seem to be indications from molecular biology data for a common ancestor regarding mechanoreceptor cell evolution between *Drosophila* and vertebrates, i.e., hair cells (Fritzsch *et al.*, 2000; see also Figure 6). Although for a long time, the balance organ had been thought to have evolved from the lateral line system, recent evidence based on multiple out-group comparison suggests that the inner ear of vertebrates evolved as a statolithic system before the lateral line system and before semicircular canals appeared.

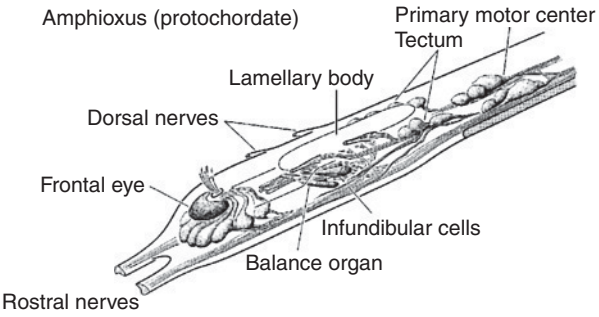

Figure 3 Amphioxus cerebral vesicle. Principal landmarks in the larval cerebral vesicle of amphioxus, showing the anterior pigment cup with the median eye, the ciliary bulb cells of the putative bilateral balance organ, and the lamellar body, which is assumed to be a pineal homologue (Th. Lacalli).

The fossil record becomes more complete only during the middle of the Paleozoic era, the Devonian period (350–400 Mya). The first record that demonstrates the existence of semicircular canals comes from jawless vertebrates, agnathan species of the Devonian and Silurian times, the ostracoderms. They possessed vertical, but not horizontal canals (Figure 4). Their vertical canals were oriented in the head as described before (Stensiö, 1927; Figure 1c). The ostracoderm labyrinth was

similar to the semicircular canal system of lampreys, the extant forms of their once-abundant ancestors. The Devonian period also marks the advent of jawed vertebrates (ganthostomes) and bony and cartilaginous fishes (osteichthyes and chondrichthyes, respectively; Figure 5). We know nothing about the labyrinth structure of the immediate ancestors of these newly appeared animals, but their modern successors display a new acquisition: horizontal semicircular canals. Thus, the vertebrate labyrinth now spans all three dimensions of physical space. The circumstances that led to the development of a horizontal semicircular canal system are unknown, but its presence most certainly introduced distinct advantages for the detection of three-dimensional space in comparison to the four canal system in the Agnatha. The acquisition of horizontal semicircular canals coincides with the expression of the vertebrate-specific gene *Otx1* (Fritzsch and Beisel, 2001, 2003; Figure 6). Knock-out mutants that do not express *Otx1* do not develop horizontal semicircular canals (Fekete, 1999). One could speculate that the appearance of horizontal semicircular canals, allowing an optimal solution, i.e., best and most economical (Gould, 1977), high signal-to-noise-ratio (Robinson, 1982; 1985; Graf, 1988) for movement detection in three-dimensional space constituted one prerequisite for the success of vertebrates later on in phylogeny. At any rate, it certainly provided one further advantage.

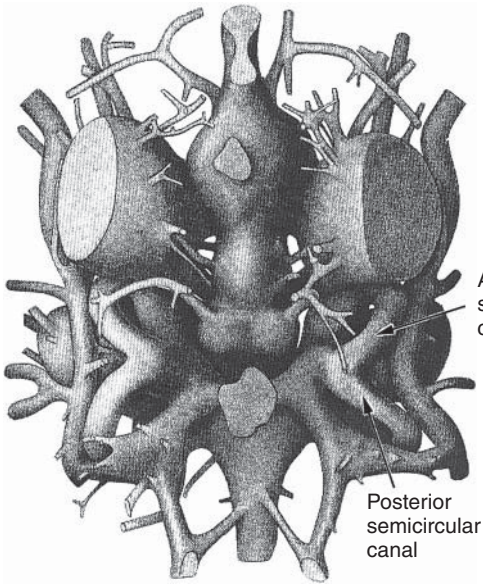

Figure 4 Photograph of a wax model of the cranial cavity of the fossil ostracoderm, *Kiaeraspis auchenaspidoides*, including labyrinths with anterior and posterior semicircular canals (Stensiö, 1927). Note diagonal orientation of the canals similar to the situation in extant vertebrates. Horizontal canals have not yet appeared.

Interestingly enough, there are two main lines of labyrinthine development in the surviving radiations, namely what we will refer to as the bony

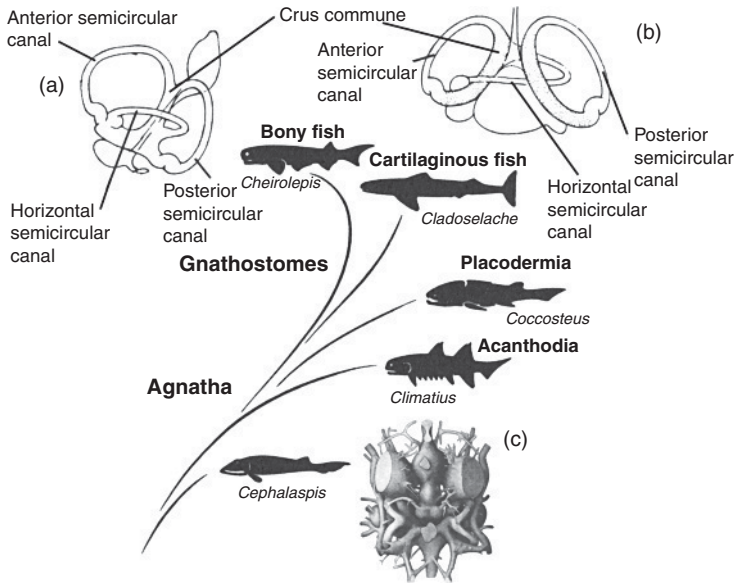

Figure 5 Phylogenetic relationship of early agnathans and gnathostomes (bony fishes, cartilaginous fishes, placoderms, and acanthodians) (Colbert, 1980), including prototypical vertebrate labyrinth characteristics. a, Human labyrinth (Werner, 1960). b, Shark labyrinth, *Chlamydoselachus* (Werner, 1930). c, Ostracoderm labyrinth without horizontal canals (Stensiö, 1927). Horizontal semicircular canals appear in bony fishes and cartilaginous fishes. In bony fishes through humans, the anterior and the posterior canal form a common crus. In cartilaginous fishes, there is no common crus between the anterior and the posterior canal. All labyrinths display a similar (diagonal) orientation of the vertical semicircular canals in the head.

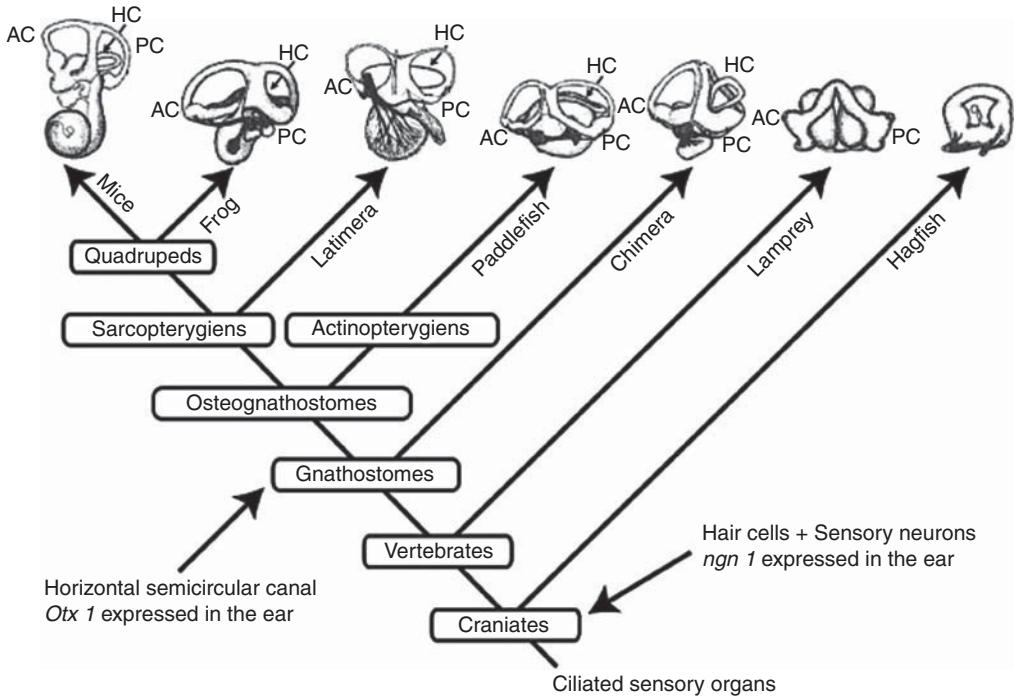

Figure 6 Morphogenic evolution of the vertebrate ear. Ciliated mechanosensory cells, so-called hair cells are now thought to be at the phylogenetic origin of the vertebrate inner ear. For the development of primary neurons, *ngn1* is necessary. One of the major morphogenetic events in vertebrate ear evolution was the appearance of horizontal semicircular canals in all gnathostomes. The development of horizontal semicircular canals coincides with the expression of the *Otx1* gene (Fritzsch and Beisel, 2001, 2003). Reproduced from Fritzsch, B. and Beisel, K. W. 2001. Evolution and development of the vertebrate ear. *Brain Res. Bull.* 55, 711 721, with permission from B. Fritzsch.

fish/tetrapod line and the cartilaginous fish line (Figure 5) (we are using the term 'bony fish/tetrapod' in the following to delineate vertebrate species between bony fish and mammals, quadrupedal and bipedal, i.e., including amphibians, reptiles, and birds). Unfortunately, no fossil record testifies to the labyrinth structures of earlier radiations that became extinct (e.g., acanthodians, placoderms).

In viewing a typical vertebrate labyrinth of the bony fish/tetrapod line, in this case a human labyrinth (Figure 5a), we observe that it consists of three canals, one anterior, one posterior, and one horizontal canal. Typically for this type of labyrinth, the anterior and the posterior canal form a so-called common crus; that is, they share a segment of their circular structure (Werner, 1960; Lewis *et al.*, 1985). The typical cartilaginous fish labyrinth, in this case from a shark (Figure 5b) also possesses anterior, posterior, and horizontal canals that display the same orientation in the head as the bony fish/tetrapod labyrinth type. However, there is no common crus between the anterior and the posterior canals. The posterior canal is separate and has a communication with the sacculus, whereas a common crus-like structure is formed between the horizontal and the anterior canals (Daniel, 1928; Werner, 1930; Baird, 1974). This particular difference in labyrinth structure between bony fishes and cartilaginous fishes leads to the intriguing question of whether the phylogenesis of horizontal canals was monophyletic or polyphyletic in vertebrates.

29.3.3 Comparative Anatomy

A great variety of movement and position detectors, so-called statocysts, are found in invertebrates (Bullock and Horridge, 1965; Markl, 1974), and we will introduce only the most pertinent examples here.

The statocysts of the fast-moving squid and cuttlefish (Figure 7a1) include grooves, which, similar to the vertebrate semicircular canals of vertebrates, direct endolymph flow toward a sensory crista with a cupula (Stephens and Young, 1978). These invertebrate canals, so-called tonoids, are oriented in space in a roughly orthogonal three-dimensional planar arrangement (Budelmann, 1977; Stephens and Young, 1978; Figure 7a2). Four canals on each side can be distinguished, which are oriented approximately in the main planes of the body, in contrast to the vertebrate arrangement (Budelmann, 1977; Simpson and Graf, 1985; Figure 7a3). Sensory receptors detect movements in the transverse plane of the body, with

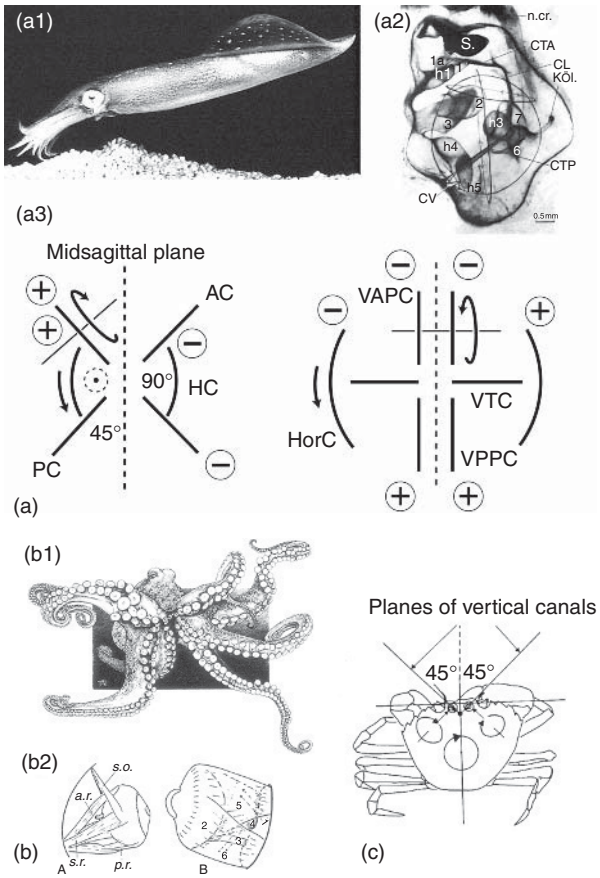

Figure 7 Convergent evolution of movement detection systems. a, Squid (a1), photo of a squid. The animal propulses itself rapidly backward by ejection of a jet of water. (a2), Retouched photograph of a squid statocyst indicating the orientations of the toroid planes (Stephens and Young, 1978). (a3), Comparison of the diagonal vertebrate semicircular canal system, and the principal axes system of squids. The squid system also fulfills the three criteria of orthogonality, bilateral symmetry, and push pull operational mode. However, instead of six canals, it has eight toroid structures, and the on-directions of the sensory receptors are just about opposite that of vertebrates. Nevertheless, the squid movement detection system functions according to the same operational principles as the vertebrate semicircular canal system. b, Octopus (b1), drawing of an octopus. Despite its seemingly amorphous body structure, the octopus possesses a well-defined three-dimensional movement detection system similar to vertebrates. b2, Comparison of the extraocular muscles in a shark, left, and in the octopus, right. Note similar diagonal spatial arrangement in the two animals (Packard, 1972). c, Crab, spatial arrangement of the semicircular canal system in crabs. Although there are only four canals in crabs, the vertical canals are oriented just like the anterior canals of vertebrates, as are the horizontal canals (Fraser, 1981).

excitation occurring during ipsilateral upward roll movements, in the longitudinal plane of the body, with receptors detecting pitch-up and pitch-down movements, and in the horizontal plane with receptors being excited during contraversive rotation (Figure 7a3, right). Some receptors also detect linear accelerations (Stephens and Young, 1978). The squid semicircular canal system can thus monitor three-dimensional angular accelerations just like the idealized vertebrate semicircular canal system with bilateral symmetry, orthogonality, and push–pull operational mode. Orthogonality of the semicircular canals would provide an optimal signal-to-noise ratio (Robinson, 1982, 1985), but in order to achieve paired orthogonality, the vertical semicircular canals need not necessarily be arranged in the familiar diagonal fashion. The one (and only) alternative arrangement is pairs in coronal, parasagittal, and horizontal planes as shown in Figure 7a3 (right). The squid/cuttlefish semicircular canal system could thus be termed a principal axes system, in contrast to the diagonal vertebrate arrangement.

In the octopus (Figure 7b1), the sensory receptor organ on each side consists of nine subsections, and is divided into three main planes, which are approximately orthogonal to each other (Young, 1960). The arrangement of the subsections suggests an angular acceleration detection system similar to that of vertebrates (Young, 1960; Budelmann, 1977). Interestingly, the extraocular muscle arrangement of the octopus resembles closely that of lateral-eyed vertebrates (Packard, 1972; Figure 7b2).

The third type of semicircular canal system introduced here in invertebrates of interest is found in crabs (Figure 7c), which possess one horizontal and one vertical toroid structure on each side. Depending on the species, these toroids can either be open or can form a closed canal system (Sandeman and Okajima, 1972; Sandeman, 1983; Fraser, 1981). In freely moving crabs, the horizontal canals are held earth horizontally, and since the horizontal and the vertical canals are close to orthogonal, the vertical canals are nearly vertical. Each vertical canal lies at an angle of 45° to the midsagittal plane in a configuration comparable to that of the anterior semicircular canals in vertebrates. Although there is only one vertical canal on each side, each one responds preferentially to movements about orthogonal axes and thus the canals of crabs are collectively capable of accurately transducing three-dimensional angular accelerations (Fraser, 1981).

Comparison of vertebrate and invertebrate solutions about how to build movement-detection systems shows a remarkable uniformity to an idealized three-dimensional geometry of optimal decomposition of all given rotation vectors. The semicircular canal systems of vertebrates and invertebrates are thus prime examples for convergent evolution.

29.3.4 Ontogeny and Phylogeny of the Labyrinth

The labyrinth develops from an enlargement of the ectoderm, the otic placode, which invaginates to form the so-called otocyst (for details see Rinkwitz *et al.*, 2001; Romand and Varela-Nieto, 2003). A number of genes and induction molecules play a role for the complicated morphogenesis of the labyrinth. There are genes that are necessary for the differentiation of various organ and system developments and some that are labyrinth-specific. Many genes work in parallel or are redundant. Gene duplication, or multiplication of genes during the progress of evolution has to be taken into consideration as well (Fritzsch *et al.*, 2000). The differentiation of the main structures of the labyrinth is guided by independent genes, which will be introduced in the following.

Although many vertebrate genes are homologous with *Drosophila* genes, the vertebrate labyrinth is a development of chordates and without precedent in other animal groups. Flies do not possess balance organs *per se*, but rely on relative movement of body parts (halteres) to orient in gravity. Homologies with other animal groups seem to be restricted to the development of receptor cells, which transform mechanical stimuli into electrical impulses (mechano electrical transduction). The receptors of the labyrinth are important examples for the general question of the origin of mechanoelectrical transduction at the level of receptor cells. For many years, evolutionary biologists believed that the labyrinth was derived from the neuromast cells of the lateral line organ of aquatic vertebrates. Meanwhile, however, functional interrelations between the pressure receptors of the nematode *C. elegans* and the sensory bristle receptors and proprioceptors of the fruit fly *Drosophila*, on one hand, and vertebrate hair cells, on the other hand, have been described (Fritzsch *et al.*, 2000; Fritzsch and Piatigorsky, 2005). The description of a mechanoelectrical transduction channel in *Drosophila* and *C. elegans* points to an early development of a mechanoelectrical receptor in evolution. The original receptors might have consisted of a cilia-like structure, including support cells. Thus, receptor cells seem to have been an important evolutionary component for the development of the sense of balance of vertebrates, but it was not the structure *per se* that led to the macroscopic expression of analogous sense organs. Interestingly, inner ear hair cells develop without involvement and influence of the neural crest, which normally guides the development of most of the sensory neurons of the peripheral nervous system of chordates.

The actual morphogenesis of the ear is governed by numerous genes, which also play a role in the development of lungs, kidneys, and extremities. Embryogenesis and morphogenesis occur during particular periods in ontogenesis, when certain genes are switched on or off, and when certain organs and characteristics are being developed. The development of sense organs is embedded into the general process of structurization and position specification. In this process, proneural genes will be activated, which are determining the precursors of the elements of sensory organs, such as support cells, glia, and portions of the actual sensory cells. Two mechanosensory basic helix-loop-helix (*bHLH*) genes are expressed in the ear, *Neurogenin 1* (*ngn1*) and *mammalian atonal homologue 1* (*Math1*). In insects, *atonal* (*ato*) is important, whose vertebrate homologue *Math1* is indispensable for the development of hair cells. Knock-out mutants without *Math1* develop support cells and primary neurons, but no hair cells. For the development of primary neurons, another *bHLH* homologue is required, i.e., *ngn1*, one of three so-called neurogenin genes.

For further labyrinth development, the so-called *FGF* and *FGFR* genes play an indispensable role (FGF, fibroblast growth factor; FGFR, tyrosine kinase receptor family). In particular, *FGF19* seems to be a fundamental element for the induction of ear development in chickens, which is activated together with *Wnt8c*. The interplay between FGFs and FGF receptors in vertebrates seems to induce the budding out of the growth zones of lungs, extremities, and the ear placode. With regard to inner ear development, the receptor FGFR-2(IIIb) is essential for the development of the semicircular canals, the endolymphatic duct, and the cochlea. Besides the *FGF* genes, *BMP*, *Pax*, *POU*, and zinc-finger genes were shown to be present in the ear. *POU4f3* knock-out mutants form a labyrinth with hair cells that are later lost. Missing the *Pax2* gene results in an ear without cochlea; however, with semicircular canals and otoliths (Fekete, 1999; Fekete and Wu, 2002).

While all genes described above have been shown to exist in insects, some vertebrate-specific genes are noteworthy, such as the above-mentioned *Otx1*, which regulates the development of the horizontal semicircular canals (Figure 6). Vertebrates without horizontal canals do not express this gene in the ear. Finally, the gene *mindbomb* should be mentioned, which plays a role during the development of the inner ear and the central nervous system. This gene is important in the context of the regeneration of

inner ear hair cells, which has been demonstrated in fishes and birds. The *mindbomb* gene seems to induce the transformation of the precursors of hair cells into support cells. Knock-out mutants produce an abundance of hair cells, but no support cells (Fekete, 1999).

Some homeotic genes also play a role for inner ear differentiation. Inactivation of *Hoxa1* produces various malformations of the inner ear and results in only the development of an epithelial cyst (Fekete, 1999).

29.4 Effectors of Sensory Input: Extraocular Muscles

29.4.1 The Extraocular Muscle Apparatus

The extraocular muscle apparatus can be considered as a prime example for the efficiency of biological systems. The spatial orientation of the extraocular muscles, in particular, illustrates in an almost ideal fashion how evolution solved a complicated problem of sensorimotor transformation.

The six extraocular muscles move the eye in a reference frame that corresponds to the spatial geometry of the vestibular semicircular canals, i.e., the typical diagonal, 45° off the midsagittal plane orientation of vertical canals, is reflected in the pulling direction of the vertical eye muscles (Helmholtz, 1910; Alpern, 1962; Figure 8). The vertical eye muscles are superior rectus (SR), inferior rectus (IR), superior oblique (SO), and inferior oblique (IO); the horizontal eye muscles are lateral rectus (LR) and medial rectus (MR). These anatomical designations give the impression of a distinct separation between straight and oblique eye muscles. In the true sense of the word, only LR and MR are straight eye muscles, whereas all vertical eye muscles, including SR and IR, are in reality oblique muscles. The illustrated example of Helmholtz's drawing demonstrates this fact very clearly (Figure 8). Unfortunately, an idealized, but erroneous figure by Bell (1823) has dominated the literature and clouded the understanding of spatial coordination of eye movements.

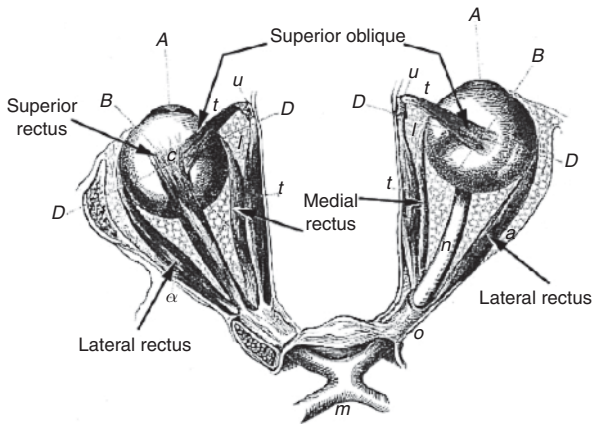

Figure 8 Spatial orientation of extraocular muscles (Helmholtz, 1910). Note diagonal orientation of vertical eye muscles, e.g., SR and SO.

29.4.2 Innervation of Extraocular Muscles

At the peripheral level of the six extraocular muscles, there are no gross differences between any of the vertebrate species, except that hagfishes do not possess eye muscles. However, at the central organization, distinct differences become evident. Across species, three patterns of eye muscle innervations can be distinguished:

1. The lamprey pattern where two eye muscles are innervated by ipsilateral (IO, IR), and one by contralateral (SR) oculomotor neurons, one by trochlear motoneurons (contralateral, SO), and one by the abducens nucleus (ipsilateral, LR).
2. The elasmobranch pattern with two ipsilaterally (IR, IO) and two contralaterally projecting (SR, MR) oculomotor motoneuron populations, one trochlear motoneuron population (contralateral, SO), and one abducens motoneuron population (ipsilateral, LR).
3. The bony fish/tetrapod pattern with three ipsilaterally (IR, IO, MR) and one contralaterally projecting (SR) oculomotor nucleus neuron population, one trochlear motoneuron population (contralateral, SO), and one abducens motoneuron population (ipsilateral, LR) (Figure 9; see also Fritzsch, 1998).

The lamprey pattern thus has only five extraocular eye movers, lacking the equivalent of a MR muscle (Fritzsch *et al.*, 1990). The main difference between the bony fish/tetrapod and the elasmobranch pattern is the positioning of the MR motoneurons, with MR motoneurons addressing a contralateral eye muscle in elasmobranchs, just as SR and SO motoneurons do (Figure 9b), and an ipsilateral placement with respect to the muscles it innervates in animals of the bony fish/tetrapod line (Figure 9a). This difference in motoneuron placement needs to be further investigated and reflected upon regarding vestibulo-oculomotor reflex connections (see below).

In evolutionary history, the MR muscle in elasmobranchs is thought to have evolved from a split of the dorsal rectus (SO) of ancestral agnathans, whereas it was derived from a split of the rostral rectus (IR) in the ancestors of the

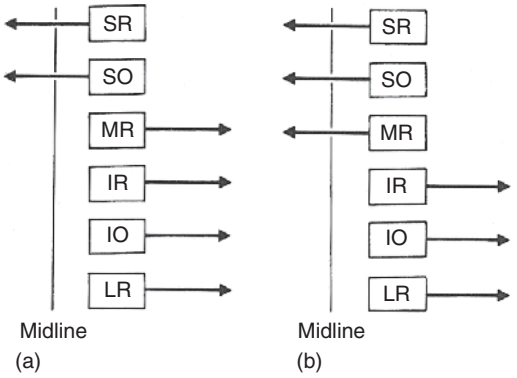

Figure 9 Oculomotor neuron projections in vertebrates of the bony fish/tetrapod line (a) and in elasmobranches (b). Note difference in MR motoneuron placement projecting ipsilaterally in bony fish/tetrapods and contralaterally in elasmobranchs. IO, inferior oblique; IR, inferior rectus; LR, lateral rectus; MR, medial rectus; SO, superior oblique; SR, superior rectus.

osteognathostomes (Nishi, 1938). Such a scenario would also explain the different motoneuron placements in the two vertebrate radiations.

There was also an idea that lungfishes actually possessed an elasmobranch innervation pattern, which would bring them taxonomically close to elasmobranchs (von Bartheld, 1992). This has now been shown not to be the case (Puzdrowski and Morshedi, 2003; Graf, unpublished observation). Clearly, lungfishes, at least the examined species, the African lungfish, *Protopterus dolloi*, shows a clear bony fish/tetrapod innervation pattern, with an ipsilaterally projecting MR subpopulation.

29.4.3 Ontogeny and Phylogeny of the Extraocular Muscles and Their Innervation

Although the geometric arrangement of the extraocular muscles is basically identical in all vertebrates that possess eyes, the horizontal eye muscles seem to have followed slightly different evolutionary paths in elasmobranchs and the bony fish/tetrapod line. Embryonically, the MR muscle seems to arise from the dorsal part of the premandibular head cavity in elasmobranchs; in other vertebrates it comes from its ventral part (see Graf *et al.*, 2002). This difference in embryonic origin may be a concomitant explanation for the contralateral versus ipsilateral placement of MR motoneurons in elasmobranchs when compared to bony fish/tetrapods. Other differences exist regarding abducens motoneurons. Abducens motoneurons originate in embryonic rhombomeres 5 and 6 in most vertebrates (lamprey, teleosts, birds, reptiles) (Gilland and Baker, 1993), exclusively from rhombomere 5 in frogs (Straka *et al.*, 1998) and mammals (Gilland and Baker, 1993), but only from rhombomere 6 in elasmobranchs (Gilland and Baker, 1992).

Abducens motoneurons are surmised to be somatic, originally being part of a series of homologous spinal-like nerves. Thus, the abducens nerve would have simply invaded a position once foreign to it (for a review of the pertinent literature, see Baker, 1992). According to this argument, the abducens would therefore not belong to the branchiomotor category. The special role of abducens motoneurons (and abducens internuclear neurons) is also underlined by the inhibitory transmitter employed by afferent vestibular and reticular neurons, i.e., glycin. In oculomotor and trochlear motoneurons, the inhibitory transmitter is GABA. Oculomotor myoblasts are thought to be derived from the premandibular region, trochlear myoblasts from the mandibular region.

The abducens nucleus is the only extraocular motor nucleus inside the *Hox* gene-expressing region, its expression being under the control of *Hoxb3*. The other extraocular motor nuclei are found around the brainstem–midbrain isthmus, with the trochlear nucleus originating in rhombomere 1. Development of the trochlear and the oculomotor nuclei seems to be primarily governed by two molecules, i.e., wingless (*wnt*) and engrailed (*en*).

29.4.4 Vestibulo-Ocular Connectivity

We described the three-dimensional geometry of the sensory periphery, the semicircular canals, and its related motor effectors earlier in this article. Clearly, there is a similarity between these geometries. The pulling directions of the horizontal eye muscles correspond with the orientation of the horizontal semicircular canals, that of the vertical recti with the orientation of the ipsilateral anterior semicircular canal, and the pulling directions of the oblique eye muscles are in line with the orientation of the ipsilateral posterior canal (Figures 10 and 12). We find this orientation principle from fish to humans.

The conservation of the coincidence of the spatial geometries of semicircular canals and eye muscles during vertebrate evolution is also accompanied by a conservation of the principal neuronal connections for the production of compensatory eye movements (VOR) from fish to humans. Within this framework, excitatory connections are formed between the anterior canal and the ipsilateral SR and the contralateral IO muscles, between the posterior canal and the ipsilateral SR and the contralateral IR muscles, and between the

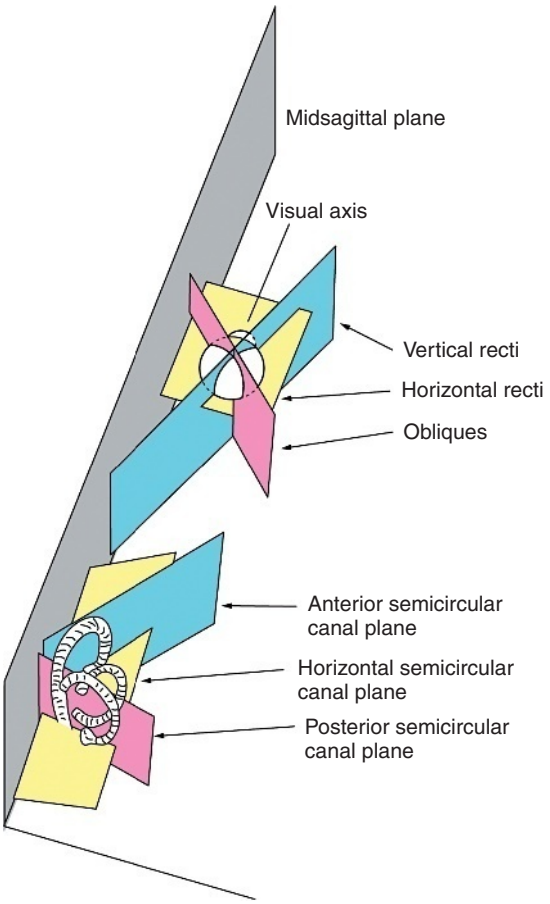

Figure 10 Three-dimensional orientation of semicircular canal planes and extraocular muscle pulling directions in humans. Note alignment of certain eye muscle pulling directions with particular canal planes, forming an intrinsic reference frame system.

horizontal canal and the ipsilateral MR and the contralateral LR muscles. Since the antagonists to these muscles will have to relax at the same time, we observe the existence of inhibitory connections to these antagonists arriving from the same semicircular canals (Figure 11). This innervation scheme has been termed the elementary VOR arc (Lorente de Nó, 1933) or the three-neuron arc (Szentágothai 1943, 1950) by the pioneers working in this field of research. The three neurons involved in this reflex arc are the primary vestibular neurons, the second-order vestibular neurons, and the respective extraocular motoneurons (Figure 11).

The development of the brainstem vestibular nuclei is under the control of a number of *Hox* genes, whose interactions are not yet completely understood (for details see Baker, 1998).

Compensatory eye movements following labyrinth stimulation in lampreys can be induced in any direction, although these animals do not possess horizontal semicircular canals or an equivalent of a MR muscle (Rovainen, 1976). The details of the neuronal connectivities underlying this behavior still need to be worked out.

While vestibulo-oculomotor connectivities have been elaborated in detail in the bony fish/tetrapod line (Figure 11), we are still lacking a definite answer as to the exact nature of the horizontal canal connections in elasmobranchs. Of particular interest are the special horizontal eye movement pathways in light of the contralaterally placed MR motoneurons in these animals.

Horizontal conjugate eye movements are produced by the simultaneous contraction of the LR muscle in one eye and the MR muscle in the other eye. In animals of the bony fish/tetrapod line, the decussating internuclear pathway from the abducens nucleus to the MR subdivision provides the necessary neuronal link between the two motoneuron populations (Figures 11c and 12a; Highstein and Baker, 1978; Carpenter and Baton, 1980; Highstein *et al.*, 1982). Since MR motoneurons in elasmobranchs are located contralateral to their respective muscles, they are found on the same side as the co-activated LR motoneurons. Therefore, we hypothesized that in these animals the organization of the horizontal VOR circuitry may be similar to that of the vertical systems, where one second-order vestibular neuron class links either the anterior or the posterior canal to two co-activated extraocular motoneuron populations (so-called yoke muscles) (Uchino *et al.*, 1980, 1982; Graf *et al.*, 1983; Graf and Ezure, 1986). In such a scenario, one horizontal second-order neuron would contact both LR and MR motoneurons to mediate conjugate eye movements in the horizontal plane (Graf and Brunken, 1984; Figure 12b). However, recent evidence suggests the existence of a contralaterally projecting internuclear pathway, besides other connectivities (Graf *et al.*, 2002; Figure 12b).

29.4.5 Lateral- and Frontal-Eyed Animals

Head movements in animals with different interocular angles, e.g., the extreme examples of rabbits and humans, seemingly require different compensatory eye movements. For instance, a head movement about the naso-occipital axis in a rabbit results in vertical eye movements, in a human, in torsional eye movements. In fact, if the reference frame is tied to the optic axis, such a difference is observed. However, if the reference frame is linked to the head, no difference occurs.

We had in fact elaborated the requirements necessary for compensatory eye movements in lateral, and frontal-eyed animals (Simpson and Graf,

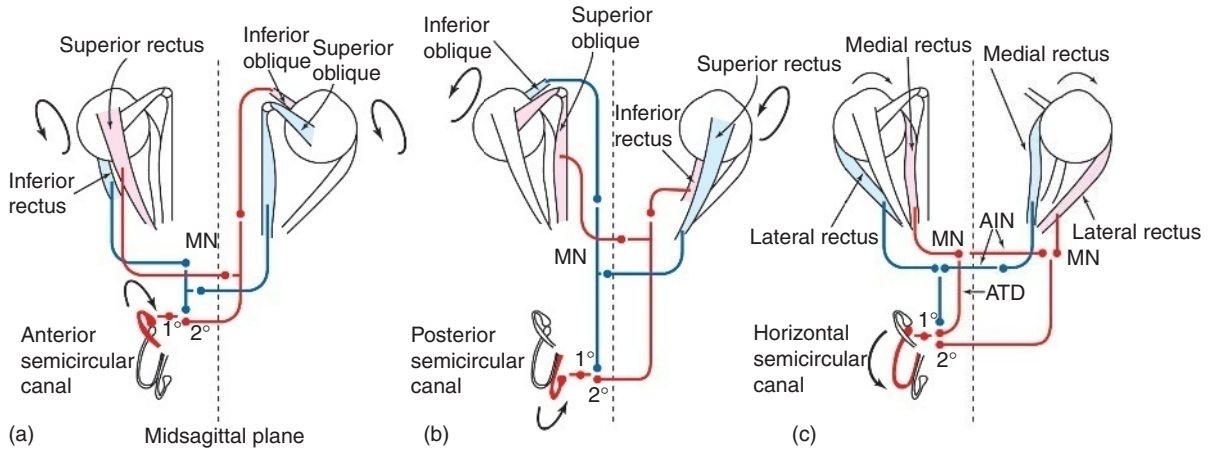

Figure 11 Spatial coordination of compensatory eye movements. Corresponding elements are illustrated in the same colors (red and blue). Semicircular canals and extraocular muscles form a three-dimensional intrinsic reference frame system for the production of VORs. The reflex arc consists of three neurons, the primary neuron (1°, vestibular nerve), the second-order vestibular neuron (2°, vestibular nucleus neurons), and the oculomotor neuron (MN, in oculomotor, trochlear, and abducens nuclei). Excitatory connections are shown in red, inhibitory connections are shown in blue. Contralaterally projecting vestibular neurons are in general excitatory, ipsilaterally projecting ones inhibitory. The respective semicircular canals (a, anterior canal; b, posterior canal; c, horizontal canal) and their efferent nerve pathways are marked in red. The on-directions of the semicircular canals are illustrated by thick black arrows. The connectivity of the horizontal system has a few peculiarities, such as an ipsilaterally projecting excitatory connection, the ascending tract of Deiters (ATD), and the abducens internuclear neuron pathway (AIN).

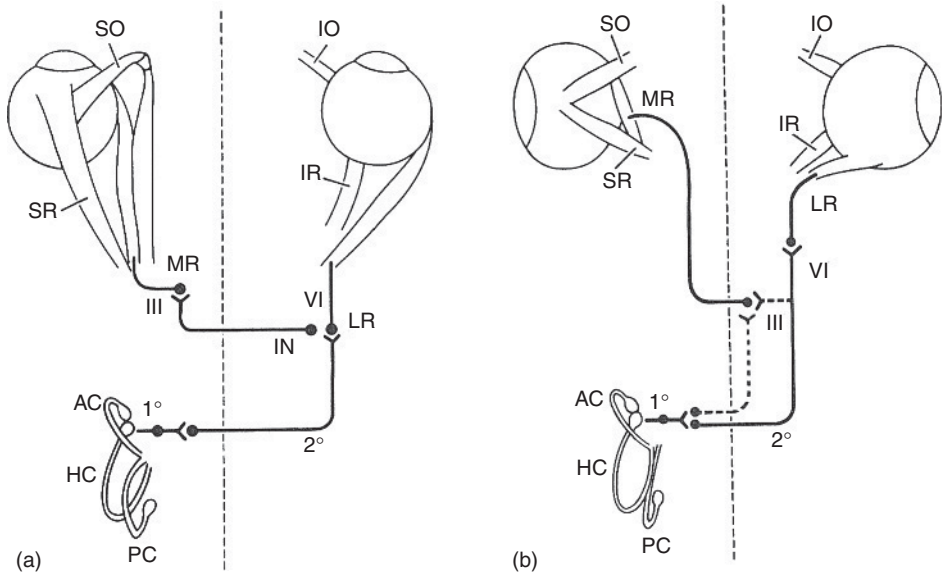

Figure 12 Schematic representation of vestibulo-ocular organization in vertebrates of the bony fish/tetrapod line (a) and elasmobranchs (b), including horizontal canal pathways. Note alignment of canals and related yoke muscles (left anterior canal, AC, with left SR, and right IO; left posterior canal, PC, with left SO, and right IR; left horizontal canal (HC) with left MR and right LR. The difference between the two prototypical vertebrate systems occurs in the horizontal reflex pathways. In vertebrates of the bony fish/tetrapod line (a), the connectivity to the lateral rectus muscle is of a three-neuron arc nature (vestibular afferent, 1°, second-order vestibular neuron, 2°, LR motoneurons in the abducens nucleus, VI), while an additional neuron, the abducens internuclear neuron (AIN) is inserted into the link to the co-activated MR muscle (MR motoneurons in the oculomotor nucleus, III). The three-neuron arc nature of the horizontal canal pathway in elasmobranchs (b), in particular the second-order vestibular neuron connectivity to LR motoneurons and MR motoneurons (2°) in the oculomotor nucleus, is hypothetical (Graf and Brunken, 1984). This fact is symbolized by the indication of the pathway in broken lines. Similarly, the nature of the contralaterally projecting internuclear pathway is not yet clear (Graf *et al.*, 2002).

1981, 1985; see also Ohm, 1919). There is no difference in the principal central nervous reflex connectivity, but subtle changes in eye muscle kinematics resulting from small changes in the insertion of vertical eye muscles during the course of evolution and the process of frontalization of the eyes.

29.5 Effectors of Sensory Input: Head–Neck Muscles

29.5.1 The Head–Neck Movement Reference Frame

Naturally, the system of the head–neck muscles used to perform head movements is more complex than that of the eye muscles, not only because of the far greater number of muscles involved (approximately 20 muscle pairs), but also because of the additional postural control functions these muscles have to fulfill. By contrast to the extraocular muscles, which do not have any postural function, one major task of head–neck muscles is to assure an upright head posture. Without the support function of the head–neck muscles, the head could not be balanced at labile equilibrium on top of the cervical vertebral column. Although the cervical vertebral column *per se* is relatively rigid, it has to be held upright, nevertheless, together with the head.

We were able to demonstrate the existence of a vestibular-based reference frame system also within the head–neck muscle system (Schaefer and Meyer, 1992; Graf *et al.*, 1997). However, the kinematic characteristics of the head–neck muscles are complex, and several muscle groups may cooperate and co-contract to perform a particular movement. Thus, the intrinsic geometry of the head–neck reference frame may not have become immediately obvious, although the very first systematic experiments by Flourens in the first half of the nineteenth century (Flourens, 1825, 1828) already pointed out its existence. These experiments involved selective transsections of semicircular canals in pigeons who subsequently performed movements in the plane of the lesioned canal. These could be eye–head, or even whole body movements (see also Suzuki and Cohen, 1964).

29.5.2 Vestibular Output and Postural Control

Some of the earliest motor control systems of vertebrates are the tectospinal and the vestibulospinal pathways. Tectospinal connections underlie visually based orienting and control mechanisms. Vestibulospinal pathways essentially provide tonic postural and balance control. This function can be impressively demonstrated following ablation of one entire labyrinth (hemilabyrinthectomy; see Schaefer and Meyer, 1974) or components thereof (de Waele *et al.*, 1989; Graf *et al.*, 1992). In essence, the horizontal semicircular canals provide the straight-ahead direction of the head, whereas the utricles assure the upright posture of the entire head–neck ensemble in the midsagittal plane, at least in birds and mammals. The sacculi seem to play a similar role regarding lateral tilt displacements of the head (Graf *et al.*, 1992). In this context, we have to mention that mammals in general possess a vertical cervical vertebral column, regardless of bipedal or quadrupedal locomotion (Vidal *et al.*, 1986; Graf *et al.*, 1995; Figure 2). The transition to bipedalism from quadrupedalism in mammals thus requires bringing the thoracic vertebral column into an upright position and modifications at the cervicothoracic junction and the lumbar level, but not within the cervical vertebral column or the atlanto-occipital articulation.

When considering the intrinsic geometry of the head–neck apparatus in the midsagittal plane, we observe that at resting position, mammals keep the articulations of the head–neck ensemble at the atlanto-occipital articulation and the upper cervical vertebral column in extreme flexion, and at the lower cervical and the upper thoracic vertebral column at extreme extension (Graf *et al.*, 1995; Figure 2b). At these endpoints, the head–neck ensemble takes on an intrinsic geometry that only has to be oriented into the correct direction by the vestibular system (Vidal *et al.*, 1993).

Since the cervical column is quite rigid, it also cannot be bent easily laterally. Lateral tilt of the entire head–neck ensemble in quadrupeds happens via rotation of vertebras at the cervicothoracic junction. Again, vestibular input provides the correct upright orientation (Graf *et al.*, 1992, 1995).

The intrinsic and semi-self-supporting architecture of the cervical column is a conserved feature in evolution, as some pertinent dinosaur findings have shown (dal Sasso and Signore, 1998). With regard to a general organization of postural mechanisms, vestibular circuits and their intrinsic three-dimensional coordinates are thought to provide a blueprint for a number of sensory and motor systems, and sensorimotor transformations (Cohen *et al.*, 1965; Schaefer *et al.*, 1975; Simpson *et al.*, 1981; Simpson and Graf, 1981, 1985; Graf, 1988; Graf *et al.*, 1988; Leonard *et al.*, 1988).

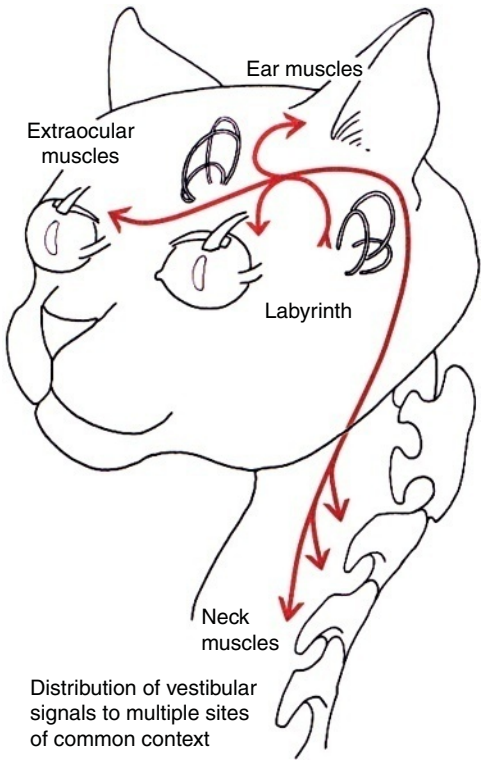

Figure 13 Schematic of vestibular neuron connectivity to motor centers of related architecture and common behavioral context. The shared neuronal pathway to eye, ear, and neck muscles would provide an economical distribution of an identical motor control signal to motoneuron pools involved in orienting behavior.

29.5.3 Vestibulocollic Connectivity

Within the vestibulocollic reflex connectivities, we find equally stereotypic innervation patterns as in the vestibulo-ocular circuitry (Wilson and Maeda, 1974; Shinoda *et al.*, 1994, 1996, 1997; Graf *et al.*, 1997). The only difference is that we are dealing with more muscles. Another indication for the similarity of reference frame systems for eye and head movements was indicated by the existence of vestibulo-ocular-spinal neurons (Graf and Ezure, 1986). These neurons would transmit their signals to oculomotor and spinal-motor centers at the same time (Figure 13). Thus, the same spatial information meaningful for the oculomotor system must carry a meaningful message for the spinal-motor system as well.

29.6 The New Wave of Vestibular Interest

The intriguing geometry and three-dimensionality of the vertebrate labyrinth has fascinated scientists since the beginning of modern science, i.e., Scarpa (1789), and numerous comparative studies have dealt with the expression of labyrinthine structures in basically almost all known vertebrates (Gray, 1907; Retzius, 1872, 1881, 1884; Werner, 1960; Lewis *et al.*, 1985). While all these studies used invasive methods to visualize ear structures, modern imaging methods have now opened a way to study them noninvasively in living tissue (Archer *et al.*, 1988; Spoor and Zonneveld, 1995); fossilized heads have also become accessible to large-scale investigations (Spoor *et al.*, 1994, 2002, 2003; Wittmer *et al.*, 2003; Clarke, 2005). These possibilities led to a number of interesting morphological discoveries that added to the vast data set already available.

In general, there were no surprises regarding the spatial orientation of the semicircular canals. These followed the familiar pattern (see Figure 1c), although some researchers seemed to be surprised by it (Spoor *et al.*, 1994). A number of authors also sought to make use of their new investigative tool to reinterpret the functional context of the vestibular system by putting it into the sole context of locomotion (Spoor *et al.*, 1994, 2002, 2003; Wittmer *et al.*, 2003). These authors argued that the dimensional morphology of the semicircular canals gave an indication about the locomotor capabilities of their owners. Thus, conclusions were drawn as to the point of effective bipedalism in certain hominids (Spoor *et al.*, 1994), or the agility of Neanderthal man (Spoor *et al.*, 2003). We have argued against such interpretations based on a number of known facts and characteristics of the vestibular system (Graf and Vidal, 1995). In essence, the former authors had based their arguments largely on the size differences in the circumference of semicircular canals within one species and across different species. However, canal fluid dynamics affecting sensitivity are also largely governed by the lumen of the canal, i.e., its cross section. Furthermore, to base locomotor activities solely on peripheral morphology means ignoring any well-known adaptive mechanisms at the receptor level, ion channel dynamics, and above all, the vast apparatus of the neuronal processing machinery that make use of vestibular signals from the brainstem and cerebellum to the cortex. Focusing on locomotion alone also ignores all the other important and vital functions subserved by the vestibular system, notably compensatory eye movements and perceptual mechanisms. Without compensatory eye movements, in particular, we would not be able to have unblurred vision during any movement. In addition, during active movements, a number of postural reflexes become suppressed, which is reflected in

elimination or attenuation of vestibular movement signals in the vestibular nuclei (McCrea *et al.*, 1999; Roy and Cullen, 2001). The arguments of Spoor *et al.* (2002) and Wittmer *et al.* (2003) have been forwarded to explain the behavior of cetaceans and pterosaurs, proposing a link between apparent extreme aquatic and aerial acrobatic capabilities of these animals, respectively. Although these findings received wide acclaim in the popularizing science literature (Stokstad, 2003; Unwin, 2003), the vestibular argument again did not take into consideration all aspects of vestibular function or the entirety of a biological system. Against the aerial capabilities of pterosaurs could be brought forward, for instance, the size and shape of their cerebellum, given that the cerebellum plays an eminent role in motor coordination. Pterosaur cerebella resemble closely that of certain bats (Baron *et al.*, 1996), and bats are not the very best flyers. As we have seen, postural control, locomotion, and eye movements are closely related to vestibular output, and there is a lot more to consider than meets the eye at first glance.

29.7 Conclusions

The evolution of the sense of balance of vertebrates and analogous systems in invertebrates suggests a number of important features of brain operations. We also observe conserved vestibulomotor organizations and circuitry in vertebrates after the development of one optimal solution, when arrangements have been preserved throughout subsequent vertebrate history. Compared to the many developments of eyes, for instance, the estimate is that eyes have been invented 40–65 times in evolution, only two basic types of three-dimensional movement detectors have been retained, the diagonal ones of vertebrates, octopus, and crabs, and the principal axes ones of squids. Each one of the two possibilities constitutes an ideal physical solution, with an optimal signal-to-noise ratio.

An additional important characteristic of central nervous operation seems to be that peripheral mechanisms are employed to simplify central operations. Such an operational principle has been ideally demonstrated in the common reference frames of the vestibulo-oculomotor system, including the central nervous connectivity. Thus, the workload of the brain is decreased in favor of animal economy and presumably higher-order operations (learning, perceptive functions, etc). When considering how the brain works, we have to look into similarities among apparent differences of expressions or behaviors. Disregarding obvious similarities of sensorimotor operations across species would mean disregarding one significant aspect of brain operation.

When viewing the particular example of the vestibulo-oculomotor systems across species, the conserved nature of the arrangement in its geometry, and to a large extent, its embryologic development is quite striking. Modern methods will hopefully enlighten us in the future, where traditional methods have failed and fossil records are absent. However, the early appearance of a viable and up-to-date conserved vertebrate vestibulo-oculomotor system, in tandem with systems of similar geometry in certain invertebrates may suggest that close to ideal physical solutions developed early in vertebrate history, onto which more advanced functions were added as a result of environmental pressure, or whatever circumstance, such as smooth pursuit or vergence eye movements. Finally, the initial function of the vestibulo-oculomotor system may well not have been to move the eyes, but to hold them still with respect to the environment in order to stabilize the visual world (Walls, 1962).

Acknowledgment

This work was supported by a grant from the European Union (QLK6-CT-2002-00151: EUROKINESIS).

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30 Evolution of Gustation

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Glossary

cyprinid	Pertaining to freshwater fish. All fish in this family are egg layers.
papillae	A bump occurring in various animal tissues and organs. Taste buds are found in circum vallate, foliate, and fungiform papillae.
taste buds	Small sensory organs that con tain gustatory receptor cells, basal cells, and supporting cells. Taste buds in humans are found in the epithelia of the tongue, palate, and phar ynx. They are innervated by the chorda tympani nerve (a branch of the facial nerve), the glossopharyngeal nerve, and the vagus nerve.
taste cells	Neuroepithelial cells found in taste buds.
tetrapod	A vertebrate animal having four feet, legs, or leglike appendages.
TRP channels (transi ent receptor channels)	Calcium permeable channels. They can be gated by a variety of chemical and physical stimuli. A subpopulation of taste cells contain TRPM5 channels and many pain fibers contain TRPV1 channels, which are activated by capsaicin, the com ponent in chile pepper that produces a burning sensation.

30.1 Introduction

The sense of taste allows mammals to discriminate between nutrient-rich stimuli and aversive, potentially toxic compounds. In vertebrates with a developed central taste system, it is fundamental to appropriate feeding behavior and survival (see The Evolution of Taste Systems). We show that the development of the mammalian brain made possible the formation of complex associations in the gustatory–reward cortices between the perceptual features of taste stimuli and the internal, physiological state of the organism. Supported by an intricate circuitry containing several distributed interacting pathways, mammalian feeding behavior became adaptive and efficient.

30.2 Peripheral Taste System

In vertebrates, the sense of taste is mediated by specialized epithelial cells arrayed in specific sensory end organs, the taste buds. Taste buds first appear phylogenetically coincident with the vertebrate lineage. In contrast, in invertebrates, the sense of taste is mediated via bipolar sensory neurons that have a distal process reaching the surface of the epithelium and a central process extending directly into the central nervous system, indicating a nonhomology with respect to vertebrates (Finger and Simon, 2000).

Several morphological features of taste buds are shared throughout the vertebrate lineage. They consist of proliferative basal cells, centrally situated elongated cells, and flattened edge cells that form the lateral boundary of the taste bud and the transition to extragemmal epithelium (Murray and Fujimoto, 1969; Finger and Simon, 2000). Taste buds occur mainly within the oropharynx but can also be found in some species, in the epiglottis and lips. In some cases, they are found across the entire body surface (see below). Nevertheless, in all cases, at specific regions of the taste bud the epithelial (taste) receptor cells make synapses with primary sensory neurons from the facial (VII), glossopharyngeal (IX),

and vagal (X) cranial nerves (CNs) (Norgren, 1990; Finger and Simon, 2000). In most species, taste buds have the overall shape of an onion and can contain 20–100 cells, with widths ranging from 30 to 100 mm (Duncan, 1964; Finger and Simon, 2000).

In tetrapods (all of which have tongues), taste buds are located on the tongue as well as in the mouth and throat (Butler and Hodos, 1996). In some amphibians, such as the frog, the tongue is a soft organ covered by 400–500 scattered fungiform papillae, most of which contain disk-shaped taste buds with no pores (Rapuzzi and Casella, 1965), comprising distinct supporting cells that do not synapse onto sensory fibers (Osculati and Sbarbati, 1995). In birds, some avian taste buds are situated deep in the epithelium and have a long taste pore, called the taste canal (Ganchrow and Ganchrow, 1987). It is not clear whether this could be generalized to all birds, but in general they seem to have fewer taste buds than tetrapods (Butler and Hodos, 1996). Taste buds in bony and cartilaginous fishes contain three distinct cell types (elongated cells bearing small microvilli, elongated cells bearing a thick microvillus, and serotonergic basal cells) that synapse onto either other taste cells or sensory nerve fibers (Finger and Simon, 2000). In some fishes, taste buds are located in areas other than the mouth and throat. For example, in cyprinids (which include carps and goldfishes), taste cells are present across the entire body surface, allowing these animals to taste their environment while searching for nutrients (Butler and Hodos, 1996).

In mammals, taste buds normally comprise a collection of 50–100 elongated epithelial cells and a comparatively smaller number of proliferative basal cells (Kruger and Mantyh, 1996). Each taste bud contains several distinguishable types of elongated taste cells, based on morphological and biochemical features, first revealed by Murray (1973) in his studies on rabbit taste receptor cells. Among these features, one way to characterize these taste cell types is to study which proteins they express. Briefly, type I cells express GLAST, a glial glutamate transporter (Lawton *et al.,* 2000), suggesting glial function; type III cells can be characterized by the expression of SNAP25, a synaptic membrane protein, which indicates transmission of information to the central nervous system (Finger, 2005). Studies suggest that this is the only cell type that forms synapses and the transmitter is ATP (Finger *et al.*, 2005). Type II cells are especially interesting in that they express the entire transduction cascade for sweet, bitter, and umami chemoreception, including the downstream transduction-related molecules phospholipase Cβ2 (PLCβ2) and IP3R3 (Miyoshi *et al.,* 2001). The downstream product of these transduction pathways appears to be the TRPM5 channel (see Figure 1 and Perez *et al.*, 2002; Zhang *et al.*, 2003).

In any event, these different cell types express taste receptors on the apical surface corresponding to the considered five basic modalities of mammalian gustatory senses: sweet, sour, bitter, salty, and umami (often described as the taste of protein, as elicited by monosodium glutamate and 5′ nucleotide monophosphate) (Scott, 2005; see Figure 2).

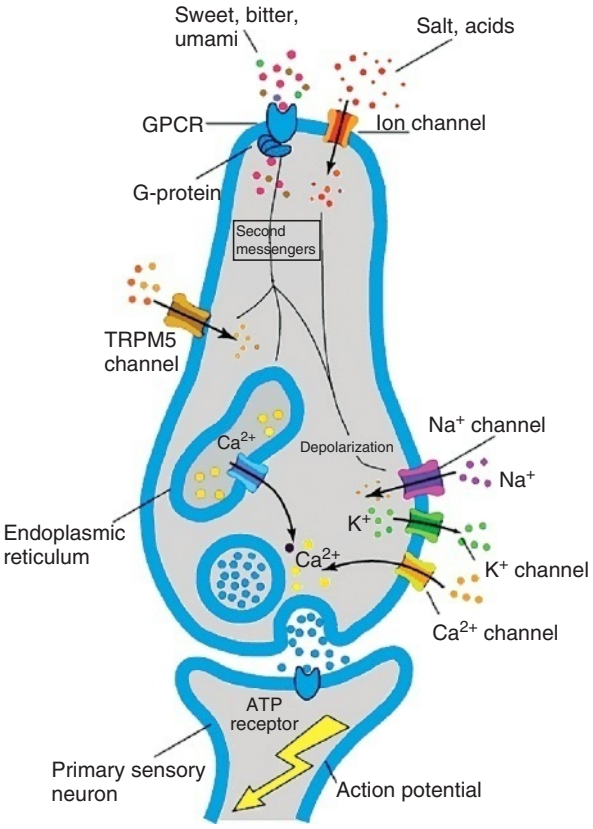

Figure 1 Schematic representation of sensory transduction in taste cells. Ion channels detect the presence of salty (NaCl) and sour (HCl) tasting compounds, whereas G-protein-coupled receptors respond to umami, sweet, and bitter tasting compounds (see Figure 2). All of these receptors are located in the apical domain of taste cells, which is separated from the basolateral domain by tight junctions. The components of the internal signaling cascade that is coupled to taste receptor molecules (including G-proteins and associated second-messenger molecules) are also preferentially expressed in the apical domain. Voltage-gated Na^+, K^+, and Ca^{2+} channels mediating the release of neurotransmitter from presynaptic specializations at the base of the cell onto sensory fibers are located in the basolateral domain, as well as the endoplasmic reticulum, which is also involved in regulating Ca^{2+} intracellular concentration. Communication between taste cells and primary sensory fibers is mediated by the neurotransmitter ATP and possibly serotonin. Another channel that is involved in G-protein-coupled receptor-mediated responses and taste cell depolarization is TRPM5.

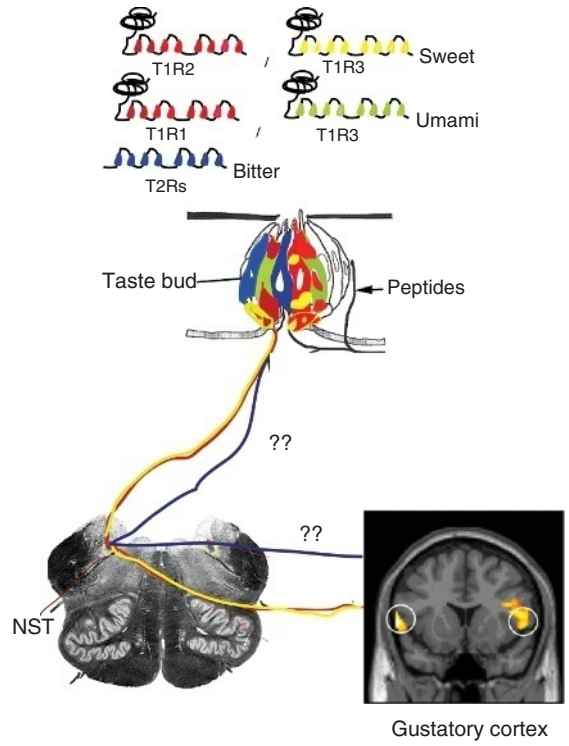

Figure 2 Diagram depicting families of taste receptors recognizing sweet, bitter, and umami substances. The heteromer T1R2/T1R3 responds to compounds that produce a sweet taste (e.g., sucrose, glucose), whereas T1R1/T1R3 responds to receptors that are activated by compounds that produce the umami taste (e.g., monosodium glutamate). The T2R family recognizes compounds that produce bitter taste sensations (e.g., caffeine, quinine). These signals seem to be transduced separately to the central nervous system through specialized gustatory neurons (labeled lines) that elicit different behaviors. Illustrated are these neurons' projections to the solitary nucleus and its tract (NST) and further projections to the primary gustatory cortex. This separation appears to persist at higher levels in the gustatory pathway although more definitive evidence is needed (question marks). The peptidergic general sensory perigemmal neurons are also shown. These neurons have receptors for molecules such as capsaicin (TRPV1) and provide information regarding the pungency of foods.

Salty (NaCl) taste uses the amiloride-sensitive sodium channel, ENaC (Lindemann, 2001). Other salts may permeate taste cells via a TRPV1 splice variant (Lyall *et al*., 2004). Sour taste, which is represented by the hydronium concentration, uses a variety of pathways depending on whether it is a strong or weak acid (Lyall *et al*., 2004; DeSimone *et al*., 2001; Caicedo *et al*., 2002). The other standard modalities are mediated by G-protein-coupled receptors. Sweet and umami detection are mediated by the T1R receptor family. Three genes, T1R1, T1R2, and T1R3, control their expression in taste cells. Evidence indicates that T1R receptors function as heterodimers, in that the T1R1/T1R3 combination in rodents is broadly tuned to amino acid detection (in humans it is narrowly tuned to glutamate) and T1R2/T1R3 to sweet detection (Li *et al.,* 2002). The T2R receptor family, comprising approximately 30 members, is known to be necessary and sufficient for the perception of bitter taste (Mueller *et al.,* 2005). T2Rs are of high behavioral relevance, since they mediate the detection and consequent rejection of potentially poisonous or toxic substances. Sequence polymorphisms between T2Rs of mice (Kim *et al.,* 2003) and nonhuman primates (Parry *et al.,* 2004) with respect to those expressed in humans were linked to different bitter sensitivities in these species. Species-specific sensitivities for sugars have also been shown (e.g., mice vs. human; Zhao *et al.,* 2003). This indicates ongoing evolutionary diversification of T1R and T2R receptors and a role in dietary adaptation and nutrient selection (Parry *et al.,* 2004).

30.3 The Mammal Central Taste System

30.3.1 Anatomy

Rodents and primates (including humans) constitute the most studied cases of central gustatory processing. The chemosensory information from CN VII primarily involves the sense of taste, whereas CNs IX and X convey chemosensory information that drives the swallowing and gaping reflexes (Markison *et al*., 1996). Also, general sensory fibers from CNs V, IX, and X provide textural and thermal responses as well as information from irritating chemosensory stimuli. In all these species, CNs VII, IX, and X transmit electrical signals that convey the chemical properties and quantity of tastants to the rostral division of the nucleus of the solitary tract (NST) of the medulla, the principal visceral sensory nucleus of the brainstem. In the rat, second-order fibers (i.e., NST afferents) project ipsilaterally to the gustatory parabrachial nuclei (PBNs) in the pons, proceeding then to the parvicellular part of the ventroposterior medial nucleus of the thalamus (VPMpc). In primates, the NST projection fibers bypass the PBN only to join the central tegmental tract and synapse directly into the VPMpc (Pritchard *et al*., 2000), whereas the PBN seems to be dedicated to convey general visceral information (e.g., from the vagus) to specialized thalamic nuclei including VPM (Pritchard *et al*., 1989, 2000).

Thalamic afferents then project (reciprocally) to the gustatory cortex (Scott and Plata-Salaman, 1999). In the rat, it was found that parabrachial fibers reach some forebrain areas including the

lateral hypothalamus and the central nucleus of the amygdala, giving gustatory information direct access to motivational and reinforcement-related structures including the dopaminergic system (through direct projections from the central nucleus of the amygdala; Fudge and Haber, 2000).

The primary taste cortex in macaques can be defined in terms of VPMpc afferents (Scott and Plata-Salaman, 1999). Pritchard *et al*. (1986) have studied the efferent projections of the VPMpc of the monkey, *Macaca fascicularis*, with tritiated amino acid autoradiography. Two discrete cortical areas were characterized as a target of VPMpc projections. First, labeled cells were located in the ipsilateral insular–opercular cortex adjacent to the superior limiting sulcus and extending as rostrally as the caudolateral orbitofrontal cortex. Moreover, further projections were located within the primary somatosensory cortex (SI), in the precentral gyrus subjacent to the anterior subcentral nucleus (i.e., a precentral extension of SI). This area is anterior to the VPM projection sites representing somatosensory information and is adjacent to or overlaps with cortical somatotopic sites for the face and oral cavity (Jain *et al*., 2001). Thus, this area might be a target of VPM and VPMpc projection fibers and thus implement the convergence in the cortex of the somatosensory and gustatory aspects of stimuli delivered in the mouth (see below).

Scott and Plata-Salaman (1999) define the anterior limit of the primary taste cortex in the macaque as the junction of the orbitofrontal and opercular cortices, from which it extends 4.0 mm posteriorly. The mediolateral extension is defined ~16–19 mm lateral to the midline in an average adult macaque. The dorsal limit is defined as ~6 mm above the lateral fissure. The insular cortex, in the depth of the Sylvian fissure, has been divided into four rostrocaudal subdivisions (Cipolloni and Pandya, 1999): the most rostral portion has been designated the insular proisocortex; adjacent to it is the agranular subdivision of the insula, followed caudally by the dysgranular and the granular insular areas. In these terms, the VPMpc nucleus projects to the opercular and insular regions of the granular and dysgranular insula and extends to adjacent agranular portions of the insula.

One of the projections from this primary taste cortex is to the central nucleus of the amygdala where gustatory information reaches the basal forebrain, lateral hypothalamus (Scott and Plata-Salaman, 1999), and dopaminergic cells in the substantia nigra pars compacta and ventral tegmental area (Fudge and Haber, 2000). Fibers also project anterior to the dysgranular caudolateral orbitofrontal region (which is defined as a secondary taste cortical area by Baylis *et al*., 1995). This transition zone, including the more anterior parts of the primary taste cortex and the adjoining caudolateral orbitofrontal cortex, was also named area G by Carmichael and Price (1996). Taste neurons in the caudolateral orbitofrontal cortex form connections laterally with visual areas in the inferior temporal cortex and, importantly, converge with more medial cells receiving projections from primary olfactory cortex, which have implications for the perception of flavor. Taste-responsive cells in the caudal orbitofrontal cortex project to the caudate nucleus, where taste information is distributed throughout the striatum, and lateral hypothalamus (Öngur *et al*., 1998; Scott and Plata-Salaman 1999), which in turn communicates directly with the central nucleus of the amygdala. The central nucleus of the amygdala in turn projects back to the NST (Price and Amaral, 1981). The described circuit could then form a complex neural network integrating information about the identity of individual tastants with their hedonic and motivational properties.

30.3.2 Electrophysiology

In rodents and monkeys, taste cells have been sampled across the central gustatory pathway by electrophysiological techniques and this may reveal some species-specific features. For example, in rats, NST taste-responding cells seem to be modulated by physiological need and satiety signals (e.g., gastric distention; Glenn and Erickson, 1976). However, NST taste cells in primates are unaffected by satiety, as shown, for example, by reversing the incentive value of glucose in a sensory-specific satiety type of experiment (Yaxley *et al*., 1985). This apparent distinction between the rodent and primate cases might be partially accounted for by the fact that in primates NST projection fibers bypass the PBN, where visceral and physiological information could be preferentially processed.

Top-down regulation is an important feature of taste processing in that stimulation of the hypothalamus or the central nucleus of the amygdala can modulate responses to tastants in NST and parabrachial nuclei (Cho *et al*., 2002; Li *et al*., 2005). This is significant since both the amygdala and the hypothalamus receive projections from cortical taste areas and could thus work as an intermediate for cortical modulation of taste processing at the brainstem level. Notice that these top-down pathways also exist in primates (Price and Amaral, 1981).

In the primates, despite its name, only a small proportion of cells in the primary taste cortex do

actually respond exclusively and consistently to taste stimuli (Scott and Plata-Salaman, 1999; ~6.5%), whereas a higher proportion (~23%) responded during tongue or jaw movements, for example. This suggests that the primate primary taste cortex might be encoding simultaneously taste and oral somatosensory properties of (intraoral) stimuli. These recording studies then constitute an early indication that multisensory encoding might occur in the primary taste cortex.

In the primary gustatory cortex (in primates, including both frontal opercular and dysgranular insula), the responses of taste-related neurons are multisensory and are more broadly tuned than in NST and VPMpc (Sewards and Sewards, 2001). Interestingly, in the rodent case, Katz *et al.* (2001, 2002) have shown that when time is accounted for as a source of variability, the taste specificity of the responses increased from approximately 10%, when only the average activity is considered, to 41% of the recorded gustatory cells, suggesting that encoding of temporal information is a central feature of taste processing. In this regard, Katz *et al.* (2002) have also shown that neurons that exhibit synchronous activity may also contribute to the identification of tastants.

Single-cell recording studies of the secondary taste cortex (orbitofrontal cortex) were able to evidence more clearly the distributed and multimodal characteristics of taste processing in primates. The role of the primate orbitofrontal cortex in reward processing has been consistently established by a number of different lines of evidence. In nonhuman primates, there is strong evidence at the single-neuron level that the orbitofrontal cortex responds as a function of the reward value of taste (Rolls *et al.*, 1989), olfactory (Critchley and Rolls, 1996), and visual stimuli (Critchley and Rolls, 1996). This shows that vision, a sensory modality especially developed in primates, can also provide inputs for association with taste perceptual information (see Primate Brain Evolution).

In the specific case of neurons responding to the reward value of taste stimuli, neurons in the macaque monkey orbitofrontal cortex have been shown to respond in a sensory-specific satiety manner (Rolls *et al.*, 1989). In addition, reward-related learning and expectation appear to be represented at the single-neuron level in the primate orbitofrontal cortex (Schultz *et al.*, 2000), probably involving the midbrain dopaminergic system. Thus, the findings detailed above provide evidence that a part of the primate taste cortex could support the simultaneous encoding of several sensory features of taste stimuli, including stimulus identity, multisensory combinations (olfactory, somatosensory), and reward value (see The Evolution of the Somatosensory System).

30.4 Functional Neuroimaging

Single-cell recording studies are limited to a relatively small number of samples from a single cortical area. To understand the dynamics between multiple brain areas representing taste–reward pathways, one may use bundles of electrodes implanted in each area (Nicolelis *et al.*, 2003). This field is in its infancy with respect to gustatory processing. However, advances in human functional neuroimaging techniques, such as functional magnetic resonance imaging (fMRI; for a description of its physiological basis, see Logothetis *et al.*, 2001), allowed for more general descriptions of taste processing in humans.

Human studies indeed confirmed that gustatory areas homologous to those of primates (as defined by anatomical studies) are responsive to unimodal taste stimuli in humans, including the anterior insula/frontal operculum, the orbitofrontal cortex, and the amygdala (Small *et al.,* 1999; O'Doherty *et al.,* 2001; de Araujo *et al.,* 2003a). This includes responses to glucose, NaCl (O'Doherty *et al.*, 2001), umami (de Araujo *et al.,* 2003a), caffeine, and citric acid (Schoenfeld *et al.,* 2004). In particular, the de Araujo *et al.* (2003c) study revealed activations for taste (sucrose) in all homologous areas in the ascending central taste pathway receiving first- or second-order projections from the VPMpc: the frontal operculum/insula complex, the orbitofrontal cortex, the amygdala, and the ventral forebrain, which most likely included anterior parts of the hypothalamus (see Figure 3).

Studies with human subjects provide evidence that gustatory cortices not only respond to the major perceptual categories of taste, but also support the encoding of the multisensory aspects of taste stimuli. In a study using taste and retronasal olfactory stimuli (and their combinations), de Araujo *et al.* (2003c) have shown that taste and olfactory inputs in the human brain converge in particular in the far anterior (putatively agranular) insular cortex. This region of the far anterior (agranular) insula is close to the part of the insular cortex where it adjoins the caudal orbitofrontal cortex.

A homology between the rodent and primate cases with respect to the central anatomy of taste and olfactory integration has been previously suggested and, thus, it is being proposed here that this homology would extend to humans to encompass at least three mammal species. In fact, Shi and Cassell (1998) reported that in rats both the granular and

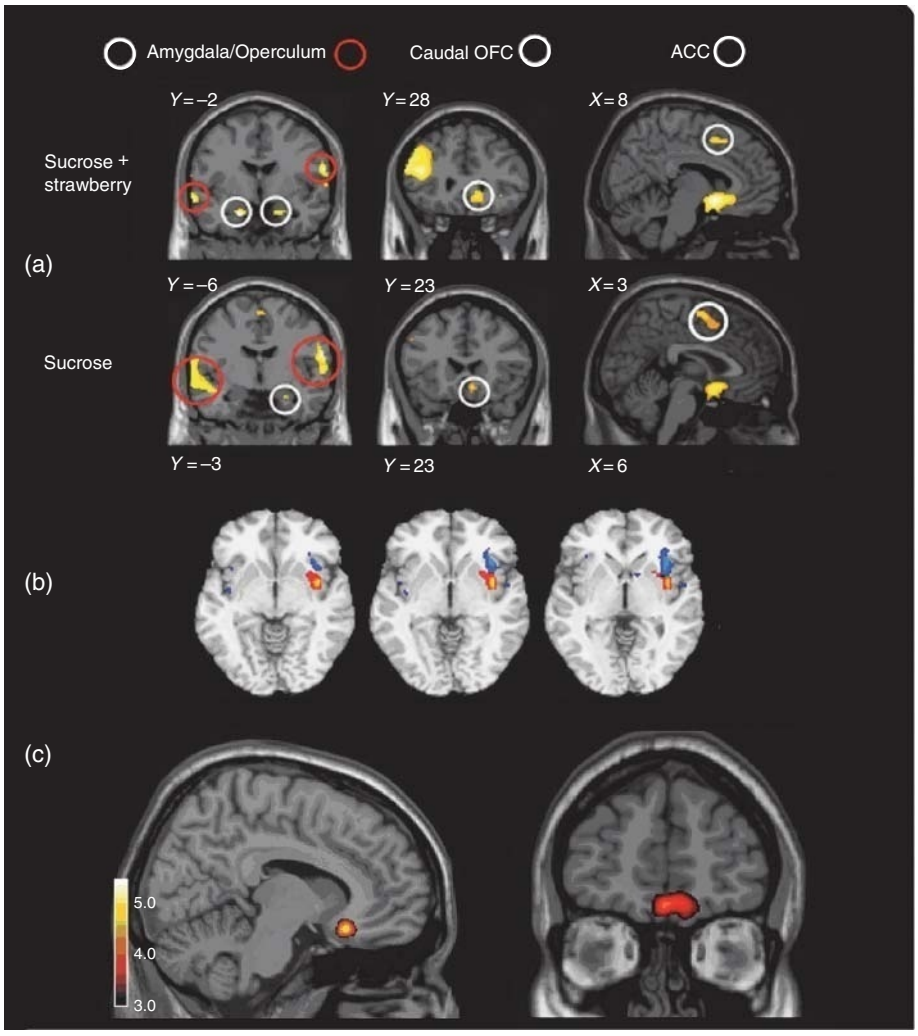

Figure 3 Increases in activity level in human gustatory brain areas as detected by functional magnetic resonance imaging. a, Activations produced by 0.5 M sucrose (bottom row) and sucrose combined with strawberry odor (top row) were observed in most of the central gustatory areas: insular/operculum, medial (rostral and caudal) orbitofrontal cortex, amygdala, and forebrain (which might include the hypothalamus and some parts of the thalamus). This shows that cortical gustatory areas support multisensory maps involving taste representations. b, Insular areas of the human brain responding to both pure tastants (sucrose) and water in the mouth (blue) and correlating with hydration states (red). c, Left, medial orbitofrontal cortex region, where it borders the subgenual cingulate cortex, that responds to water in the mouth only when thirst is present, thus indicating representations in the taste cortex of the internal state of the organism. Right, an anterior medial orbitofrontal cortex area in which activity correlates with the subjective pleasantness of a taste/olfactory mixture. Adapted from de Araujo, I. E., Kringelbach, M. L., Rolls, E. T., and McGlone, F. 2003b. Human cortical responses to water in the mouth, and the effects of thirst. *J. Neurophysiol.* 90, 1865 1876 and de Araujo, I. E., Rolls, E. T., Kringelbach, M. L., McGlone, F., and Phillips, N. 2003c. Taste olfactory convergence, and the representation of the pleasantness of flavor, in the human brain. *Eur. J. Neurosci.* 18, 2059 2068.

the dysgranular zones in the posterior insula are part of the gustatory cortex (see also Cechetto and Saper, 1987). Moreover, based on the projection patterns among the granular, dysgranular, and agranular parts of the rat insula (as well as on the projection patterns from the VPMpc; Cechetto and Saper, 1987), Shi and Cassell (1998) claimed that the dysgranular insular cortex constitutes a secondary taste association cortex (in contrast to the lower-order granular zone). They further hypothesized that the agranular part of the insular cortex is a tertiary taste association cortex supporting flavor perception (given its afferent projections from the olfactory bulb and piriform/endopiriform cortices). Sewards and Sewards (2001) proposed thus that this homology between the rodent and primate cases holds also for the secondary (dysgranular) and tertiary (agranular) taste association area. In particular, the agranular insula and the adjoining caudal part of the orbitofrontal cortex would support flavor perception given the convergence of olfactory and taste inputs in these areas. Further regions of

taste/retronasal odor convergence found in this neuroimaging study (de Araujo *et al.*, 2003c) include the amygdala, the ventral forebrain, and the anterior cingulate cortex, which is targeted by regions of the anterior insula, possibly including taste cortex (Vogt and Pandya, 1987). The findings above thus indicate that several parts of the central taste system allow for combinations between taste and odor to form flavor percepts; moreover, its anatomical bases seem comparable across distinct mammal species.

As mentioned, it has been found in monkeys that a representative number of neurons in the primary taste cortex respond to oral somatosensory/motor stimulation (Scott *et al.*, 1986; Ogawa, 1994). In fact, it has been shown in humans (de Araujo and Rolls, 2004) that activation of the anterior insular (putative primary) taste cortex by oral viscosity stimuli occurs in such a way that brain activation in this region was proportional to the log of the viscosity of the oral tasteless stimuli (carboxymethyl cellulose), providing evidence of somatosensory/gustatory integration in the primary taste cortex. It is known that in more posterior regions of the insular cortex in owl monkeys (Jain *et al.*, 2001), the caudal part of the face representation in area 3b extends anterior beneath the central sulcus and above the upper bank of the lateral sulcus. The representation of the oral cavity is located rostral to this region extending to the orbitofrontal cortex (Manger *et al.*, 1996; Jain *et al.*, 2001). The de Araujo and Rolls (2004) study used quantitative variation of texture features of intraoral stimuli by manipulating viscosity and found activation of the midinsular and anterior insular cortices.

Another example of activations in the human primary taste cortex that are independent of the major perceptual categories of taste is activations to water in the mouth, when subtracted from activations produced by artificial saliva at the same viscosity (de Araujo *et al.*, 2003b). This corroborates previous electrophysiological studies in macaques showing that water in the mouth activates neurons in the primary taste cortex in the anterior insula and adjoining frontal operculum (Scott *et al.*, 1986; Yaxley *et al.*, 1990). Thus, not only the stimulation of taste receptors by prototypical tastants, but also substances generally relevant to behavior and survival, seem to elicit responses in the mammal gustatory cortices.

In fact, to guide feeding behavior and maintain energy homeostasis, mammal brains must not only represent the sensorial aspects of an intraoral stimulus, but also combine these with the internal state of the organism, in that they must ascribe the stimulus motivational value. Human studies also provide evidence that the reward value of taste is represented in the gustatory cortices, in particular in the orbitofrontal, insular, and anterior cingulate cortices. Small *et al.* (2001) found that the caudomedial part of the orbitofrontal cortex and a region of the midinsula represent the changing reward value of a food eaten to satiety. Interestingly, the same pattern of responses was found in responses to water in the mouth at different levels of hydration: activity in the mediocaudal orbitofrontal cortex and midinsula is modulated by the physiological state (thirst) of the body (de Araujo *et al.,* 2003b).

The finding that the midinsula and adjoining posterior insular areas respond to water in the mouth in a (thirst) state-dependent way is in agreement with the viscerotopic map of the rat insular cortex as proposed by Cechetto and Saper (1987). Their results suggest an anterior–posterior distribution of visceral representations in the rat insula, with special visceral (taste) projections situated preferentially in more anterior areas, whereas general visceral (including gastric mechanoreceptor-responsive and cardiopulmonary units) were distributed more posteriorly and dorsally. The human insula might thus reproduce such topography by combining special visceral (taste) and general visceral inputs in insular regions.

In addition to the current motivational value of a taste stimulus, the secondary taste cortex, the orbitofrontal cortex, and the adjacent anterior cingulate cortex area also represent the degree to which subjects ascribe reinforcing properties to gustatory-related stimuli. For example, correlations with consonance and pleasantness ratings for the smell and taste combinations were found in a medial anterior part of the orbitofrontal cortex (de Araujo *et al.,* 2003c), the pleasantness ratings for a food eaten to satiety were correlated with activity in the medial orbitofrontal cortex (Small *et al.,* 2001), and the (subjective) rewarding properties of water in the mouth under different hydration states were correlated with activity in the medial orbitofrontal cortex and in the far anterior cingulate cortex (de Araujo *et al.,* 2003b). Moreover, the orbitofrontal cortex is also involved in encoding the reward value of visual signals predicting taste stimulus receipt. In a classical conditioning paradigm, where a previously neutral cue was associated with receipt of glucose, expectation of the pleasant taste produced activation in particular in the amygdala and orbitofrontal cortex (equivalent results were found for a cue predicting receipt of an unpleasant taste, saline; O'Doherty *et al.*, 2002), also evidencing the ability of the human cortex to associate visual and taste representations.

The evidence described above indicates that the mammal taste cortex can serve a more general

purpose other than simply representing the end line for ascending taste information. It seems rather that this is a byproduct of a more general function, namely, to encode information about stimuli relevant for survival, be they taste stimuli (sugars), nutrient-rich stimuli with particular textures (fat), or clean water. Thus, it should be involved in generating behavior through back-projections to the noncortical regions of the taste system, such as the hypothalamus and the brainstem.

30.5 The Mammal Taste System in the Context of Vertebrate Evolution

Mammals first appeared approximately 210 Mya during the first interval of the Mesozoic era, approximately at the same time as crocodiles and dinosaurs (e.g., Rougier and Novacek, 1998). A characteristic feature of all living mammals is a 1–3 mm thick, multilayered sheet of neural tissue situated between more lateral olfactory areas and medial hippocampal areas, the isocortex (Northcutt and Kaas, 1995). Although there are different views on how the mammalian isocortex might have evolved from their nonmammalian ancestors (e.g., outgroup vs. recapitulation hypotheses; Northcutt and Kaas, 1995), it seems clear that it resulted in more complex cortical processing and much higher associative power. Aboitiz *et al*. (2003), for example, argued that the mammalian isocortex appeared by means of a dorsalizing effect during the early development of the pallium of the first mammals. This would have resulted in the formation of a hippocampal–dorsal cortex circuit supporting complex olfactory-based representations of space. The ability to form such complex representations and to use them to guide behavior would be then a hallmark of mammalian evolution.

In fact, when compared to other tetrapods, the multilayered cortex seems to account for most of the specificity in mammalian sensory processing. Comparative data on gustatory processing are very scarce. It nevertheless seems clear that in nonmammalian tetrapods, CN fibers provide taste-related information to the ascending gustatory pathway arising in the nucleus of the solitary tract that then projects to the parabrachial region, which in its turn projects extensively to the forebrain, as in the case of the lizard *Varanus exanthematicus* (Ten Donkelaar and De Boer-Van Huizen, 1981). The forebrains of reptiles and mammals are similar in that the dorsal surface of their cerebral hemisphere is formed by a pallium with three major segments: an olfactory (laterally situated) cortex, a limbic cortex (dorsomedial), and an intermediate cortical tissue that in the mammal case corresponds to the isocortex, but in reptiles and birds consists of part of the dorsal cortex and the dorsal ventricular ridge (Ten Donkelaar, 1999) (see Vertebrate Olfactory Subsystems and their Evolution). In any case, in all tetrapods, gustatory information (as well as other modalities) reaches the telencephalon, and the intermediate pallial segment receives sensory projections from the thalamus and contains modality-specific sensory (presumably including gustatory) areas in reptiles, birds, and mammals (Ten Donkelaar, 1999).

If the ascending gustatory pathway is homologous from the CN fibers up to thalamic (forebrain) level in several classes of vertebrates (tetrapods), the possibility remains that mammal-specific gustatory cortices could be heterogeneously structured across different mammal species. There is, nonetheless, evidence to the contrary. That is, all mammals seem to have a primary somatosensory area and homologous adjoining fields (Kaas, 1980). In addition, homologous limbic, orbital, and lateral gustatory fields can also be found in different mammal species (Northcutt and Kaas, 1995; Preuss, 1995), unlike, for example, some prefrontal regions specific to primates such as the dorsolateral prefrontal cortex (Preuss, 1995). In particular, when comparing the connection patterns of the rat insular cortex with those on the insular cortex of cats and monkeys, Guldin and Markowitsch (1983) suggested that on the basis of thalamocortical connections, the insular cortex is a heterogeneous structure with homologous subdivisions in each of these species, including a separate gustatory (somatosensory) insular region. Likewise, basal mammals seem to possess structures supporting higher-order taste-related cortical areas homologous to higher mammals, such as the orbital fields of the hedgehog tenrec (*Echinops telfairi*; Radtke-Schuller and Künzle, 2000).

The strongest indication that the mammal central gustatory system is conserved across different species comes from molecular genetic studies performed on mice by Charles Zuker, Nicholas Ryba, and colleagues (Mueller *et al*., 2005; Zhang *et al*., 2003; Zhao *et al*., 2003). As mentioned, mice and humans show different sensitivities for some sweet stimuli, such as aspartame, which cannot be recognized by mice. In this regard, mice engineered to express the human T2R homologous gene in place of the mice T2R gene develop a preference for aspartame, recognized as a sweet compound (Zhang *et al*., 2003). This seems to indicate that when different mammal species are provided with receptors for the same class of ligands, then behavior (e.g., avoidance/approach) is controlled through an innate, homologous dedicated (Sugita and Shiba, 2005) neural circuitry.

In summary, based on the available neurophysiological data from mammals and especially humans, it seems that gustatory processing has also benefited from the mammal-specific development of cortical layers supporting higher-order, cross-modality associations. This would allow tastants and other biologically relevant intraoral stimuli to be represented in multisensory maps, whose processing is distributed across different regions of the cortex. Information about the physical properties of a given compound will be combined with information about the internal physiological state of the organism. Information about the physiological state of the organism is carried by visceral inputs to the taste cortices (as in the case of conditioned taste aversion; Garcia *et al*., 1955) and by indexes on the animal's current fluid and energy status (as provided by specific hypothalamic regions responsive to changes in levels of hormones – leptin, insulin, angiotensin; e.g., Niswender *et al.,* 2004). These cortical sensory–visceral maps will then generate, through back-projections to the brainstem mediated by hypothalamic and amygdalar areas, a large repertoire of complex behaviors regulating food intake and body weight that is unique to mammals.

Acknowledgments

This work was supported in part by Philip Morris International Inc., Philip Morris External Research Program, and NIH grant DC-01065.

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31 The Evolution of the Somatosensory System

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Glossary

areas This term is often used to describe separate subdivisions of the brain and neocortex. In the neocortex different areas generally have a number of identifying features, such as unique appearance in histological stains (cytoarchitecture), unique connections to other areas, unique cellular responses, and result in specific deficits following damage. Some well known cortical areas include primary somatosensory cortex (S1), primary visual cortex (V1), and primary auditory cortex (A1).

cortical barrel A circular region of the neocortex visible in various histological stains of the somatosensory area in rodents where touch information from a single whisker projects. First recognized by Woolsey and Van der Loos (1970) in mice.

cortical magnification The relative size of a representation, or processing area for a sensory input, in the cortical map. This generally refers to the larger representations of behaviorally important sensory inputs as compared to less important inputs. A common example in humans is the large area of cortex devoted to processing touch information from the hand relative to other, larger body parts (such are the leg or back) that have a proportionally much smaller representation in the cortex.

cytochrome oxidase A mitochondrial enzyme. Processing brain tissue to reveal the distribution of this enzyme often reveals different subdivisions, particularly in the neocortex. Cortical barrels can be seen in the distribution pattern of this enzyme (Figure 1).

Eimer's organ A small (40 80 μm) swelling in the nasal epidermis of talpid moles that contains and orderly array of mechanoreceptors used for tactile discriminations. Similar to a push rod in montotremes.

electrosensory/ electroreception Electroreception is the ability to detect weak electric fields in an aquatic environment through dedicated sensory organs (electroreceptors). This sense is sometimes used by predators (e.g., sharks) to detect the small electric fields given off by prey.

neocortex The outer six layered sheet of brain tissue in mammals where much of the information from sensory receptors projects. Often shortened to 'cortex' in discussions of the mammalian brain. Many investigators prefer the term 'isocortex' to avoid the implication of an invalid phylogenetic sequence suggested by the term 'neo'.

mystacial vibrissae The large, mobile whiskers on the face of a rodent.

ocular dominance column	Stripes of cortical tissue in layer 4 of primary visual cortex that receive input from the lateral geniculate nucleus, relayed from primarily only one eye. Each stripe is generally bound by similar stripes representing the opposite, contralateral eye.
receptors	In this context, 'receptors' refers generically to the sensory organs and nerve endings that receive and communicate sensory information from the environment. More specific modality designations include mechanoreceptors, photoreceptors, electroreceptors, etc.
saccade	A saccade is a sudden, jerky movement. The term 'saccade' is most frequently used in reference to an eye movement. In the visual system a saccade is the characteristic sudden movement of the eye that positions different parts of a visual scene on the retinal fovea.
sensory representation	Generally refers to a topographic map of primary afferent inputs to the central nervous system (CNS). In the case of the somatosensory system, the sensory representations reflect the distribution of mechanoreceptors in the skin, and as such they form a 'map' of the body surface that can be identified in neocortex by recording the activity of nerve cells in response to stimulating the skin.
somatosensory cortex	The area of neocortex that receives and processes touch information from mechanoreceptors on the body.
tactile fovea	The descriptor draws an analogy between the high resolution retinal fovea in the visual system and the high resolution part of the star nosed mole's nose used for detailed, tactile investigations of object of interest. A similar analogy with the visual system has been made in the auditory system of bats, where an 'auditory fovea' is said to represent the most important echolocation frequencies.

31.1 Introduction

The somatosensory system provides a rich source of diversity for revealing principles of mammalian brain evolution. At the same time, it is daunting to consider the number of different aspects of mammal bodies that have changed in the course of evolution and often challenging to identify examples of brain specializations that can be confidently attributed to specific sensory adaptations. Consider, for example, the vast difference in brain size between shrews – that resemble ancestral mammals in many respects – and humans, that have only recently emerged on the evolutionary landscape (Figure 1).

This comparison highlights some of the challenges to deciphering mammalian brain evolution, as the differences between shrew and human brains may parallel the differences between the small brains of early stem mammals and the larger and more complex brains found in many modern lineages. The comparison of a human brain to a shrew brain seems appropriate as an introduction because it not only illustrates a range of mammal brain sizes, but also because insectivores hold a particularly important historical position in theories of mammalian brain evolution. Fossil evidence indicates that the earliest ancestral mammals had brains and bodies similar to those of modern insectivores, particularly shrews that have little neocortex (Kielan-Jaworowska, 1983, 1984). As a result, a number of theories of brain evolution have been based on the premise that modern insectivore brains resemble those of ancestral species (Lende, 1969; Glezer

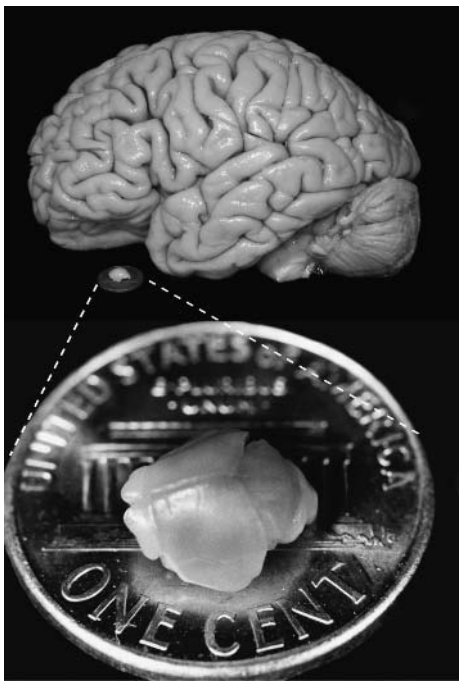


Figure 1 An adult human brain compared to the brain of a shrew. The upper panel shows the two brains at the same scale, with the shrew brain resting on a penny for scale. The lower panel shows the shrew brain enlarged.

et al., 1988; see Deacon, 1990, for review). This historical trend was bolstered by early recording experiments in hedgehogs (Lende and Sadler, 1967) and moles (Allison and Van Twyver, 1970) which indicated that insectivore cortex was poorly differentiated with overlapping cortical subdivisions. However, recent investigations of insectivores (Catania, 2000a) have revised our conception of these species as primitive mammals with poorly organized brains and thus historical theories of brain evolution based on early investigations of insectivores need to be reconsidered.

Before developing theories for how somatosensory cortex may have evolved in different lineages, it is first essential to describe what is known about the products of evolution. What are the major differences in brain organization observed across different species? What facets are unique to particular lineages, and what has been conserved across taxa? What solutions to sensory processing have recurred in the course of evolution and may thus illuminate constraints on the ways brains can be modified?

Although the brains of only a small percentage of extant mammals have been examined in detail, recent investigations of somatosensory cortex have expanded our understanding of brain organization in mammals that range from standard laboratory rats (Remple *et al.*, 2003) to monotremes (Krubitzer *et al.*, 1995; Krubitzer, 1998) and marsupials (Beck *et al.*, 1996; Rosa *et al.*, 1999; Huffman *et al.*, 1999; Catania *et al.*, 2000) representing important branches of the mammalian radiations. By considering the organization of cortex in selected species, it is possible to draw some general conclusions about how cortical organization has changed in the course of mammalian evolution.

In addition to our growing understanding of brain diversity across species, a number of recent advances in the ability to modify gene expression during the course of development have allowed investigators to mimic the process of brain evolution in the laboratory. Thus, on a small scale, some of the diversity that is observed across species can be generated within species by manipulating gene expression (Fukuchi-Shimogori and Grove, 2001). This in turn suggests potential mechanisms by which brains may have been modified in the course of evolution (Rakic, 2001).

Finally, in discussing the evolution of somatosensory areas in the brain, it is important to simultaneously consider the mechanosensory periphery. After all, the main function of the somatosensory cortex is to process information from these receptors and there is an intimate association between the sensory periphery and the central nervous system (CNS) during the course of both development and evolution.

31.2 How Have Brains Changed in the Course of Evolution?

31.2.1 Areas May Be Added to the Processing Network

There is still much disagreement and uncertainty regarding the organization of cortex and the identity of areas in many of the most intensively investigated species (see Kaas, 2005). However, it is nevertheless clear from comparative studies that larger brains differ significantly from the smaller brains in living mammals, and by extension that larger brains of modern species differ from the small brains of ancestral species that gave rise to these lineages (see Jerison, 1973; Kaas, 1987a, 1987b, 1995, 2005; Krubitzer, 2000). This is exemplified by comparing the shared cortical areas between shrews and humans (Figure 2). Shrews are particularly interesting because many of them represent the lower size range for the mammalian body and brain (see Schmidt-Neilsen, 1984). Shrews are also particularly interesting because fossil evidence indicates that early mammals also had small brains with little neocortex. Thus, understanding constraints on the organization of a small neocortical sheet may help us infer how early mammalian cortex was organized.

Shrew brains were found to have only a few cortical sensory areas with sharp borders as determined from both electrophysiological and histological evidence (Catania *et al.*, 1999). These areas include primary and secondary somatosensory cortex (S1 and S2), primary visual cortex (V1), primary auditory cortex (A1), and motor cortex (M1). Human brains also contain these same subdivisions in similar relative position in the cortex (i.e., V1 is caudal in cortex, A1 is lateral, M1 is most rostral). This comparison demonstrates two important and very general findings in mammals. First, diverse mammal species share a number of cortical areas in common. Second, larger-brained mammals tend to have more cortical subdivisions. The greater number of intervening cortical areas is not illustrated in Figure 2 for humans, but can be appreciated from the schematic in Figure 3, which illustrates the number of cortical subdivisions in a shrew compared to the estimated number of cortical subdivisions in a macaque. Whereas shrews have only five known cortical areas with little room for additional subdivisions (Catania *et al.*, 1999), macaques are thought to have over 50 different areas

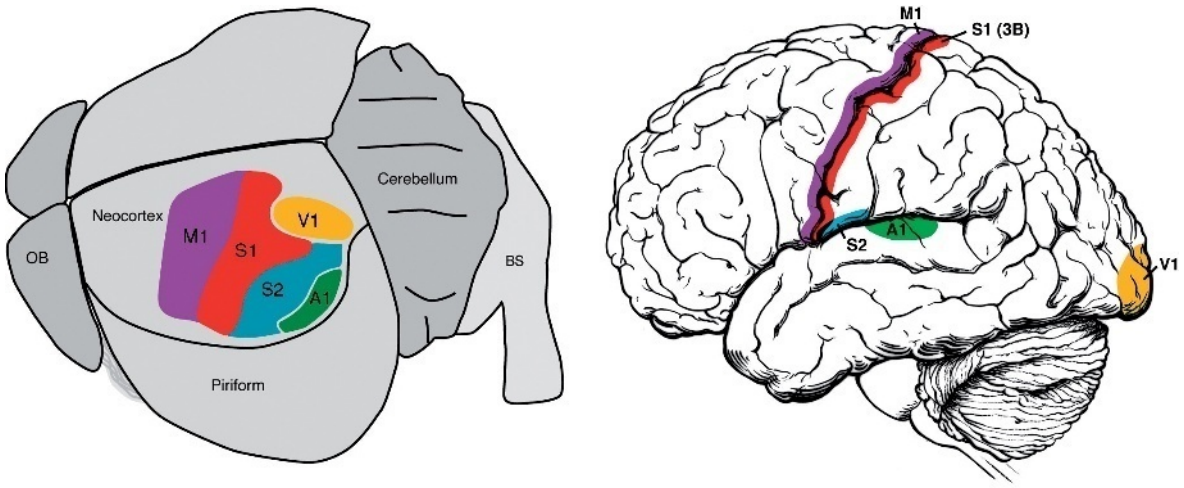


Figure 2 Shared cortical areas between a shrew and a human. Left side shows a shrew brain and cortical areas, including primary somatosensory cortex (S1), secondary somatosensory cortex (S2), primary visual cortex (V1), primary auditory cortex (A1), and primary motor cortex (M1). The same (homologous) cortical areas are depicted in the human brain on the right. Human have many additional cortical areas that are not illustrated, whereas shrews have little room for additional cortical subdivisions. OB, olfactory bulb; BS, brainstem.

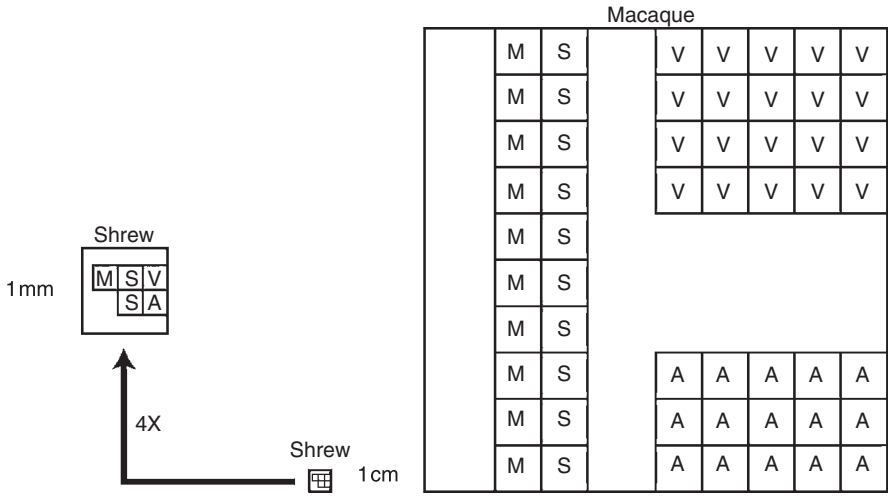


Figure 3 A schematic representation of cortical organization in a small-brained (shrew) and large-brained (macaque monkey) mammal. Shrews have as little as 0.15 cm^2 of neocortex, whereas macaques have roughly 72 cm^2 a 480-fold difference. Humans, with approximately 800 cm^2 of neocortex, do not fit on the figure, but have neocortex with over 5000 times the surface area of a shrew. Given that shrews are similar in size and habits to ancestral mammals, there has clearly been a tremendous enlargement of cortex in many mammalian lineages. In addition to getting larger, the internal organization of cortex has changed as well. Many cortical subdivisions have been added in larger-brained mammals, and this can be appreciated by comparing the enlarged shrew brain (far left) to the macaque brain. The letters denote visual (V), auditory (A), somatosensory (S), and motor areas (M). Shrews have only a few cortical subdivisions, whereas macaques have many. The illustration is not intended to show the relative size or location of cortical areas. Reproduced from Catania, K. C. 2004. Correlates and possible mechanisms of neocortical enlargement and diversification in mammals. *Int. J. Comp. Psychol.* 17, 71 91.

(see Kaas, 1995 for review) and additional areas will almost certainly be identified in macaque cortex.

This observation is perhaps not surprising; however, it does raise additional questions regarding brain scaling and evolution. It is clear that large-scale changes to brain organization have occurred in many mammalian lineages – for example, in the primate and carnivore orders that have more cortical subdivisions than smaller-brained rodents and insectivores (Kaas, 1982). Greater numbers of cortical subdivisions are often considered to be an important underlying substrate for increased intelligence and behavioral complexity. Yet, it is difficult to separate factors related to brain scaling from

those related to increased processing ability. For example, as brain areas increase in size, local connections must increase in length to maintain a similar degree of global connectivity. Such increases in lengths of axons and dendrites must be accompanied by increases in their diameters in order to maintain similar conduction times between cells (Ringo *et al*., 1994). The main point is that increasing the size of a brain and its cortical areas includes many engineering challenges and thus some cortical areas may become subdivided simply to maintain the status quo (Kaas, 2000).

In addition, it is often difficult to confidently identify a particular brain specialization related to increased behavioral complexity or processing ability when comparing distantly related species, such as insectivores and primates, as some traits may be most common in a given lineage without an obvious adaptive value. This has been termed the taxon level effect (Pagel and Harvey, 1989). One way to more confidently identify specializations related to a particular behavioral or sensory ability is to look in closely related mammals of similar brain and body size, in which only one dimension of a sensory system has changed in a particular member of the group.

31.2.2 The Star-Nosed Mole – A Case Study in Somatosensory Evolution

Comparing the somatosensory systems of different mole species provides what might be considered a natural experiment in the elaboration of the mechanosensory portion of the nose and corresponding representations in the brain. Unlike most other mammals, moles use the skin surface of the snout – rather than vibrissae – to explore their environment through touch. But the degree of elaboration of the nose and associated sensory organs differs greatly across species. Consider, for example, the eastern American mole (*Scalopus aquaticus*) in Figure 4. This species is a more generalized mole that resembles the kind of ancestral condition from which the star-nosed mole evolved (Catania, 2000b). How does brain organization differ between star-nosed moles and the less specialized but closely related eastern American mole?

Microelectrode recordings from the brain of *Scalopus* reveal a somatosensory cortex similar in many general respects to that found in the star-nosed mole (Figure 5a). A relatively large S1 contains a representation of the body with caudal body parts (tail and hindlimb) located medially in cortex and the face and nose represented more laterally. As in star-nosed moles, a relatively large S2 is found as a mirror image of S1 in more lateral and caudal cortex. This basic layout of two relatively large

Figure 4 Comparison of two mole species. a, The eastern American mole (*S. aquaticus*) is the least specialized mole resembling the probable ancestral condition for moles (Catania, 2000b). b, The star-nosed mole (*Condylura cristata*) is the most specialized mole with a snout consisting of 22 mechanosensory appendages.

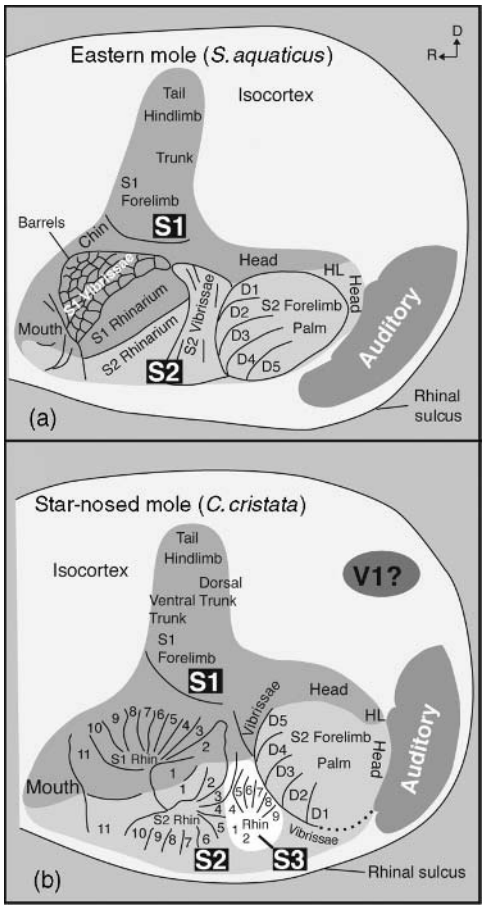


Figure 5 The results of recent investigations of cortical organization in moles. a, The eastern American mole has two somatosensory areas, primary (S1) and secondary (S2) somatosensory cortex, which include visible barrels much like those identified in rodent cortex. b, The star-nosed mole has three representations of the star (S1, S2, and S3). These areas are visibly reflected as a series of modules in flattened sections of cortex processed for cytochrome oxidase.

somatosensory areas, S1 and S2, is also found in other moles species (Catania, 2000c) and in the sister group to moles, the shrews (Catania *et al.*, 1999; see Figure 2). Thus, moles and shrews generally have two representations of the nose in lateral cortex. However, star-nosed moles have three representations of the star (Catania and Kaas, 1995) in lateral cortex (Figure 5b). The most parsimonious interpretation of these observations is that star-nosed moles have independently evolved an extra representation of the star.

This finding is from very closely related species that differ little in body weight and brain size, and it supports the conclusion that the addition of a new area to the cortical network is an important substrate for more efficient processing of sensory inputs. The most obvious difference between star-nosed moles and other moles is the elaboration of the somatosensory star with a corresponding increase in innervation density accompanied by more complex foraging behaviors (e.g., foveation movements of the star – this is discussed in Section 31.2.4). As a result, star-nosed moles are one of the fastest and most efficient of mammalian foragers (Catania and Remple, 2005) and the larger number of cortical representations of the star may facilitate this ability, perhaps through the parallel processing of different facets of touch information.

31.2.3 Behaviorally Important Areas Are Magnified in the Brain

Figure 6 illustrates cortical magnification of important sensory surfaces in the naked mole-rat and the star-nosed mole showing how the most behaviorally important sensory surfaces take up a disproportionate area of cortex. This feature of cortical maps has been documented since the pioneering studies of Adrian (1943) and Woolsey *et al.* (1942), in which it was noted that parts of the body that have the greatest tactile acuity have the largest cortical projection zones. Cortical magnifications have since been described for different sensory systems in diverse species, and this phenomenon makes for striking imagery. However, the relationship between sensory surface size and cortical representational area also raises important and fundamental questions about brain organization and evolution. Namely, how do the most important sensory surfaces acquire the largest territories in the brain?

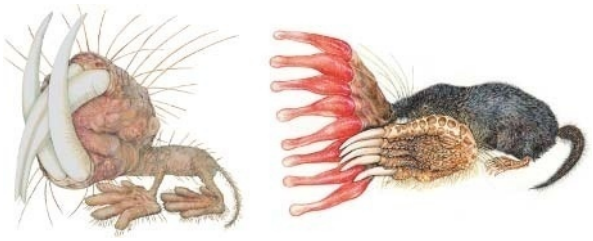


Figure 6 Cortical magnification in naked mole-rats and star-nosed moles. These schematics illustrate the relative proportions of the somatosensory cortex taken up by representations of different body parts in each species. Surprisingly, the naked mole-rat devotes much of its cortex (30% of S1) to the representation of the incisors. In contrast, star-nosed moles devote a huge portion of their somatosensory cortex to the representation of the star.

Early investigations of this relationship in rodent barrel cortex revealed a direct linear correlation between the size of a cortical barrel (the area representing a whisker) and the innervation density of the corresponding whisker (Welker and Van der Loos, 1986). This result suggested that cortical representational area could be, in general, proportional to the innervation density of the sensory surface projecting to any given area of cortex. Such a relationship would explain the expanded representations of important areas of the skin, retina, and cochlea that had been described in a number of species. At the same time, this finding suggested that there was no 'cortical component' to cortical magnification and that this parameter could be predicted without even examining the brain, simply by determining the relative innervation density of a sensory surface. Lee and Woolsey (1975) recognized this possibility and suggested that cortical representations are more appropriately described by a "peripheral scaling factor" than a "cortical magnification factor."

Of course, another possibility is that cortical representational area is not proportional to number of inputs from the periphery, and instead important sensory inputs could project to a larger area of cortex than less important inputs. This has been the subject of considerable historical debate in the visual system of primates, where some studies suggest that the large cortical representation of the retinal fovea simply reflects the number of retinal ganglion cells projecting from the retina (Drasdo, 1977; Wassle *et al.*, 1989, 1990), whereas other studies indicate that ganglion cells projecting from the fovea have a disproportionately large representation in cortex (Malpeli and Baker, 1975; Myerson *et al.*, 1977; Perry and Cowey, 1985; Silveira *et al.*, 1989). The weight of most recent evidence supports the contention that important inputs in the visual systems of primates are indeed overrepresented in the cortex (Azzopardi and Cowey, 1993). However, the few studies that have addressed this issue and the conflicting results from different studies in the

primate visual system highlight the difficulty of making these determinations in most sensory systems. This is a case where the particularly specialized sensory system of the star-nosed mole has provided new insights as a result of its anatomical specialization. But before describing how star-nosed moles can shed light on visual system organization, it is necessary to outline the parallels between the star-nosed mole's somatosensory system and the visual systems of sighted mammals.

31.2.4 A Somatosensory Fovea in the Star-Nosed Mole

Although the nose of the star-nosed mole is a tactile sensory surface, there are a number of behavioral and anatomical similarities between the mole's sensory system and the visual systems of other species. This is most obvious from observations of star-nosed mole behavior (Catania and Remple, 2004). The entire star is used for the detection of relevant stimuli in the environment, but once an object or food item of interest is detected, the nose is shifted in a saccadic manner for detailed investigations with the touch fovea. There are 11 finger-like appendages on each side of the star, and the ventral-most, 11th pair constitutes the fovea. The other appendages take up a much greater surface area and act as the 'tactile periphery' in a manner analogous to the peripheral visual receptors of the retina.

Because one small area of the skin surface is the behavioral focus of the star, we can address the question of whether the most important inputs from a sensory array are allocated extra territory in the cortex, or alternatively whether the sizes of each cortical representation are simply proportional to their innervation density. This question is relatively easy to answer in the star-nosed mole because of the favorable anatomical organization of the sensory system. It is possible to quantify three different parameters: (1) the number of sensory organs on the star, (2) the number of primary afferents innervating the each appendage of the star, and (3) the area of primary somatosensory cortex devoted to each appendage (Figures 7a–7c). It is also possible to accurately measure the cortical representation of the star because of the histologically visible reflection of the appendage representations as a series of modules in somatosensory cortex. This aspect of star-nosed mole brain organization is discussed in more detail in the next section.

Because these different parameters can be measured in star-nosed moles, a number of interesting comparisons can be made. First, it is possible to consider the relationship between innervation density (number of nerve fibers) and the number of sensory organs (Eimer's organs) on the skin surface of each appendage (Figure 7d). This comparison shows that the number of nerve fibers and the number of Eimer's organs co-vary almost precisely for appendages 1–9. However, for appendages 10 and 11, there are more fibers per sensory organ. This reflects the higher acuity of this behaviorally important sensory surface. But does this account for the cortical magnification of the fovea, as suggested by studies in rodent barrel cortex? Figure 7e shows this comparison (average area of cortex per afferent for each appendage of the star) clearly indicating that the higher innervation for the fovea area of the star does not account for the cortical magnification of the fovea. Instead, star-nosed moles devote a greater average area of cortex to the most important afferents from the 11th appendage of the star (the tactile fovea) and conversely a smaller average area of cortex to the representations of the afferents from remaining 10 appendages (Figure 7e). Thus, the favorable anatomy of star-nosed mole's sensory system has allowed for the quantification of variables that are difficult to measure in many species and these findings may reflect a common relationship between sensory surfaces and the cortex in mammals. For example, the degree of cortical overrepresentation of the inputs from the fovea of the star is similar to the degree of overrepresentation of the retinal fovea in primates (Catania, 1995; Azzopardi and Cowey, 1993).

Finally, the subdivision of the star-nosed mole's sensory system into fovea and periphery is a remarkable example of the convergent evolution of similar features across disparate sensory systems. It suggests this organizational scheme is a general solution to designing a high-resolution sensory system. The most familiar and common example of a fovea-periphery organization is of course found in many visual systems of diverse mammals; however, auditory systems can have an acoustic fovea as well. This has been demonstrated in a number of studies by Suga and colleagues (Suga and Jen, 1976; Suga, 1989) for mustached bats (*Pteronotus parnellii*). Mustached bats emit an echolocation call that includes a narrow frequency range around 60 kHz that is particularly important for detecting the acoustic evidence of wing-beats caused by flying insect prey. A large proportion of the hair cells in the bat's cochlea are tuned to this important echolocation frequency and a large territory of the bat's A1 is devoted to processing sounds at this frequency. Thus, mustached bats have an acoustic fovea, and they have the acoustic equivalent of a saccade as well. This is necessary because returning

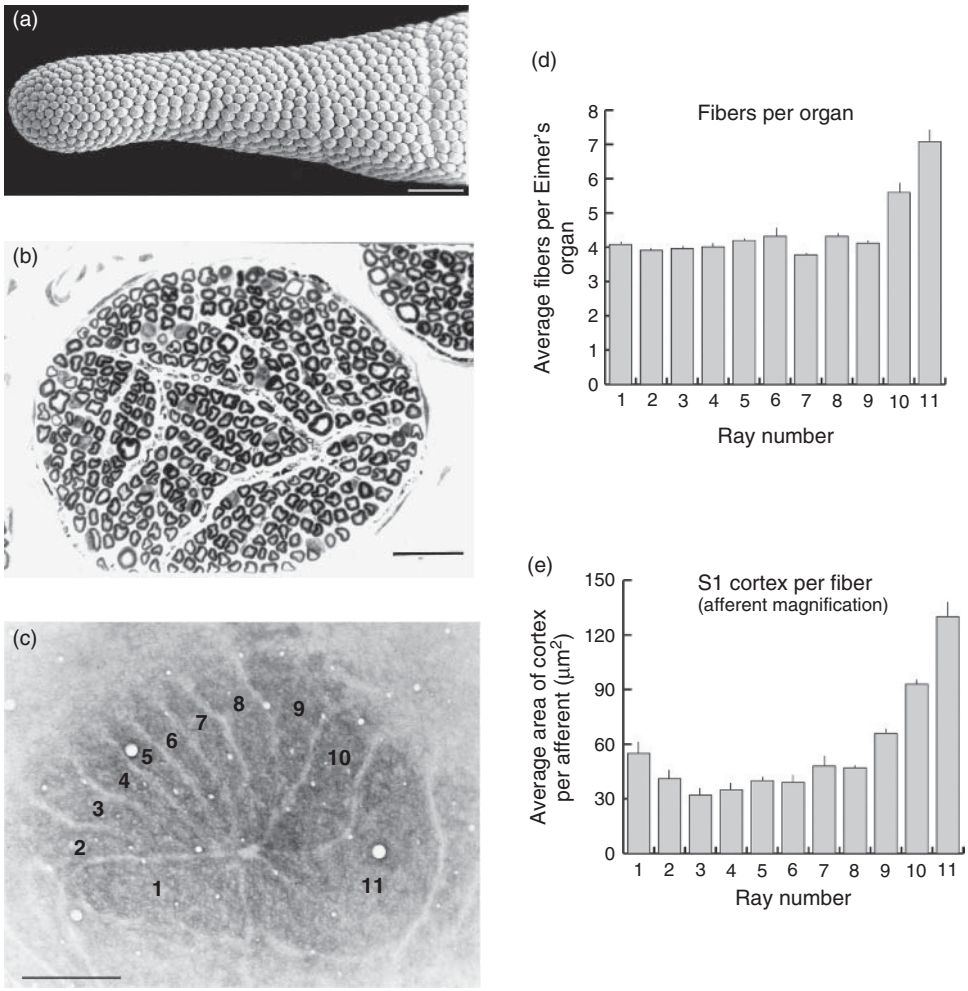


Figure 7 Quantification of the number of sensory organs, innervating nerve fibers, and representational area of the star in primary somatosensory cortex. a, A single appendage of the star under the scanning electron microscope showing the many visible sensory organs (Eimer's organs) covering the skin surface. b, A thin section of tissue showing a small portion of the many myelinated afferents supplying an appendage of the star. c, A portion of the cortex of a star-nosed mole that has been flattened and processed for cytochrome oxidase to reveal the primary somatosensory representation of the star. The area representing each appendage is visible as a separate subdivision. d, A graphic representation of the ratio of fibers (afferents) innervating each appendage per sensory organ on each appendage (or ray) of the star. e, The average area of cortex devoted to the primary afferents for each appendage (ray) of the star. Scale bars: a, 250 μm; b, 20 μm; c, 500 μm. Reproduced from Catania, K. C. and Kaas, J. H. 1997c. Somatosensory fovea in the star-nosed mole: Behavioral use of the star in relation to innervation patterns and cortical representation. *J. Comp. Neurol.* 387, 215 233.

echoes are often Doppler shifted to different frequencies depending on the speed of the bat and its target. To compensate for these Doppler shifts, bats are constantly shifting the frequency of their outgoing pulses to 'focus' the returning echo on the high-resolution area of the acoustic fovea. This behavior, called Doppler shift compensation (Schnitzler, 1968), is surprisingly similar to a saccade in the visual system.

The most well-developed visual systems, somatosensory systems, and auditory systems, all exhibit a fovea-periphery organization. An obvious benefit of this design is the conservation of neural processing area in the brain and innervating nerve fibers at the level of the sensory periphery. For example, making the entire sensory system high resolution would require a massive enlargement of the nerves carrying information to the brain, and a corresponding enlargement of the cortical areas processing the inputs. The ultimate result would be a staggering increase in brain size. It is far more efficient to devote a large part of the computational area of the brain to a small part of the sensory system (the retinal, tactile, or acoustic fovea) and then move that area around like a spotlight to analyze important stimuli (or in the case of bats, move the frequency of echolocation

pulses to ensure that Dopplar shifted echoes can be analyzed by the fovea).

31.2.5 Modules Represent Sensory Surfaces in Diverse Species

Woolsey and Van der Loos (1970) made the discovery of modules in the somatosensory cortex that represented the important facial vibrissae, or whiskers, in mice. They described cylindrical groupings of cells that were most easily seen in sections of the cortex cut parallel to the cortical surface. Electrophysiological recording of neuronal responses revealed that each barrel corresponded to the cortical representation of a single whisker on the face. This finding was remarkable because it revealed a visible reflection of a somatosensory map and at the same time provided a useful model system for exploring many details of mammalian brain organization and development. Cortical barrels were also considered to provide anatomical support for the columnar hypothesis of cortical organization, which suggests that cylindrical columns of interconnected neurons are the fundamental organizational unit of neocortex.

From the time since cortical barrels were first described, a number of investigations of cortex have revealed cortical subdivisions, or modules, related to sensory specializations in diverse species. Star-nosed moles provide one of the more dramatic examples of this relationship. Figure 8 shows details of star-nosed mole cortex. In the case of star-nosed moles, the receptors represented in cortex are part of an elongated skin surface, rather than a hair surrounded by a ring of mechanoreceptors as found in rats and mice (see Rice *et al.*, 1993). As described previously (Figure 5b) electrophysiological recordings from the cortex of star-nosed moles reveal three representations of the star in lateral cortex. When sections of the flattened cortex are cut parallel to the cortical surface and processed for cytochrome oxidase (Wong-Riley and Carroll, 1984) three different maps of the star are visible (Figure 8b). Each of these maps represents the entire contralateral star and each cortical module representing an appendage takes the from of elongated wedge.

The representations of the appendages of the star-nosed mole differ from cortical barrels of rodents in a number of ways. First, the representations of the appendages consist of elongated stripes of cortical tissue, rather than circular barrels. Second, the representation of the tactile fovea is greatly expanded in cortex relative to the size of this appendage on the star. As outlined above, the representation of this appendage reflects the behavioral importance of the fovea, rather than the innervation density of the sensory surface. Finally, in the star-nosed mole's cortex multiple maps of the sensory surface are uniquely visible. Three different somatosensory areas, S1, S2, and a new area we

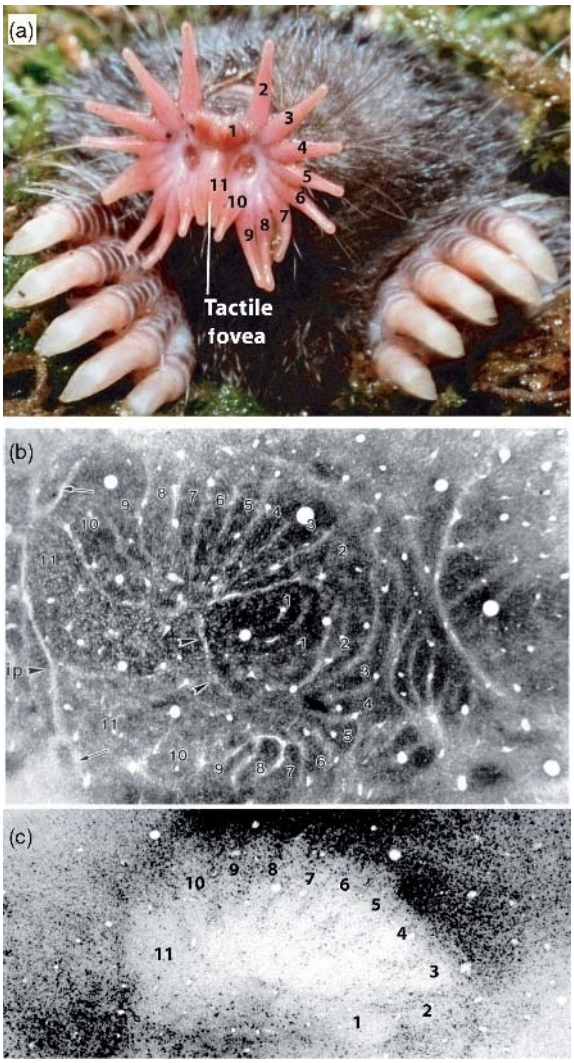


Figure 8 The unusual mechanosensory star and its corresponding cortical representation in the star-nosed mole (*C. cristata*). a, A star-nosed mole emerges from an underground tunnel showing its large forelimbs and the 22 fleshy appendages that surround each nostril. The 11th appendages on each side act as the somatosensory fovea and are used for detailed tactile investigations. b, A section of flattened cortex revealing all three cortical representations of the star (S1, S2, and S3 see Figure 5b) visible as a series of modules, each representing an appendage from the contralateral star. c, An example of the specificity of callosal connections around the S1 star representation. Cells and terminals are concentrated in the septa between appendage representations and surrounding the star representation but are absent from the centers of each cortical stripe. Reproduced from Catania, K. C. and Kaas, J. H. 2001. Areal and callosal connections in the somatosensory cortex of the star-nosed mole. *Somatosens. Mot. Res.* 18, 303 311.

have termed S3, contain modules representing individual appendages.

From these observations, one can conclude that cortical modules are not constrained to form traditional columns, as suggested from barrels. It is also clear that insectivores may have exceptionally well-organized and complex cortical representations. This result is in stark contrast to results from early investigations of insectivores, including moles (Allison and Van Twyver, 1970), which suggested they had overlapping cortical areas with poorly defined topography. In this regard it is significant that the modules in the star-nose mole's cortex have different connections than the septa between modules. For example, tracer injections into the cortex reveal that callosal connections terminate selectively in the septa between cortical modules, whereas intercortical connections terminate primarily within modules (Figure 8c).

A different kind of modular representation of a sensory surface is found in the eastern American mole (*S. aquaticus*). This mole has an unusual and sensitive forelimb consisting of an oval palm and heavily clawed digits. In S2 of this species cytochrome oxidase processed sections of cortex reveal a modular reflection of the forelimb. This cortical pattern appears just like the large clawed hand that it represents (Figure 9). Tracer injections into the spinal cord of the eastern American mole show that the modular forelimb representation is also the location of dense areas of corticospinal projecting neurons (Catania and Kaas, 1997a). These examples of different kinds of connections to different parts of cortical modules in moles support the general conclusion that different parts of cortical modules may be the selective substrate for the distribution of specific cortical circuitry (Chapin *et al.*, 1987; Koralek *et al.*, 1990; Fabri and Burton, 1991; Hayama and Ogawa, 1997; Kim and Ebner, 1999).

In addition to cortical modules discussed above in rodents and insectivores, investigations of cortical organization in the duck-billed platypus have provided a different example of modules representing

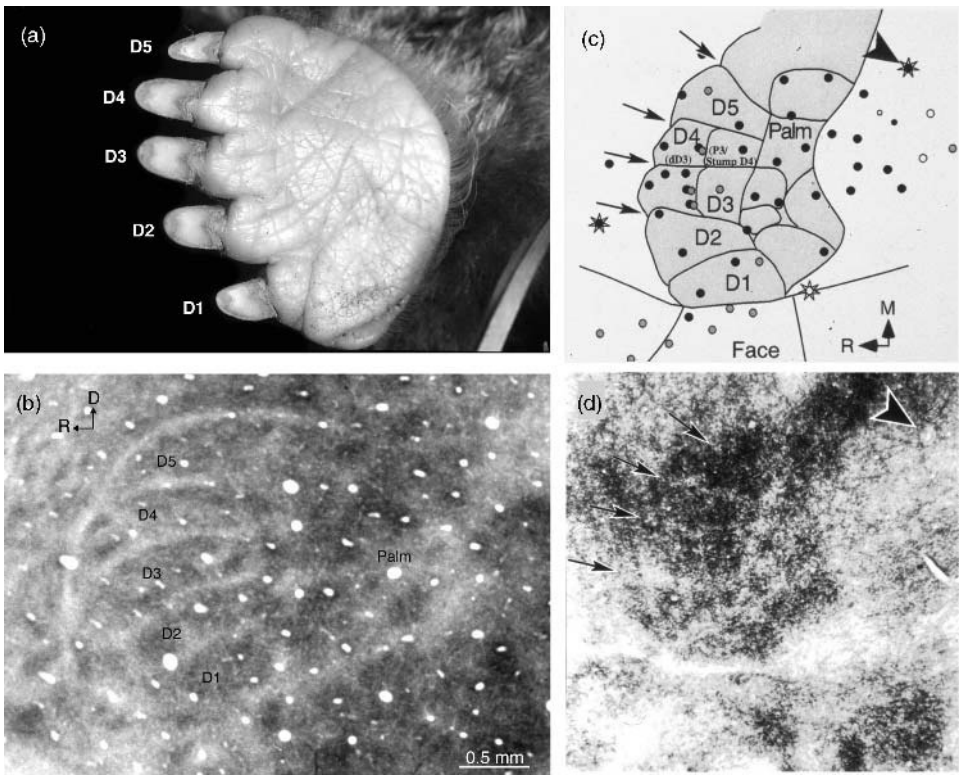


Figure 9 The representation of the eastern mole forelimb (*S. aquaticus*) and the hand of an owl monkey (*Aotus trivirgatus*). a, The large clawed forelimb of the mole. b, The cortical representation of the mole's forelimb as revealed by sections processed for cytochrome oxidase. The representation appears just like the large clawed hand it represents. c, A reconstruction of cortical recordings from an owl monkey showing the relative location of areas that responded to the digits (D1 D5) and the palm. d, Histological sections from the corresponding area of S1 (area 3B), in the same owl monkey, showing the cortical modules that represent the fingers and palm of the hand (arrowhead marks microlesions). b, Reproduced from Catania, K. C. and Kaas, J. H. 1997a. The organization of somatosensory cortex and distribution of corticospinal neurons in the eastern mole (*Scalopus aquaticus*). *J. Comp. Neurol.* 378, 337 353. d, Reproduced from Jain, N., Catania, K. C., and Kaas, J. H. 1998. A histologically visible representation of the fingers and palm in primate area 3b and its immutability following long-term deafferentations. *Cereb. Cortex* 8, 227 236.

sensory surfaces. The platypus bill contains tens of thousands of mechanoreceptors and electroreceptors (Manger and Pettigrew, 1996). Microelectrode mapping of the platypus somatosensory cortex has revealed a large S1 representation of the bill in lateral cortex (Krubitzer *et al.*, 1995). Flattened sections of cortex processed for cytochrome oxidase reveal alternating stripes of cortical tissue with dark and light regions representing higher and lower amounts of chronic neuronal activity. The dark areas represent the projection zones for mechanosensory information whereas the light zones represent the projection zones for combinations of mechanosensory and electrosensory information. Thus, S1 in the platypus contains receptor specific subdivisions very similar to the alternating bands of cortex representing rapidly adapting and slowly adapting mechanoreceptors in S1 of primates (Sur *et al.*, 1981), cats (Stretavan and Dykes, 1983), and raccoons (Rasmusson *et al.*, 1991). This anatomical arrangement of different sensory inputs in the platypus cortex is also reminiscent of ocular dominance columns representing inputs from the different eyes in primate area 17 (Hubel *et al.*, 1976).

The examples of cortical modules described above are from a range of particularly specialized mammals, and this raises the question of how common such representations of tactile sensory surfaces are across species and whether such findings have implications for primate and human brain organization. Relatively recent findings in primates suggest there are similar organizing principles for mechanosensory inputs across these diverse species. Jain *et al.* (1998) examined flattened cortex of three different primate species processed for myelin (Gallyas, 1979) and identified myelin-dense cortical modules representing the mechanoreceptors of the digits and palm in S1 (Figures 9c and 9d). Thus, the cortical representation of the primate hand, like the representation of rodent whiskers and the mole's star, is visibly reflected in flattened sections of cortex (see also Qi and Kaas, 2004). These findings indicate that large- and small-brained mammals share common developmental principles that segregate maps in similar ways. They also suggest there is a ubiquitous instructional role for the sensory periphery in guiding the formation of central representational maps.

31.2.6 The Sensory Periphery Guides Aspects of Cortical Development

The finding of histologically visible cortical maps of sensory surfaces that reflect the details of mechanoreceptor topography raises the question of how somatosensory areas become matched to the sensory periphery. Because the development of the somatosensory system begins with the skin surface and ends at the cortex (see Killackey *et al.*, 1995) there is opportunity for the sensory surface to instruct the cortex. Evidence for such an instructive role of the sensory periphery comes from the somatosensory system of rodents where it has been shown that early damage to a whisker disrupts the formation of the corresponding cortical barrel (Andres and Van der Loos, 1985; Woolsey, 1990).

A different but related kind of evidence comes from strains of mice bred for variations in the whisker pattern. Van der Loos and Dorfl (1978) noted that strains of mice born with extra whiskers on the face also developed extra barrels in the cortex in the appropriate topographic location. They argued that it was unlikely for a single mutation to have simultaneously altered the entire sensory system from whisker to barrel, but rather a mutation acting at the level of the early developing skin surface was more likely to have been communicated to the subcortical nuclei and then to the developing cortex. Similar results have more recently been reported in star-nosed moles, where wild-caught animals have an unusually high rate (5%) of extra or missing nasal appendages. The different nose configurations are invariably reflected in the cortical maps (Catania and Kaas, 1997b).

Although Van der Loos and Dorfl made a compelling argument, they could not entirely rule out the possibility of a single genetic modification simultaneously and independently altered the brain and the whiskers of mice. Recently, however, their interpretation of an instructive role for the skin surface has received strong support from investigations in which altered whisker patterns were induced during embryonic development by transfecting the epidermis of mice with a virus containing the patterning gene Sonic hedgehog (Shh). This manipulation resulted in the formation of extra whiskers on the face, and later extra barrels in the cortex (Ohsaki *et al.*, 2002). However, in this case the genetic change was clearly restricted to the skin surface, supporting the hypothesis that the skin surface instructs the later-developing cortex.

Another possible role for the periphery in guiding the formation of cortex may be found in the timing of developmental events. For example, the retinal fovea in primates develops earlier than the peripheral retina, and inputs from the fovea have a preferentially magnified representation in cortex (as previously described). Similarly, the tactile fovea in star-nosed moles develops earlier than the more peripheral parts of the star. This can be

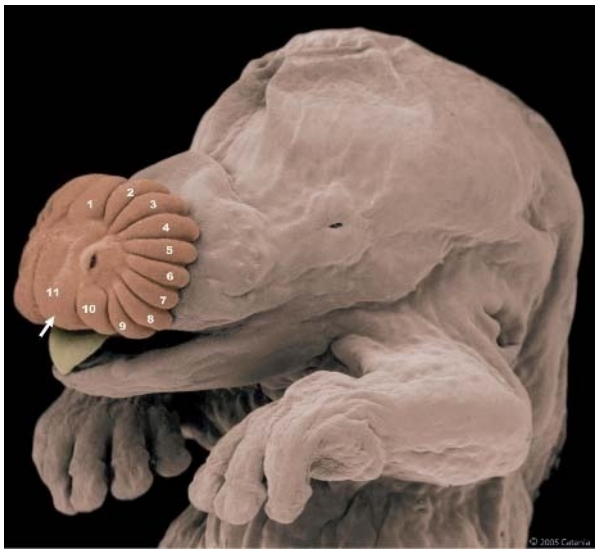


Figure 10 An embryonic mole showing the developing star. The appendages are numbered 1 11, as in adults. Note however, the relatively much larger area of the star taken up by the 11th, foveal appendage (arrow) at this early stage of development compared to appendage 11 in adults (see Figure 8a). Examination of this development sequence (Catania, 2001) reveals that the fovea leads the development of the star, and this may allow afferents from the developing fovea to capture a larger area of cortex (e.g., Figure 7e) in a competition for cortical territory. Photo copyright 2005 Catania.

appreciated by examining embryonic (Figure 10) and adult (Figure 8a) star-nosed moles and comparing the size of the 11th appendage (the tactile fovea) at these different stages. The 11th appendage takes up a far greater proportion of the star in embryos than it does in adults. More detailed investigations of this relationship (Catania, 2001) reveals that the tactile fovea leads the development of the star, such that it grows large early, has the largest innervated sensory surface in embryos, and develops sensory organs (Eimer's organs) before the peripheral appendages of the star (appendages 1–10). Yet later in development the peripheral appendages grow larger than the fovea, until in adults the 11th appendage is dwarfed by the rest of the star (Figure 8a).

The early development of these important sensory surfaces may give them an advantage in a competition for cortical territory during development. Evidence for this possibility comes from studies of the primate visual system. When one eye is sutured shut and deprived of visual input during critical periods of development, ocular dominance columns related to that eye are greatly reduced in size compared to the open eye (Hubel *et al.* 1977). Activity dependent expansions have also been documented for the somatosensory system, where the most active regions of barrel cortex undergo the greatest amount of growth during development (Riddle *et al.*, 1993; Purves *et al.*, 1994). These studies suggest that the most active inputs during critical periods of development have a competitive advantage in capturing representational space in the cortex.

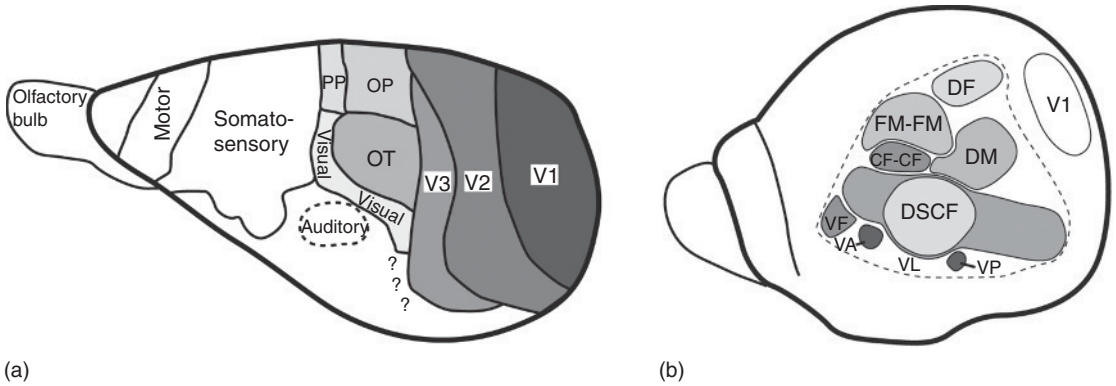
So far, I have highlighted some of the evidence for changes that may have commonly occurred in the course of the evolution of the somatosensory system. These include the magnification of behaviorally important areas of sensory maps, the addition of new areas to cortical networks, the formation of a fovea-periphery organization for high-resolution sensory systems, and the subdivision of areas into modules representing segregated sensory surfaces in the periphery. In this last section I will outline some ideas for potential mechanisms by which some of these changes may occur.

31.3 What are the Mechanisms of Evolutionary Change?

31.3.1 Levels of Organization

The examples outlined above for the somatosensory system suggest two different levels of organizational change in the evolving neocortex that may be altered by two different mechanisms. The first level involves alterations of details of cortical representations that stem from the developmental link between the sensory periphery and the brain. Evidence for this possibility comes from a number of sources as outlined in the previous sections, including surgical alterations to the whiskers that change barrel patterns in mice, strains of mice bred with supernumerary whiskers that have extra barrels in the cortex, wild-caught star-nosed moles with extra appendages on the star and extra representational stripes in the cortex (indicating this occurs in natural populations), and evidence that changes in the timing of developmental events at the sensory surface may have an important impact on cortical development.

A second level of organizational change is the addition of completely new areas to the cortex, in the form of new maps of the sensory periphery. Evidence for this kind of change comes from comparative studies that illustrate the variation in numbers of cortical subdivisions in differently specialized species. The extra somatosensory area in star-nosed moles (Figure 5) compared to other mole species provides one example that can be confidently attributed to the elaboration of the somatosensory system. Other examples include



(a) (b)

Figure 11 Cortical organization in megachiropteran and microchiropteran bats, demonstrating visual and auditory specializations, respectively. a, Summary of cortical areas in the megachiropteran flying fox (*Pteropus poliocephalus*). This fruit-eating species relies heavily on vision and this is reflected in the proportion of cortex devoted to vision and the number of corresponding visual areas. Roughly half of the cortex is taken up by a series of at least six visual areas (shaded areas) and a number of additional areas are likely to be found in more rostral-lateral cortex. b, Summary of cortical areas in the microchiropteran mustached bat (*Pteronotus parnellii*). In contrast to megachiropteran bats, microchiropteran bats have reduced visual systems and depend heavily on echolocation to navigate and locate flying prey. This is reflected in the organization of their neocortex which is dominated by a network of eight or more auditory areas (shaded areas) that largely process information in the frequency range of returning echolocation pulses. These closely related species provide an example of how cortex has evolved in parallel with the more complex visual and auditory abilities of each respective species. a, Data from Rosa, M. G., Krubitzer, L. A., Molnar, Z., and Nelson, J. E. 1999. Organization of visual cortex in the northern quoll, *Dasyurus hallucatus*: Evidence for a homologue of the second visual area in marsupials. *Eur. J. Neurosci.* 11(3), 907 915. b, Data from Suga, N. 1989. Principles of auditory information-processing derived from neuroethology. *J. Exp. Biol.* 146, 277 286.

comparison of the many visual areas in highly visual megachiropteran bats to the few visual areas in echolocating microchripteran bats, and conversely comparison of many auditory areas for processing echos in the microchiropteran bats to the few auditory areas in nonecholocating, megachropteran bats (Figure 11).

31.3.2 Potential Mechanisms of Change

As described above, the intimate developmental relationship between receptor arrays and cortical maps suggests that many changes to cortical areas in the course of evolution may initially occur simply by altering the body. It seems likely that cortical and subcortical areas of the brain are flexible enough to accommodate changes to the sensory periphery that may provide a selective advantage. For example, the expansion of a sensory surface allows for a greater area of the environment to be investigated per unit time. This is presumably the selective pressure that drove star-nosed moles in the direction of enlargement of their mechanosensory snout relative to other species. In support of this possibility, star-nosed moles eat relatively small prey items compared to other species of moles, and this requires locating more prey per unit time to satisfy metabolic requirements (Catania and Remple, 2005). The evidence of supernumerary appendages in some moles suggests a simple mechanism by which such an expansion of the sensory surface can occur – that is, change the star locally and the sensory processing areas will accommodate the alterations through a developmental cascade.

Yet changes to cortical areas to accommodate different configurations of a sensory surface may have important, negative consequences for sensory processing (Figure 12). In this respect, a cortical area may be challenged in the same general ways that have been outlined for increasing the size of the entire brain (Deacon, 1990; Kaas, 2000). One problem is the lengths and numbers of interconnections within a cortical area. As neurons become more widely separated, the diameters of their axons and dendrites must become greater to maintain similar conduction times between neurons (Ringo *et al.*, 1994). This in turn typically requires increases in the size of the supporting neuronal cell body in order to supply the metabolic requirements of the neurites. In addition, as the number of neurons in larger areas increases, the number of connections between neurons must increase drastically to maintain a similar degree of global connectivity between neurons within the area (Deacon, 1990). All of these changes require more space in the cortex, which compounds the problem. Thus, increasing the size of a cortical area could result in a suboptimal processing area and set the stage for the adaptive benefits of adding a new area to the cortex.

Figure 12 provides a schematic outline for how some of these changes may occur. The progressive

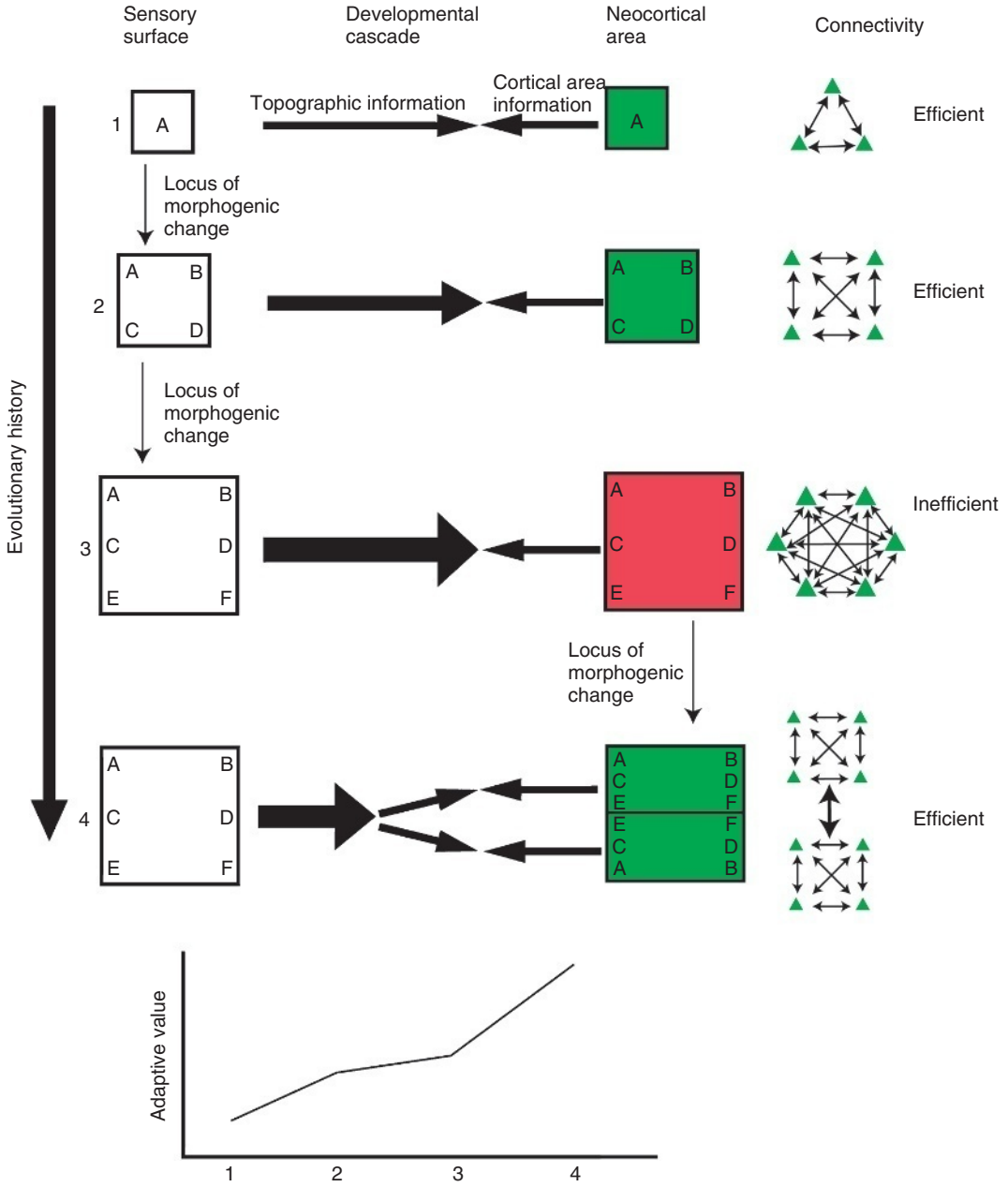


Figure 12 Schematic illustration of possible steps in the progressive evolution of a more complex cortex with new areas. Steps 1 4 represent a progression of changes in the species over successive generations. The graph at the bottom represents the proposed adaptive value of each evolutionary change for steps 1 4. In this proposal, the sensory surface leads the evolutionary process of brain reorganization through a cascade of developmental events in steps 1 3. This begins with the expansion of the sensory surface and a corresponding expansion of the representation of the sensory surface in cortex. The far right side represents the level of connectivity between neurons needed for sensory processing. Although each step is presumed to provide a net advantage (lower panels), by step 3, the cortical processing area is strained and no longer processing the information at peak efficiency. This sets the stage for step 4, during which the cortical area is duplicated (through developmental mechanisms centers in the cortex see text for example) allowing for the two smaller areas to efficiently process information. Although not illustrated, the two areas are now free to specialize in processing different facets of sensory information and this is considered to be part of the adaptive value of this step (lower panel).

evolution of a sensory system is illustrated from top to bottom of the figure. The initial stages (1–3) reflect the progressive elaboration of the sensory surface (left side) and the corresponding expansion of the representation in cortex (right side) through a developmental cascade. The graph at the bottom illustrates the proposed adaptive value of each change. Initially the developmental changes to the sensory surface are accommodated by the later-developing brain, and there is a steep rise in adaptive

value (1–2). However, at some point (3) the cortical area is no longer at an optimal size for processing information from the sensory surface (as illustrated in red). Although the expansion of the sensory surface has still resulted in an increased net adaptive value for the sensory system as a whole (bottom panel), the stage is now set for the addition of a cortical area to optimize sensory processing. With the addition of a cortical area (4) there is another steep rise in adaptive value of the sensory system (4). Although not illustrated, the ability for the two daughter areas to specialize for processing different facets of sensory information may provide the most important advantaged for sensory processing.

There seems to be ample evidence from both experimental manipulations of development and naturally occurring variants that steps 1 and 2 can occur. That is, changes to the sensory system localized to the sensory periphery may cause alterations of the representations in the CNS. However, evidence for variation in the number of cortical areas is much less obvious, and must usually be inferred and reconstructed from comparative studies across species. Although there may be some ongoing variations in numbers of cortical areas in a given species, so few brains are processed and examined in detail for any species that the chances of such variants being identified are small. This can be contrasted with variants in body parts and sensory systems that can be readily identified by simply examining an animal's body (e.g., Van der Loos and Dörfl, 1978). As a result, the potential mechanisms for altering cortical area number are most readily deduced from laboratory investigations of patterning-gene expression.

Recent investigations and manipulations of gene expression patterns in developing mouse cortex suggest some of the mechanisms that control cortical area position, orientation, and number (Cecchi, 2002; Fukuchi-Shimogori and Grove, 2001; Ohsaki *et al.*, 2002; O'Leary and Nakagawa, 2002). These investigations have revealed graded expression of patterning proteins in the developing cortex that can be manipulated to cause predictable alterations in the positions of entire cortical subdivisions. One growth factor in particular – FGF8 (a member of the fibroblast growth factor family) – has been the focus of a number of recent studies. FGF8 is normally expressed at the rostral pole of the developing neocortex. In a landmark experiment, Fukuchi-Shimogori and Grove (2001) introduced a second source of FGF8 at the caudal pole of developing mouse neocortex. When they later examined the adult somatosensory cortex in these mice, some individuals had generated a partial mirror-image duplication of the S1 barrel field (Figure 13) that presumably was supplied by its own set of thalamocortical axons (O'Leary and Nakagawa, 2002).

This experiment has profound implications because the generation of a new, mirror-image representation of a sensory surface has clearly occurred many times in the course of mammalian brain evolution. Thus, addition of a new FGF8 source to developing cortex produces a phenotype in the laboratory that mimics a common product of cortical evolution.

Long before genetic manipulation of patterning genes was possible, previous investigators of mammalian cortical diversity had suggested that sudden duplications of cortical areas might occur as

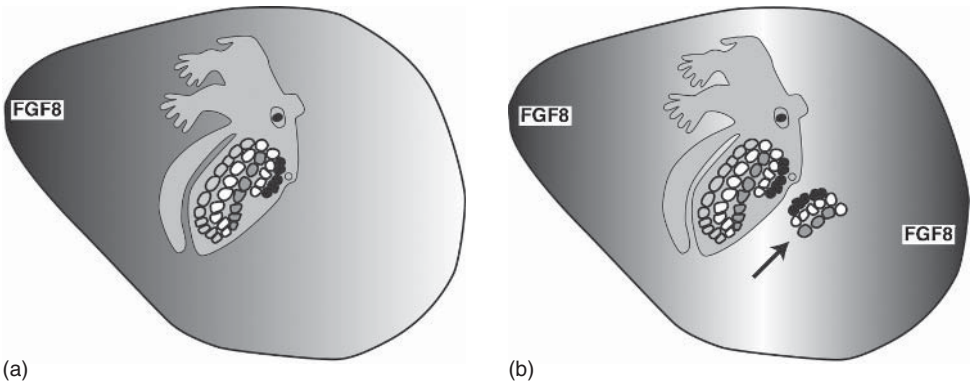


Figure 13 Schematic illustration of recent experiments that have induced the partial duplication of the cortical barrel field by adding a new source of FGF8 to the caudal part of developing cortex. a, FGF8, a member of the fibroblast growth factor family, is normally expressed rostrally in developing cortex. b, When a second source of FGF8 was introduced by electroporation during fetal development, adults were later found to have a partially duplicated barrel field (arrow). This results suggests a mechanism by which mirror image duplications of a cortical area might occur in the course of mammalian evolution. Reproduced from Fukuchi-Shimogori, T. and Grove, E. A. 2001. Neocortex patterning by the secreted signaling molecule FGF8. *Science* 294, 1071 1074.

mammalian brains evolved (Allman and Kaas, 1971; Kaas, 1982). The idea was that a new area could then become specialized to perform new functions while releasing the original area from some of its functions. There are a number of attractive features to this theory of cortical elaboration. Meristic changes – or alterations to a standard part – are a common mechanism of evolutionary change that has been well documented from the level of genes (see Ohno, 1970) to entire body parts (Raff, 1996). That this can occur for cortical areas seems likely in light of the recent findings of Fukuchi-Shimogori and Grove (2001).

In addition to this recent evidence from FGF8 expression, there are a number of considerations related to cortical area organization that suggest duplication of an area may be an efficient mechanism for expanding cortical functions. For example, such a mechanism (dependent on chemical gradients) would likely result in mirror-image maps (see Figure 13b and Catania, 2004), as observed for the supernumerary barrel representation in mice with an extra FGF8 source. Most adjacent cortical areas are mirror images of one another and share a congruent border (Kaas, 1982). This results in areas that are more topographic as a group, than non-mirror-image areas (i.e., neighbor relationships are maintained at, and across the congruent border between areas). It seems likely that such topographic representations are a particularly efficient configuration of cortex. Such an organization groups neurons that interact together, reducing fiber lengths and minimizing conduction delays. Topographic representations may also facilitate detection of movement and the refinement of acuity through center-surround receptive field configurations.

An alternative possibility for cortical elaboration is that cortical areas slowly fission by gradual separation. However, this seems less likely, as the result would be two daughter areas with the same (non-mirror image) orientation – and this is seldom observed. In addition, areas that gradually separate from one another would pass through a very nontopographic and presumably less efficient intermediate stage. Finally, if chemical gradients play a major role in the positioning and orienting of cortical areas during development, gradual separation of two areas may be difficult to achieve and the resulting, non-mirror-image representations may be difficult or impossible to code with chemical gradients (Catania, 2004).

31.4 Conclusions

Investigations of specialized mammals reveal a number of clear trends in mammalian brain evolution. This includes the expansion of the representations of behaviorally important sensory surfaces, the subdivision of cortical areas into modules representing parts of a sensory surface, and the addition of entirely new cortical areas to the processing network. Surgical alterations of sensory surfaces during development and the discovery of natural variations in sensory arrays suggests that many of the changes to the representations in the cortex may occur simply as a result of changes to the sensory surface that are communicated centrally by a developmental cascade. However, larger-scale changes in brain organization, such as the addition of new cortical areas to the processing network, require alterations of gene expression that are centered in the developing brain. The most recent advances in manipulating the expression of patterning genes in the cortex suggest mechanisms by which areas may be added to the cortex. These findings support some long-standing theories for how the brains of ancestral mammals may have evolved to produce the diversity of cortical configurations observed in modern mammalian lineages.

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32 Somatosensory Specializations of Flying Mammals

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Glossary

boundary layer	In reference to the airflow around a wing or airfoil, the thin layer of air immediately adjacent to the surfaces where air speed is reduced by surface drag.
camber	Cross sectional profile of an airfoil with a convex curve to upper surface that provides lift.
homunculus	Schematic, two dimensional presentation of body surface representations mapped in mammalian somatosensory neocortex.
interfemoral membrane (IFM)	The segment of wing membrane between a bat's legs.
neuroethology	The study of the nervous system in context with the natural behaviors and functional requirements of an organism.
radiohumeral membrane (RHM)	The segment of wing membrane in front of a bat's arm and forearm.

32.1 Introduction

As bats represent the only mammals with true powered flight, most of this article considers the bat somatosensory system. Although bat flight has long been observed and studied, surprisingly few data exist on sensory adaptations for flight. The oversight may result from the fact that the bat's somatosensory system has seldom been considered from an ethological viewpoint and almost never considered in terms of the unique capabilities and limitations of the bat hand-wing or how the somatosensory system might support bat flight or flight-related behaviors.

Bat flight has a highly acrobatic quality based on a wing that is largely made up of an elaborated hand (Chiroptera, the order of bats, translates as hand-wing). Figure 1 shows the basic pattern of the bat wing with a membrane of thin skin stretched between body wall with elongated forelimb arm and digits that act as wing-supportive struts. In most bats, a continuation of the wing membrane stretches between the legs (the interfemoral membrane or IFM). In the transformation into an airfoil, the bat hand has become functionally closer to a quadriped's hand than a biped in the sense that the hand-wing is almost entirely used to support the body (at least during flight) with little apparent ability to grip, carry, or manipulate objects. In other mammals, dense or specialized tactile innervation is generally associated with dexterity and a need for feedback in the fine control of manipulation and grip. Surprisingly, there is considerable evidence (from studies beginning over 140 years ago) that the bat wing is densely innervated with a number of potential tactile specializations. Only in the past 20 years, however, have these features been considered in terms of their role in a hand that is specialized for flight rather than manipulation. Before reviewing the earlier and recent studies of the bat somatosensory system, it is useful to consider some of the unique characteristics and challenges of flight and foraging with a hand-wing and specific roles for tactile feedback from the wing (Zook and Fowler, 1982, 1986; Zook, 1985, 2005,

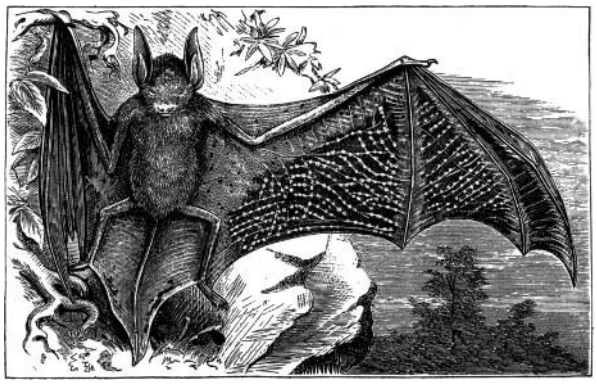

Figure 1 Nineteenth-century lithograph of *Myotis welwitschii*. Reproduced from Maxim, H. 1912. The sixth sense of the bat. The possible prevention of sea collisions. *Sci. Am. Suppl. V* 74, 148 150, with permission.

2006; see The Evolution of the Somatosensory System, The Evolution of the Basal Ganglia in Mammals and other Vertebrates, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory and Motor Cortices?).

32.1.1 Wing Camber, Boundary Flow, and Lift

Although the dexterity of the bat hand-wing is limited, the bat's limbs and hands retain sufficient joint mobility for a flying bat to adjust the overall shape of the hand-wing airfoil, or even the shape of separate wing segments. With this articulated airfoil, a flying bat can continually adjust its wings to fine-tune the lift properties of wing to enhance control of wing aerodynamics, providing for a level of maneuverability unmatched by most birds.

The shape of an airfoil is critical for generating and sustaining lift properties (specifically the camber of the airfoil, the cross-sectional contour of the wing between leading and trailing edges). (For more complete treatments of bat-wing aerodynamics, see Rayner, 1987; Norberg, 1990; Altringham, 1996.) Figure 2a shows a simplified pattern of the airflow around a cambered airfoil. The parallel lines above and below the wing represent the pattern of laminar flow found within the boundary layer of air that lies next to the wing. Lift is based on the fact that air in the boundary layer must travel farther and thus faster over the top of the airfoil than below the airfoil. With this velocity difference, air pressure above the airfoil is reduced relative to below, and lift is created. When other factors are constant (air speed, angle of wing attack, drag), lift can be amplified by increasing airfoil curvature (camber), but only as long as boundary layer flow is able to follow the curved surface. Figure 2b shows the condition of boundary layer separation, where camber has been

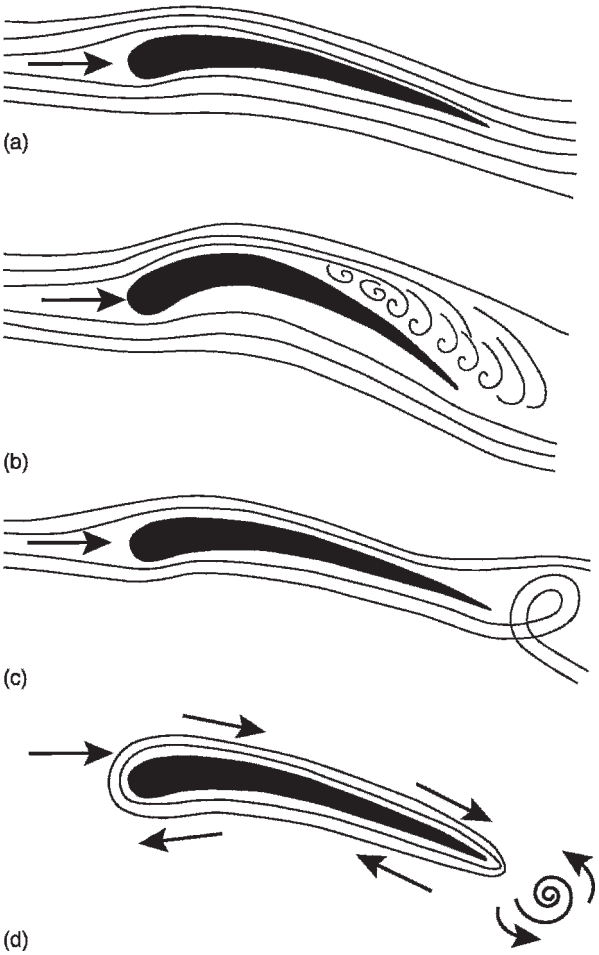

Figure 2 Boundary layer airflow patterns. The curved profiles represent a cross-sectional shape of a typical airfoil (camber), leading edge to left, trailing edge to right. a, Parallel lines represent the typical pattern of laminar airflow in the boundary layer next to wing surfaces. b, Boundary layer separation and boundary turbulence result when airfoil curve or camber is increased past the point where boundary flow can follow the curve. c, Flapping is thought to set up vortex wake patterns which develop and are shed from wing tips or from the trailing edge as shown here. d, During the wing-beat cycle, vortex theory suggests that boundary layer flow will circulate around the wing.

increased beyond the point where boundary flow can no longer follow the airfoil surface. Separation results in local turbulence in the flow and a sudden loss of lift (the wing stall point). The bat's control of wing shape allows it to adjust camber to optimize lift (and reduce stall speed). Unfortunately, the complex variables that affect boundary flow in flapping flight make estimation of boundary flow, camber, and stall difficult to judge or predict (Norberg, 1990; Altringham, 1996).

In flapping flight, the wings must provide propulsion as well as lift through changes in wing shape and angle of attack throughout the wing-beat cycle.

Vortex wake theory applied to a flapping airfoil predicts that the boundary layer flow will be pulled into the wing. Rotational currents are set up along wing surfaces, generating local vortex eddies which tend to be shed off the trailing edge of the wing (Figure 2c) or off the wing tips (Rayner, 1987; Altringham, 1996). During all or part of the wing-beat cycle, pressure gradients cause boundary flow to circulate around the wing (Figure 2d) which affect ongoing lift, drag, and propulsive forces acting on the wings (Rayner, 1987; Altringham, 1996; Norberg, 2002). The proposed somatosensory specializations of the bat wing may provide necessary feedback regarding wing lift by monitoring boundary layer flow, turbulence, circulation, and wake patterns (Zook, 1985, 2005; Zook and Fowler, 1986). The basic data to support this theory are reviewed here along with recent physiological and behavioral experiments from our lab (Zook, 2005, 2006).

32.1.2 Wing Capture of Active Prey

Although well adapted for flight (Swartz, 1998), the hand-wing's dexterity is limited by the extreme attenuation of its membrane-bound digits as well as the reduced strength and number of intrinsic muscles (Swartz *et al.*, 1996; Norberg, 2002). This functional tradeoff between flight and manipulation abilities presents a real challenge for most foraging behaviors and especially for the midair capture of flying insects. Bats may occasionally use their jaws to snatch insects out of midair, but such direct mouth capture is seldom a real option, given the size of a typical bat's mouth, the size of most prey, and the relative three-dimensional motion between pursuer and evasive prey (Webster and Brazier, 1965; Kalko and Schnitzler, 1998). While wing digits cannot be used to pick insects out of the air, the wings can be cast like nets to sweep a targeted insect out of the air (Vaughan, 1970; Kalko, 1995; Kalko and Schnitzler, 1998).

Once an insect is swept from air to the wing surface, there is still the challenge of transferring prey from the wing to the bat mouth without the ability to grasp the struggling insect during transfer (Webster and Griffin, 1962; Webster and Brazier, 1965; Zook, 2006). Furthermore, since capture generally takes place at night, and wing-gathered insects are too close for effective echolocation, transfer from wing to mouth must take place without either visual or echolocative cues (Fenton, 1990). Despite these challenges, foraging can be quite efficient, with some estimated capture rates greater than 90% (Kalko, 1995; Acharya and Fenton, 1999; Rydell *et al.*, 2002; Surlykke *et al.*, 2003). Feedback from wing cutaneous receptors could play a major role in this remarkable foraging efficiency (Zook, 2005, 2006).

32.2 Innervation of the Bat Wing

32.2.1 Early Neurohistology

As appropriate neurohistological techniques became available around the turn of the nineteenth century, a number of studies focused on the innervation of the wing (Schöbl, 1871; Sabussow, 1910; Ackert, 1914; see Griffin, 1958; Quay, 1970b, for reviews). These early studies and all that followed noted a particularly dense innervation of wing membranes, with nerve fibers concentrated along the bundles of elastin–collagen fibers that form a weblike network of prominent bands within all membranes, as shown in Figures 1 and 3 (Gupta, 1967; Holbrook and Odland, 1978). Most observers have commented on the regular series of raised domes distributed in the skin over the membrane bands. As shown in Figures 3 and 4, each dome is marked by a central hair follicle that is surrounded

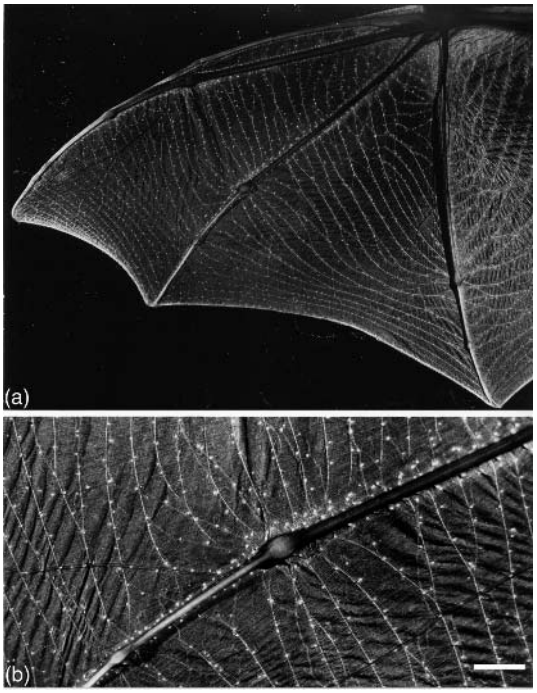

Figure 3 Photographs of transilluminated wing membranes from an *Antrozous pallidus* wing. a, Low-magnification photograph of the distal wing segments. b, Close-up view of the finger joints of the fourth digit shown in (a). Note the weblike pattern of membrane elastin collagen bands that span the wing with a tendency to converge at wing joints. Both photographs also show the typical wing pattern of single domes spaced along the bands as well as the tendency for domes to be concentrated near digits and joints. Scale bar: 2 mm. Reproduced from Zook, J. M. and Fowler, B. C. 1986. A specialized mechanoreceptor array of the bat wing. *Myotis* 23, 31 36, with permission.

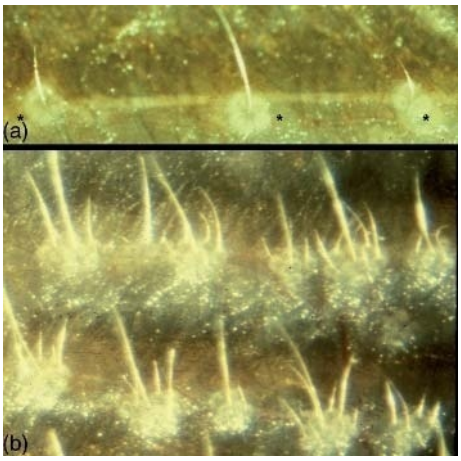

Figure 4 High-magnification photomicrographs of wing membrane domes from an *Antrozous pallidus* wing. a, A close-up view of the main wing pattern of single domes spaced along a membrane band. Asterisks mark the shadows of transilluminated domes positioned on the opposite membrane surface. b, A view of band-associated arrays of dome clusters found on both surfaces of the IFM. The average diameter of domes are 100 μm (a) and 250 μm (b).

by prominent sebaceous and apocrine glands (Schöbl, 1871; Ackert, 1914; Gupta, 1967; Quay, 1970a; Zook and Fowler, 1986; Crowley and Hall, 1994). Studies with a neurohistological focus consistently noted concentrations of nerve fibers and endings within the domes' dermis, basil epidermis, and at the hair follicle (Schöbl, 1871; Ackert, 1914; Quay, 1970b). In several microchiropteran species, free nerve endings and a number of possible terminal specializations were also reported in association with wing domes (Sabussow, 1910; Ackert, 1914).

Largely for historical reasons, none of the wing studies published before the 1980s had made an association between the rich innervation of the bat wing and possible sensory demands of flight and wing foraging. Before 1930, studies of the bat wing were dominated by the curious, but prevailing, theory that bats were able to navigate and forage in the dark due to a special tactile sixth sense of their wings. This tactile-navigation theory had been promoted by Georges Cuvier and others from the end of the eighteenth century. Touch remained the predominant explanation for the bats' blind-navigation skills for over a century, even though the Italian scientist, Lazzaro Spallanzani, had reported compelling experimental evidence of the role of bats' ears in distance navigation as early as 1795 (for reviews, see Dijkgraaf, 1949; Griffin, 1958; Neuweiler, 2000). The wing tactile theory persisted largely because, before the discovery of ultrasound and echolocation, no one could see how the ears could provide the distance sensing necessary for navigation.

In 1908, Walter Hahn came closest to associating wing tactile receptors with flight (and bat ears with navigation) when he observed that bats with grease-coated wings had trouble flying while bats with ear-plugs could fly but had trouble avoiding obstacles (Hahn, 1908). Hahn's mixed results did not deter Maxim (1912), who, in an attempt to improve ship navigation, came up with the most concrete mechanism for a tactile-based navigation sense. Maxim proposed that the bat's own wing beats generated a series of propagating, low-frequency waves – waves that could be reflected back from obstacles and processed by cutaneous receptors of the bat's wing (and face). The bat illustrated in Figure 1 appeared in Maxim's (1912) *Scientific American* article, where he introduced his design for a ship-based iceberg detector derived from the bat's supposed tactile navigation. Maxim's original caption noted: "This bat furnishes us with a very good illustration of the sensitive wing that enables a bat to send out vibrations and to receive the echo. The spots on the wing probably represent nerve centers." Although his first conjecture was off the mark, his second observation has proven quite prophetic.

Prior to these early wing studies, Merkel (1875) and Pinkus (1905) had separately described raised dome structures with a potential sensory function in other mammalian skin. These touch domes were named for their concentration of nerves and specialized terminal cells, Merkel cells, in the dome's basal epidermis (Iggo and Muir, 1969; Halata *et al.*, 2003). Merkel cells and their associated nerve terminals have come to be recognized as one of the four basic classes of mammalian tactile receptors (Smith, 1967; Iggo and Muir, 1969; Johnson, 2001; Halata *et al.*, 2003). Although not noted until later (Zook and Fowler, 1982), the bat domes bear a close resemblance to a less common form of touch dome–Merkel cell complex, a Haarscheibe, distinguished from other touch domes by a central or peripherally placed hair follicle (Pinkus, 1902; Smith, 1967).

Following the discovery of echolocation, the bat's wing received less attention, considered mainly in terms of the thin membrane's value for study of neurovascular, glandular, or lymphatic systems (reviewed in Quay, 1970a). Although the few additional histological studies mentioned the innervation of wing in passing, none directly addressed the possibility of a sensory role of the wing in flight and flight behaviors (Schumacher, 1932; Gupta, 1967; Quay, 1970b). It was not until almost 50 years later, in light of new attention on bat somatosensory neocortex in the 1980s, that wing innervation and wing domes were to be

considered in terms of flight (Zook and Fowler, 1982; Calford *et al.*, 1985).

32.2.2 Studies of the Somatosensory Central Nervous System

These cortical studies began with electrophysiological mapping of bat wing and body representation in primary somatosensory cortex (S1), and were mainly driven by evolutionary, comparative interests (Calford *et al.*, 1985; Wise *et al.*, 1986; Krubitzer, 1995). Most have been extensively reviewed elsewhere (Krubitzer *et al.*, 1993; Krubitzer, 1995) and will be summarized here only to the extent that they are relevant for flight adaptations. The majority of these studies were undertaken in species from the nonecholocating, frugivorous megachiropteran suborder of bats. The megachiropterans possess well-developed visual and olfactory systems useful for diurnal fruit foraging. As fruit eaters, their flight is not as finely tuned as it is in Microchiroptera, and tactile specializations may be less elaborate, particularly in comparison to insectivorous species which must actively pursue evasive prey in the night air. It is worth noting here that all of the primary studies reported large central neural representations of the hand-wing in Megachiroptera (Calford *et al.*, 1985; Krubitzer *et al.*, 1993; Martin, 1993; Manger *et al.*, 2001a, 2001b) as well as Microchiroptera (Zook and Fowler, 1982; Wise *et al.*, 1986).

32.3 Somatosensory Receptors of the Wing

32.3.1 Surface Features

Re-examination of the somatosensory periphery initially focused on the wings of the microchiropteran bat, *Antrozous pallidus*, and quickly began to reveal new details of the wing somatosensory system (Zook and Fowler, 1982, 1986). As *A. pallidus* is specialized as a gleaning/terrestrial foraging insectivore, two additional species were added to these studies, *Pteronotus parnellii* (an aerial/gleaning feeder) and *Eptesicus fuscus* (an aerial feeder).

The domes found on the bats' wing membranes were both more numerous and more highly organized than the Haarscheibe observed in other species (Smith, 1967). Similar to the earlier wing descriptions, domes in these species were distributed in regular arrays spaced out along wing elastin–collagen bands (Figures 3 and 4a), with additional concentrations grouped along the edges of wing bones (Figure 3). Individual domes were often spaced along the leading and trailing edges of the wing and scattered over limb bones and digits. In all three species, the density of domes per unit area varied between wing segments with a general increase toward the leading and trailing edges as well as wing tips. While dome distribution patterns were similar across the wings of all species, there is variation in dome diameter (100–400 μm), hair size, and hair length as well as specialized regional distributions (see discussion of the IFM, below). The largest domes were found in *A. pallidus*, most likely a byproduct of the exceptionally large, dome-associated glands in this desert-dwelling species.

In all species examined, domes appear to be distributed over the two sides of each wing membrane in almost mirror-image patterns. This can be appreciated in Figure 4a (*A. pallidus*), where each dome and hair projecting from the ventral surface is almost always closely opposed to a dome on the dorsal side (asterisks in Figure 4a).

The IFM was distinguished by a very different pattern, with small clusters of domes spaced out along the elastin–collagen bands of the membrane. This cluster pattern was particularly distinct over the dorsal and ventral surfaces of the IFM in *A. pallidus* (Figure 4b). Light microscopy and scanning electron microscopy showed that each IFM cluster consisted of a large, central dome with an unusually long hair surrounded by five to six smaller domes, each with a shorter, smaller-diameter hair (Zook and Fowler, 1982, 1986). The IFM clusters were also seen in the other species examined, but these were generally more randomly organized.

In their histological examination of the megachiropteran wing, Crowley and Hall (1994) found similar dome structures on the wing surfaces of several megachiropteran species. The megachiropteran domes differed in size of dome and diameter of dome hairs (respectively 2× and 5× larger) and in dome distribution across wing membranes. Although the megachiropteran wing membranes have similar elastin-band arrays, Crowley and Hall reported that megachiropteran domes were neither specifically associated with wing elastin bands nor regularly distributed across wing surfaces. There did not appear to be any special dome clustering or dome distribution on the megachiropteran IFM. Crowley and Hall did note that megachiropteran domes were particularly concentrated in lines running over elevated or projecting features of the wing, such as the larger membrane blood vessels, membrane-bound muscles, and wing bones. These particular locations may improve the mechanical isolation of domes from the surrounding wing membranes or could raise domes and dome hairs away from the wing surfaces to sample better specific regional boundary layer flow patterns.

32.3.2 Dome Neurohistology and Primary Afferent Recordings

Preliminary studies of bat wing with modern histological techniques (Zook and Fowler, 1982, 1986) revealed a large population of presumptive Merkel cells (arrowheads, Figure 5) concentrated at the basement membrane along the dome surface and surrounding the hair follicle. Following the classic description of Merkel cells (Pinkus, 1905; Smith, 1967; Halata *et al.*, 2003), these dome cells were typically large, clear cells with lobulated nuclei restricted to the epidermal basal lamina. This identification has been supported by more recent histology (Zook, 2005) showing a positive staining of these cells with both Merkel-specific quinacrine fluorescence (Nurse *et al.*, 1983) and a cell-specific antibody to the cytokeratin protein, CK20 (Moll *et al.*, 1995). Nerve fibers within the dome complex can be traced to individual Merkel cells, although free nerve endings and as yet unidentified specialized nerve terminals were identified around the dome hair follicle.

Preliminary recordings of wing dome primary afferent nerves have been made in the microchiropteran species, *A. pallidus* and *E. fuscus* (Zook and Fowler, 1986; Zook, 2005). These studies used suction and hook electrodes to isolate and record single afferent fiber activity from either a main limb nerve or from a smaller membrane nerve bundle isolated within the proximal wing membranes. Recorded afferents generally showed low-threshold responses to light touch or air-puff stimuli and could be characterized as either slowly adapting (SA) or rapidly adapting (RA) units. Both SA and RA responses could be associated with tactile stimulation of specific domes or wing regions. Afferent SA responses could be elicited by direct contact with an individual dome or from a small cluster of domes. Responses generally could not be elicited from light touch of the wing membrane surrounding a responsive dome or in between domes. Such response patterns are typical of Merkel cells in other mammals which are distinguished by their mechanically isolated, SA response patterns (Iggo and Muir, 1969; Halata *et al.*, 2003). In some wing afferent recordings, SA responses could be elicited by contact or movement of the dome hair. Still other dome-associated afferents showed primarily RA responses to hair movement. These latter afferents were particularly sensitive when air puffs were used to move dome hairs.

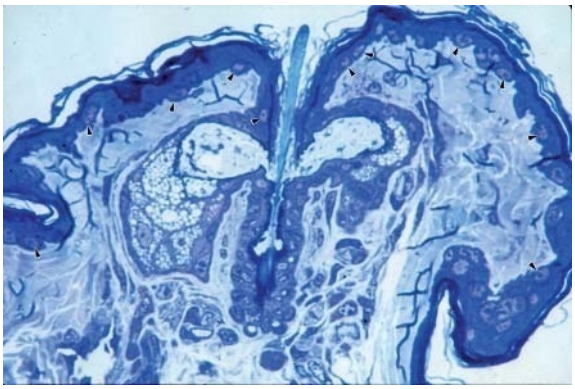

Figure 5 A high-magnification photomicrograph of a wing membrane dome in cross section. Methylene blue stain from *A. pallidus*. The darkly staining epidermis contains a number of clear cells, Merkel cells, along the boundary with the lighter-staining dermal layer. Concentrations of Merkel cells are also found encircling the centrally placed hair follicle above and below the dome's large sebaceous glands. The cross-sectional width of this dome is 120 μm.

The large-nerve recordings revealed another population of less selective RA afferents with a generalized response to membrane contact or membrane stretch. These afferents were characterized by medium to large receptive fields involving substantial portions of a membrane segment and often including a portion of a neighboring arm or digit. In many cases, these stretch-responsive afferents showed eccentric receptive fields with a point of greatest sensitivity near the convergence of multiple elastin bands at joints of the digits, wrist, or arm with a larger, less sensitive response field extending out from the side of the joint toward the middle of an adjacent wing membrane (Zook, 2005, 2006). Primary afferents serving cutaneous receptors in the IFM have not been examined.

Perhaps these studies' most interesting observation was the degree to which responses to threshold stimulation were confined to one or the other surface of a wing membrane. In other words, a given afferent might respond vigorously to the slightest contact with the dorsal membrane surface while remaining silent with even major deflections of the opposing ventral membrane surface. This surface-specific response was quite unexpected, given the extremely thin wing membrane skin that ranges in thickness from less than 0.03 mm in aerial-feeding insectivores to around 0.06 mm in many terrestrial-feeding microchiropteran species (Studier, 1972). megachiropteran species can have somewhat thicker membranes up to around 0.25 mm (Crowley and Hall, 1994). Given these dimensions, it is difficult to see how these receptors on opposing wing surfaces could be both sensitive to surface contact yet mechanically isolated from stimulation of the opposing surface.

The surface-specific response phenomenon was most dramatic in the case of the broadly sensitive,

large-field, stretch receptor population. Although afferents associated with stretch receptors responded to the slightest surface contact within its receptive field, almost all were markedly insensitive to gross stimulation of the opposite membrane surface, including gross physical deformations. The smaller receptive fields associated with dome and dome hair receptors were also more mechanically isolated from stimulation of the membrane surface directly opposing the dome than to the surrounding membrane on the same side as the dome. This uncoupling of membrane surfaces could be useful for selective discrimination of different airflow patterns over dorsal and ventral surfaces or may even be useful for improving the detection and localization of insect prey in contact with a wing surface.

The structural and physiological bases of this surface-specific receptor sensitivity have yet to be explored. It would seem likely that the limited mechanical isolation between surfaces could be enhanced by some form of mutual, contralateral inhibition within the local receptor population (Zook, 2005). Among the topics yet to be explored are the suggestion of afferent responses to directional stimulation of dome hairs and dome clusters, and the possibility of selective response of dome hairs to specific airflow or turbulence patterns.

Different types of wing hairs and pelage hair distributions have been observed over the wings of some microchiropteran species. While the forearm bones in most bats project above the membrane surface, such drag-inducing protrusions may be smoothed out by a tapered pattern of surrounding pelage hairs observed in some species, most notably in the swifter-flying Microchiroptera, where drag is a major factor (Vaughan and Bateman, 1980). A similar tapered extension of pelage hairs may smooth the transition between body and dorsal IFM (Vaughan, 1970). For slow-flying species, Vaughan and others have suggested that the projecting forearm bones or other hair patterns might be used to induce a measured degree of turbulence into the boundary air layer as a means of reducing flow separation from the wing reducing drag (Vaughan, 1970; Hill and Smith, 1984; Norberg, 2002).

32.3.3 Possible Roles of Tactile Receptors in Flight

The observed wing stretch receptor population has the most obvious potential role as a means of monitoring wing and wing membrane strain during sudden turns or extreme flight maneuvers (Zook, 2005). A number of studies have noted the fine balance struck by the wing between the need to conserve flight mass and the requirements for structural support (Studier, 1972; Swartz *et al.*, 1996; Swartz, 1998; Norberg, 2002). The ability to gauge relative strain on delicate wing elements during flight would seem crucial for all bats. A possible secondary role for stretch receptors in foraging (Zook, 1985, 2005, 2006) will be covered in the next section.

Domes and dome hairs show a number of characteristics that would make them suitable for monitoring boundary layer airflow, such as their ordered distribution across all wing surfaces as well as their mechanical isolation from the membrane and sensitivity to air stimuli. A preliminary survey across species suggests that the greatest variation in dome distributions may be found between species specialized for either slow or fast flight, which may have different requirements for monitoring boundary flow or wing vortex patterns (Zook and Fowler, 1986).

One preliminary behavioral study explored the potential role of dome hairs in flying bats (Zook, 2005). Several bats of the species *A. pallidus* and *E. fuscus* were flown in a lab setting after a depilatory cream was used to remove dome hairs from all wing surfaces. Flying bats without dome hairs showed no obvious difficulties during straight flight or when performing shallow turns. Flight performance, however, was clearly affected during sharp-angle turns. Although there were wide variations between tests and among bats, all showed abnormally large changes in flight elevation during most turns, often taking the form of an oscillating pattern of elevation changes. Following regrowth of dome hairs, normal turning behavior was re-established in each test bat, marked by a gradual rise during entry into the turn and a smooth transition through the turn (Aldridge, 1987).

Sharp turns are among the more complex of flight maneuvers, involving sudden braking, loss of air speed, and major changes in wing position and conformation (Norberg, 1985, 1990; Aldridge, 1987). Although preliminary, these hair ablation experiments suggest that dome hairs may be particularly involved in complex flight maneuvers that require rapid and precise adjustments of wing camber to maintain optimal lift. Similar wing-spanning sensory arrays would presumably be useful for birds, but in a feathered wing, sensory feedback for lift control may be obtained from extracutaneous receptors, specifically from muscle spindle populations in the avian forearm (Brown and Norberg, 1997). In bats, similar proprioceptive-based feedback could be provided by the muscle spindle afferents of the intrinsic membrane, striated muscles found within the proximal wing of many bats (Schumacher, 1932; Gupta, 1967; Crowley and

Hall, 1994). While the main role of these membrane-bound muscles may be for shaping or tensioning the membrane during flight (Swartz *et al.*, 2004), their spindle afferents might also signal local membrane disturbances, such as the ripples or fluttering that would result from boundary layer turbulence (similar to the lufting motion of a poorly trimmed sail).

Apart from a potential role in monitoring boundary flow, the clustered domes of the IFM are particularly intriguing in light of the proposed roles of this membrane in flight and aerial stability. Although the IFM is not present in all species and thus may not be essential for flight (Vaughan, 1970; Hill and Smith, 1984), it does add an additional lift surface and may aid in flight maneuvers such as an air brake during turns or as a flap for adjusting longitudinal stability (Vaughan, 1970; Norberg, 1985). Since bats have a short fuselage and lack long tails, pitch control may have to be actively maintained, possibly by curving the IFM either upward or downward to counterbalance shifts between a bat's center of mass and center of lift during different parts of the wing-beat cycle (Vaughan, 1970; Rayner, 1987). The IFM's elaborate dome arrays may provide feedback to optimize vestibulomotor reflexes controlling longitudinal stability (Zook, 2005). Wing dome hair arrays and their regional patterns need to be explored in terms of their potential interaction with vestibular and motor control of flight and flight stability in all dimensions (Horowitz *et al.*, 2004).

32.3.4 Possible Roles for Wing Receptors in Foraging

All or part of the wing receptor array could be used for locating, tracking, and assisting transfer of wing-gathered prey from initial contact point to mouth. Given their distribution, response patterns, and mechanical isolation, wing domes and dome arrays could provide fairly accurate positional feedback to aid in locating prey during manipulation and transfer (Zook, 2005, 2006). Insect movement and direction of movements across the wing surfaces could be tracked from a sequential stimulation of individual domes, dome hairs, or dome hair arrays.

The IFM is a favored site for capture in all kinds of foraging (Webster and Brazier, 1965; Arlettaz, 1996; Kalko and Schnitzler, 1998). While the IFM can be cupped more than the other wing membranes, struggling prey still cannot be pinned in place. Tactile feedback from IFM dome clusters might provide even more feedback on prey contact points or the direction of prey movement than the spaced dome arrays of the main wing. The main wing membranes of the forearm are the second most common sites for prey contact during aerial foraging. While the forearm and digits provide a longer reach for sweeping in prey than would be possible with the IFM, the forearm wing cannot be cupped for containing prey to the degree possible with the IFM (Arlettaz, 1996; Zook, 2005, 2006).

Forelimb sweeping motions may offer another advantage during capture. By continuing the wing's sweeping motion through the contact point, a bat may use the wing's acceleration and the insect's inertia to stick the insect to the wing membrane. Such an inertia-pinned insect would be easier to control during the short but critical transfer from wing to mouth. While the large receptive fields of the stretch receptors may not be useful for precisely locating wing-gathered prey, they could provide a means to monitor where the insect first contacts the wing membrane (Zook, 2005, 2006) and how well the insect is pinned to the wing contact point.

32.4 Central Somatotopic Representation of the Bat Wing: A Neuroethological Perspective

The observed peripheral tactile specializations provided an impetus for examining neocortical somatotopic representations of the wing in *A. pallidus* (Zook and Fowler, 1982). At the same time, a series of other studies were begun that mapped somatotopic representations of wing and body in several megachiropteran species, focusing primarily on the comparative and evolutionary implications of these cortical maps (Calford *et al.*, 1985; Wise *et al.*, 1986). As with most central sensory maps, these somatotopic maps generally reflect the relationships and proportions of the related sensory surface, in this case skin tactile receptors, with a disproportionate representation of peripheral structures that have particularly rich innervation. Figure 6 shows comparable schematic representations (homunculi) of bat somatotopic maps from primary neocortical area S1. Figure 6a is a homunculus redrawn from Zook and Fowler (1982), while Figures 6b and 6c represents homunculi respectively from a different microchiropteran species and a megachiropteran species (Wise *et al.*, 1986). A rat homunculus is included for comparison (Figure 6d).

Focusing on the wing representations, all four bats showed an enlarged representation of the hand with a disproportionate representation of the thumb, the only free digit of the hand-wing. All the bat maps contain representations of the remaining digits and

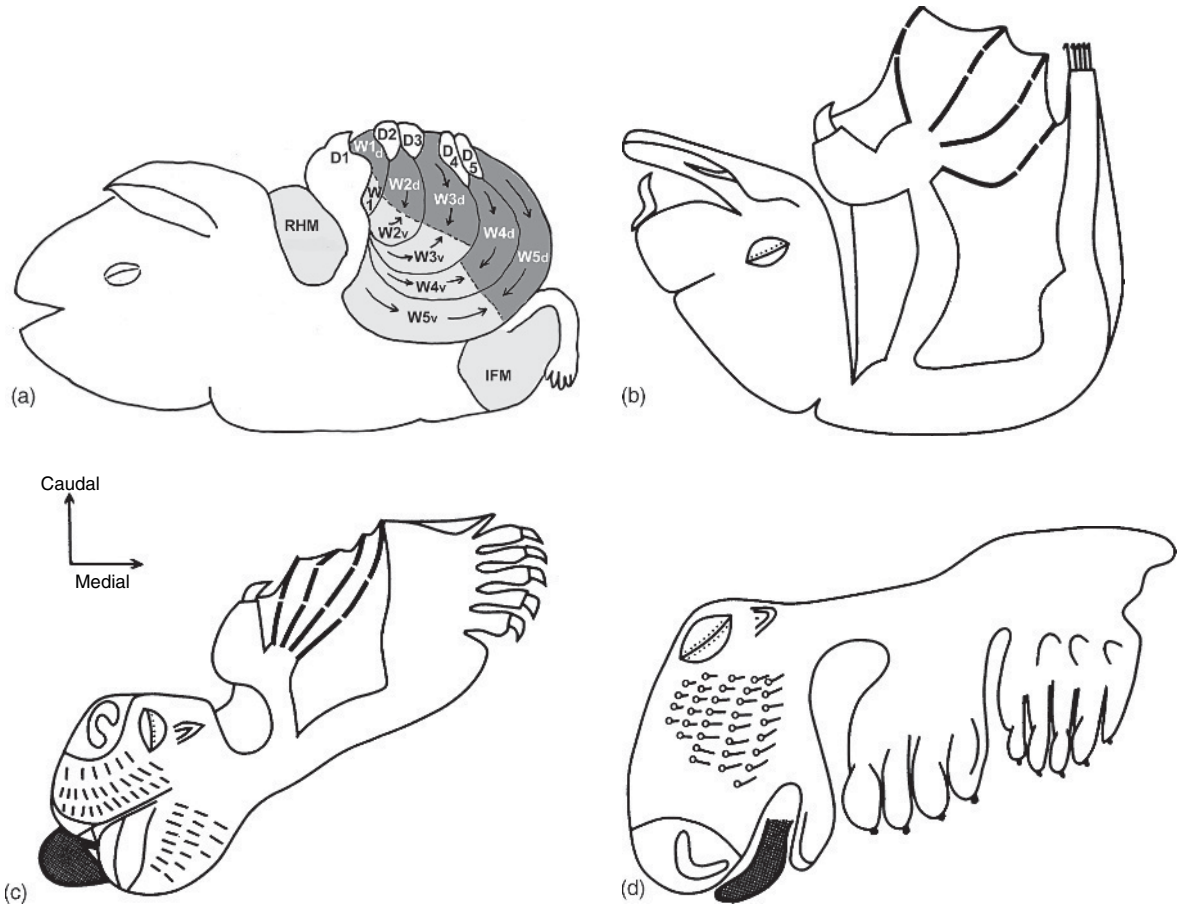

Figure 6 Schematic representations of the body surface (homunculi) from mapping studies of primary somatosensory cortex (S1) from two microchiropteran bats: a, *Antrozous pallidus* data (partly) from Zook and Fowler (1982); b, *Macroderma gigas* from Wise *et al.* (1986); and c, the microchiropteran bat *Pteropus poliocephalus* from Calford *et al.* (1985). A homunculus from the rat is shown in (d). Maps are not to scale, but are all oriented to match the position of the original cortical recordings on the surface of the cortical hemisphere. As indicated by the marker, the right side of each map is closest to the cortical midline, the left (face) side of each map is toward the lateral side of the hemisphere, while the top of each map is oriented toward the caudal pole of the brain. The bottom of each map is oriented toward the rostral pole of the brain. In map (a), the wing membranes (dark and light gray shading, labeled W1 W5) were represented somewhat separate from the digits (digits 1 5 labeled D1 D5) with a separate representation of each membrane surface (ventral light gray, dorsal dark gray). Each membrane surface representation was internally consistent, but flipped along the trailing edge (dashed lines). The arrows point the membrane maps' progression in the representation from leading to trailing edges. The ventral surface leading edge begins near the palm/D1 while the dorsal surface leading edge begins near the digits D2 D5, with both representations converging into the single trailing edge representation. RHM, radiohumeral membrane; IFM, interfemoral membrane. b d, Reproduced from Somatosensory cortical representation in the Australian ghost bat, *Macroderma gigas*, *J. Comp. Neurol.*; Wise, L. Z., Pettigrew, J. D., and Calford, M. B.; Copyright © 1986, Wiley-Liss. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

wing membranes. In the Zook and Fowler map, however, membrane segments are partially segregated from the digits and include separate representations of the dorsal and ventral forelimb membrane surfaces. The other bat-wing representations report a single wing surface with digits positioned between wing segments. It is not clear whether surface-specific responses were encountered in these latter studies.

The difference between results in the different bats may be a matter of the different techniques or species used. Most somatotopic mapping studies depend almost exclusively upon surface-stimulating probes to elicit a standard response over all skin regions and across species. In order to elicit surface-specific responses from the wing membranes, the Zook and Fowler studies used a combination of tactile probe and air-puff stimulation. Air puffs may have selectively stimulated a subset of skin tactile receptors, imposing a modality bias on the cortical maps. In other species, when the different submodalities of tactile sensation are independently mapped on cortex, the somatotopic patterns can be quite different from the more standardized, probe-based map (Dykes, 1983; Friedman *et al.*, 2004). It is also

possible that the dual-surface pattern is unique to a subset of microchiropteran bats with specific flight or foraging requirements. Of the two microchiropteran species represented in Figure 6, *A. pallidus* is a gleaning and terrestrial insectivore known for the use of passive sound localization and highly maneuverable flight (Fuzessery *et al.*, 1993), while *Macroderma gigas* is an exceptionally large carnivorous bat with a well-developed visual system and less acrobatic flight (Wise *et al.*, 1986).

One of the most striking aspects of the surface-specific membrane representation in *A. pallidus* is the internal consistency within the two membrane surface maps. The ventral surface map reflects the hand and membrane pattern seen in the other bats in Figure 6, with the membrane segments extending caudally from the hand and thumb (D1) representations. At the trailing edge of the ventral surface representation, the membrane map flips to begin representing the dorsal surface, progressing from trailing to leading edge (arrows in the two membrane surface maps begin near the representation of the leading edge and point toward trailing edge representation where the surface maps converge). The fact that peripheral separate-surface response patterns are retained to the level of S1 may indicate a degree of independent processing of the two surfaces' receptor populations.

These wing-specific features aside, the general pattern of cortical body maps were otherwise quite consistent among these bat species (Wise *et al.*, 1986; Krubitzer, 1995). The greatest variation appears in the hindlimb placement, which may reflect the difficulty in mapping this underrepresented area (Calford *et al.*, 1985). Compared to S1 cortical maps in other mammals, however, the mapped position of the bat forelimb was strikingly different. In the general mammalian pattern, the representation of the limbs projects rostrally in the brain (see the orientation bars in Figure 6) and is mapped on the homunculus ventral from the trunk and below the head with the digits projecting rostrally (Figure 6d). In the bats (Figures 6a–6c, the forelimb and digital representation extended toward the caudal brain so that the homunculi map's arm and wings project dorsally away from the trunk and above the head. This partial map reversal was first noted by Calford *et al.* (1985) and has been consistently observed in all species examined (Zook and Fowler, 1982; Calford *et al.*, 1985; Wise *et al.*, 1986; Krubitzer *et al.*, 1993). Comparisons of bat and general mammal somatosensory pathways and subcortical maps have shown that the limb reversal is set up through a series of transformations between the sensory periphery, dorsal column nuclei, and somatosensory thalamus (Martin, 1993; Manger *et al.*, 2001a, 2001b).

Calford and others have suggested that the digital reversal may be a cortical reflection of the manner that bats' hands are held at rest with digits positioned caudal to the hand and arm (Calford *et al.*, 1985; Wise *et al.*, 1986). While there is no direct evidence to support this habitual position theory, it is intriguing, as it implies a major proprioceptive influence in the ordering of these somatotopic maps. Joint proprioceptive, muscle spindle, and other extracutaneous somatosensory modalities have not been examined in bats but may all play a role in facilitating the exquisite vestibulomotor control exhibited by night-flying and night-foraging bat species (Horowitz *et al.*, 2004). Proprioceptive somatosensory modalities are likely to play a central role in night navigation, and may be critical for the bat's extraordinary ability to memorize and navigate complex flight paths without echolocation or other sensory guidance (Möhres and Oettingen-Spielberg, 1949; Griffin, 1958).

32.5 Future Prospects for Somatosensory Study of the Bat Wing

The study of the somatosensory system in mammalian flight has a history of misdirection, neglect, and lack of clear focus. Hopefully this article has given a sense of the possibilities for future study and the potential of the neuroethological approach that has proven so successful in the exploration of bat echolocation. While the focus here has been on the bat wing, cutaneous receptors of the head and face may offer an even greater range of specializations, for example, the thermal and tactile receptors in vampire bats (Kürten and Schmidt, 1984; Kürten, 1985). With regard to the wing, more data are needed, focusing on a comparative neurohistology of the chiropteran wing and a closer examination of cortical representation of the wing in a broader range of representative species. With regard to broader comparisons, there may be some interesting similarities between bat dome arrays and the manatee's array of tactile body hairs. The manatee hairs appear to function as a mammalian lateral-line system for the detection of water currents and other near-field phenomena (Reep *et al.*, 2002). In a manner similar to Maxim's proposed tactile sixth sense, a manatee's tactile hairs could serve as part of an active, remote-sensing system capable of detecting and interpreting echo patterns from pressure waves set up by the manatee's own movements.

Further study of both peripheral and central adaptations needs to establish behaviorally

appropriate stimuli to explore proprioceptive as well as cutaneous somatosensory receptor populations in terms of flight and flight-related behaviors. Although interesting in themselves, the wing dome arrays and the uncoupling of wing membrane responses and representations suggest that the main somatosensory adaptations for flight may lie in the population responses, the interplay of feedback from cutaneous and extracutaneous receptors, and their integration within the motor and vestibulomotor reflexes that optimize flight control (Horowitz *et al.*, 2004). This broader perspective of the role of somatosensory specializations in mammalian flight could lead to a greater appreciation of the sensorimotor mechanisms involved in vertebrate flapping flight as well as the role of feedback in the design and optimal control of micro air vehicles (Shyy *et al.*, 1999; Norberg, 2002).

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33 The Evolution of Motor Cortex and Motor Systems

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Glossary

autoradiograph (or autoradiogram)	An image produced on photographic emulsion or film by the radiation emitted from a radioactive isotope that has been absorbed by tissue; e.g., radiolabeled amino acids can be used to visualize neuronal tracts via axoplasmic transport.
Betz cells	The largest layer V pyramidal neurons. They are believed to comprise a large proportion of corticomotoneuronal cells.
cladogram	A branching, treelike diagram in which the endpoints of the branches represent specific species of organisms. It is used to illustrate phylogenetic relationships and show points at which various species have diverged from common ancestral forms.
corticomotoneuronal	Referring to corticospinal neurons that form monosynaptic contact with spinal cord motoneurons.
corticospinal tract	Collective term for neurons with somata located in cerebral cortex that send axons to the spinal cord via the cerebral peduncles and medullary pyramids.
cytoarchitecture	The cellular composition of a bodily structure; e.g., used to define a structurally distinctive cortical area.
eutherian	Belonging to the infraclass Eutheria, a division of mammals to which all placental mammals belong.
funiculus	One of the major white matter tracts in the spinal cord consisting of large bundles of ascending and descending fibers.
intracortical microstimulation	A technique for stimulating a small neuronal cell population

	by introduction of a microelectrode and delivery of current pulses; used to stimulate corticospinal neurons to define functional motor representations in cerebral cortex.
lissencephalic	Characterized by a smooth cerebral cortex.
microelectrode	A very small insulated wire or fluid filled micropipette used to study functional characteristics of living cells and tissues; commonly used for recording action potentials from one or more neurons, or for stimulating a small group of neurons.
prototherian	Neurologically primitive, egg laying mammals found only in Australia, Tasmania, and New Guinea; the monotremes.
striatum	The phylogenetically more recent part of the corpus striatum (neostriatum) consisting of the caudate nucleus and the putamen.
supraspinal	Origin located above the spinal cord.
tetrapod	A vertebrate animal with four feet, legs, or leglike appendages.

33.1 Introduction

The varied and complex motor behaviors exhibited by vertebrates are the result of extraordinary alterations and enhancements over hundreds of millions of years of nervous system evolution. While this brief article cannot convey a complete picture of all of the selective pressures that likely gave rise to the differentiation of specialized motor structures, we will try to highlight some of the salient features that have marked the evolution of vertebrate motor systems and the uniquely mammalian motor cortex (see The Evolution of the Somatosensory System).

33.2 The Phylogenetic History of Descending Control of Spinal Cord Motoneurons

33.2.1 The Basic Vertebrate Plan

Based upon a large number of tract-tracing studies conducted over the past few decades, it is possible to survey extant mammals and surmise the probable evolution of descending control of spinal cord motoneurons in vertebrate species. This allows us to determine what features might be unique in specific lineages (e.g., the anthropoid lineage leading to humans), or specific ecological niches, and to appreciate the parallel contribution of common selective pressures regardless of lineage. To put the mammalian motor system in perspective, and to derive general principles for the evolution of motor systems, it is first necessary to review briefly descending control of spinal cord motoneurons prior to the divergence of mammals. With the addition of new evidence, this review represents an update of an earlier survey (Nudo and Masterton, 1988).

Even in the most primitive vertebrate forms still extant, neurons in the spinal cord are influenced by axons originating at supraspinal levels. Some of these descending pathways emerged in the most primitive vertebrate forms. The application of retrograde transport techniques has demonstrated supraspinal origins of spinal fibers in a wide variety of vertebrates (ten Donkelaar, 1976; Kokoros and Northcutt, 1977; ten Donkelaar *et al.*, 1980; Smeets and Timerick, 1981; Wolters *et al.*, 1982; Forehand and Farel, 1982; Kimmel *et al.*, 1982; Kunzle and Woodson, 1983; Oka *et al.*, 1986; Prasada Rao *et al.*, 1987; Ronan, 1989; Lee and Eaton, 1991; Cruce, *et al.*, 1999; Zhang *et al.*, 2002). Table 1 summarizes these results in various vertebrate classes for some of the major descending pathways that are found in mammals. Although these data do not provide a perfectly complete picture of all descending spinal afferents, and the species that have been studied cannot be considered to be either perfect representatives or random samples of their entire class, the many cases already available display several clear similarities that shed light on the very early, premammalian history of those descending pathways now found in mammals.

As shown in Table 1, at least three major supraspinal cell groups have projections to the spinal cord in every species of vertebrate studied to date: the reticular formation (a collective term for a diverse set of neurons that reside in the midbrain and hindbrain) (Lee and Eaton, 1991) (reticulospinal tract), the vestibular nuclei (vestibulospinal tract), and the interstitial nucleus of the medial longitudinal fasciculus (interstitiospinal tract). Further, in all vertebrates yet studied, the spinal projections of each of these three cell groups travel down the cord in the ventral funiculus and each terminates in the ventromedial spinal gray (ten Donkelaar, 1976). Based upon their similar origins, trajectories, and terminations, it is probably safe to conclude that each of these three pathways is a true homologue of its respective counterpart throughout the vertebrate subphylum. If so, they can be considered to constitute a vertebrate common plan of descending spinal pathways.

In addition to these three pathways, tract-tracing studies in the most neurologically primitive

Table 1 Supraspinal cell groups with descending projections to the spinal cord in various vertebrate classes

	Agnatha	*Chondrichthyes*	*Osteichthyes*	*Amphibia*	*Reptilia*	*Mammalia*	*Aves*
Reticular formation	+	+	+	+	+	+	+
Vestibular nuclei	+	+	+	+	+	+	+
Interstitial nucleus	+	+	+	+	+	+	+
N. descending trigeminal tract	?	+	+	+	+	+	+
Raphe complex	?	+	+	+	+	+	+
N. solitary tract	?	+	+	+	+	+	+
Hypothalamus	+	+	+	+	+	+	+
Red nucleus		±	±	±	±	+	+
Cerebellar nuclei				+	+	+	+
Telencephalon				+	?	+	

+, Pathway has been positively identified in all species examined in the class; , pathway has been sought, but has not been found in any species examined in the class; ±, pathway has been identified in some, but not all species examined in the class; ?, differentiation of these structures is still unsettled in class Agnatha.

vertebrates (lampreys and hagfish; class Agnatha) have revealed spinal cord projections from brainstem neurons that may be homologous to those identified in gnathostomes, or jawed vertebrates. Despite their seemingly simple neurological organization, agnathans may possess spinal pathways originating in the nucleus of the descending trigeminal tract, the Raphe complex (many investigators include the cells identified here as the Raphe complex to be part of the reticular formation), the nucleus of the solitary tract, and the hypothalamus (Ronan, 1989). Thus, many of the cell groups that form spinal pathways in mammals were probably already in place early in vertebrate evolution. While these various cell groups became more specialized in different vertebrate classes, once established, this basic vertebrate plan was maintained throughout hundreds of millions of years of evolution, and still exists in all known living vertebrates.

33.2.2 Augmentation in Jawed Vertebrates (Gnathostomes): The Curious Case of the Rubrospinal Tract

Table 1 shows that in all vertebrate classes except Agnatha, descending spinal fibers have been identified that originate from the red nucleus and project to the contralateral spinal cord (rubrospinal tract). Although descending fibers originate from cells in the mesencephalic tegmentum in lampreys, the red nucleus is not differentiated (Ronan, 1989). It appears that all mammals and birds studied to date possess rubrospinal neurons. In contrast, in cartilaginous fish, bony fish, amphibians, and reptiles, rubrospinal neurons are present in some species and absent in others (see Somatosensory Specializations of Flying Mammals, Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?).

Variability in the presence of the rubrospinal tract in vertebrate species has generated considerable discussion regarding its functional significance. At least in mammals, the rubrospinal tract is thought to play a major role in the control of limb movements (Massion, 1988). In this regard, it is interesting that the rubrospinal tract is absent in boid snakes, caecilians (wormlike amphibians), and sharks, but present in limbed amphibians, limbed reptiles, and in rays (ten Donkelaar, 1988). Curiously, a crossed rubrospinal tract has also been described in lungfishes (Ronan and Northcutt, 1985) and goldfish (Prasada Rao *et al.*, 1987). This raises the possibility that the rubrospinal tract functions to control fins in aquatic vertebrates as well as limbs in terrestrial vertebrates.

If the rubrospinal tract emerged early in the gnathostome radiation, then it apparently became vestigial in many limbless amphibians and reptiles. If so, the disappearance of the rubrospinal tract would represent a rare exception to the rule that, once established, descending spinal pathways are maintained throughout subsequent evolution.

33.2.3 The Tetrapod Augmentation

Table 1 shows that the cerebellum (i.e., the deep cerebellar nuclei) projects to the spinal cord in amphibians, reptiles, mammals, and birds. Again, since this cerebellospinal tract has a similar origin, trajectory, and termination pattern, this tract is probably homologous across the tetrapod classes. If so, the basic vertebrate plan appears to have undergone a second augmentation with the appearance of tetrapodal vertebrates, the true amphibians. Because the cerebellum provides a major input to the red nucleus via the crossed cerebellorubral pathway, it is likely that much of the evolution of the cerebellum and the emergence of cerebellospinal fibers occurred in concert with the emergence of a differentiated red nucleus and rubrospinal tract.

33.2.4 The Mammalian Augmentation

Table 1 shows that telencephalic cell groups project to the spinal cord in amphibians, mammals, and possibly reptiles (Bruce *et al.*, 1980; ten Donkelaar *et al.*, 1981; Nudo and Masterton, 1988). However, the telencephalospinal neurons identified in amphibians and reptiles are located in the basal telencephalon, possibly homologues of amygdalospinal neurons of mammals (Nudo and Masterton, 1988). No spinal cord projections have been found in pallial (cortical gray matter) structures in any vertebrate species except for mammals. Therefore, in the lineage leading to mammals, it appears that the vertebrate common plan again was augmented with the addition of fibers originating in neocortex. Of course, this unique and possibly sole major addition to the mammalian lineage, a true corticospinal tract (CST), became more massive than any other descending pathway in some mammals.

In parallel with the enlargement of the CST, it has been suggested that the rubrospinal tract became reduced in size in the anthropoid lineage (Nathan and Smith, 1955, 1982). This would seem to contradict the consistent finding of rubrospinal neurons except in certain limbless jawed vertebrates. Though it has been rather widely accepted that the rubrospinal tract assumed a more modest role in the human lineage, comparative analysis suggests otherwise. When subjected to strict statistical tests for a variety of old and new hypotheses regarding the evolution of the rubrospinal tract and its adaptation to particular ecological niches, the number of rubrospinal neurons appears to be related to both body size and brain size of the host, with the relationship with brain size the dominant feature. Furthermore, the size of the tract was probably increased radically over the evolution of the carnivores (lineage leading to raccoons and cats) but not over the evolution of primates or rodents (lineage leading to tree squirrels), whether or not concomitant changes in brain size are held constant. Despite previous conclusions to the contrary, the number of rubrospinal neurons is positively, not negatively related to the number of corticospinal (CS) neurons (Masterton *et al.*, 1989).

33.2.5 Summary

Comparison of the major descending spinal pathways in vertebrate classes suggests that many of the pathways found in mammals can be traced to even more distant, premammalian ancestry, with several possibly as old as the entire vertebrate subphylum. The phylogenetic history of direct descending projections from brain to spinal cord along the ancestral lineage leading to mammals seems to have been marked by a series of widely spaced, steplike augmentations of the previous complement of descending tracts. The appearance of rubrospinal neurons coincides with the radiation of jawed vertebrates, but its presence is variable and is probably related to the presence of appendages. The appearance of cerebellospinal neurons coincides with the radiation of tetrapods, and seems to have been maintained in subsequent lineages. Finally, the appearance of CS neurons coincides with the radiation of mammals and the emergence of neocortex. The further differentiation of the CST in the mammalian lineage leading to humans is reviewed in a later section.

33.3 Emergence and Differentiation of Motor Cortex

33.3.1 Forebrain Motor Systems of Reptiles

Because a six-layered neocortex emerged in early mammals, there is no true homologue for mammalian motor cortex in nonmammalian species. Reptiles possess a telencephalic structure that is part of a neural circuit involved in the modulation of movement by sensory information. This structure, the anterior dorsal ventricular ridge (ADVR), receives information from the visual, auditory, and somatosensory systems and projects to the striatum, which sends efferents to descending pathways that modulate activity of motoneurons (Ulinski, 1983).

Early stimulation studies reported a motor area in dorsal cortex of turtles (*Chelydra serpentina*) in the rostrolateral subdivision or pallial thickening (Johnston, 1916). Due to its topographic position, the investigator concluded that this area represents a motor cortical area corresponding to motor cortex in the mammalian brain. Others reported a similar motor area in dorsal cortex of alligators (Bagley and Langworthy, 1926). Still others claimed to elicit motor responses in reptiles only when the striatum (most likely ADVR) was stimulated (Goldby, 1937; Koppanyi and Pearcy, 1925; Schapiro and Goodman, 1969). These early stimulation studies of the telencephalon in reptiles most likely evoked motor responses due to current spread to subcortical areas and the meninges (Peterson, 1980). Thus, to date, studies of the telencephalon in reptiles suggest that there is no motor area in the dorsal cortex comparable to motor cortex in mammals.

33.3.2 Motor Cortex in Neurologically Primitive Mammals and the Concept of the Sensorimotor Amalgam

The somatosensory-motor cortex of most mammals (e.g., primates) is not an undifferentiated mass, but is comprised of at least two distinguishable somatotopically organized areas (Woolsey, 1958). According to Woolsey, although each of these areas displays both sensory and motor characteristics, one modality predominates. Woolsey introduced the term 'somatic sensory-motor area I', or SmI, to describe the postcentral, predominantly somatosensory area of primates (and its homologues in nonprimates), and the term 'somatic motor-sensory area I', or MsI, to describe the precentral, predominantly motor area of primates (and its homologues). During the evolutionary development of somatosensory-motor cortex, clear-cut changes seem to have taken place in the cytoarchitecture, input, and output of MsI, resulting in a separate motor area. But did this separation emerge by progressive differentiation of SmI, or did a true motor cortex emerge *de novo*, adding yet another augmentation to the previous complement of descending spinal cord pathways? Studies of neurologically primitive mammals still extant may hold clues to the emergence of early motor cortex.

Endocasts of skulls in the fossil record reveal that the brains of early mammals had a small volume of lissencephalic neocortex, similar to a number of small-brained species of extant Didelphid marsupials (Jerison, 1990). The family of Didelphid opossums has long been thought to have retained the basic brain structure approximating a form that can serve as a starting point for tracing the evolution of modern mammalian neural organization, whether marsupial or placental (Smith, 1910; Loo, 1930; Edinger, 1948; Simpson, 1949, 1959; Olsen, 1959; Frost and Masterton, 1992; Frost *et al.*, 2000).

The basic scheme of a progressively differentiating somatosensory and motor cortex in mammals was proposed at least by the 1940s. In 1945, von Bonin stated: "It is of the essence of cortical organization that sensory and motor areas become divorced more and more from each other – pulled further apart as it were – as evolution proceeds" (von Bonin, 1945).

Studies using cortical surface recording and stimulation in Didelphid opossums (*Didelphis virginiana*) conducted in the early 1960s by Richard Lende seemed to provide support for this notion. Lende referred to the undifferentiated cortical area as a sensorimotor amalgam (Lende, 1963a, 1963b, 1964) based on a complete motor map of the body superimposed on a complete somatosensory map. Similar results were found in *Didelphis azarae*. In both studies, the amalgam was quite large (perhaps due to the use of surface recording and stimulation techniques), encompassing the complete length of the parietal region.

Based strictly on electrophysiological grounds, however, it is now clear that the opossum's sensorimotor amalgam is not motor cortex in the same sense as it is in other mammals, such as primates. To begin with, the somatosensory cortex of primates is not without some motor properties. Evoked motor responses from electrical stimulation in primary somatosensory cortex (S1) of eutherian mammals resemble those evoked in primary motor cortex (M1), with the exception that stimulation thresholds are significantly higher (Welker *et al.*, 1957; Woolsey, 1958; Doetsch and Gardner, 1972; Sessle and Wiesendanger, 1982). In stimulation studies of cortex in opossums (Lende, 1963a; Beck *et al.*, 1996; Frost *et al.*, 2000), movements were most often evoked using relatively high stimulating currents compared to those typically observed using microelectrode stimulation in layer V of M1 in primates (Sessle and Wiesendanger, 1982; Nudo *et al.*, 1992; Stepniewska *et al.*, 1993; Nudo and Milliken, 1996; see figures 12 and 14 in Frost *et al.*, 2000, and figure 9 in Nudo *et al.*, 1996). This disparity in current thresholds for evoked movements suggests that the electrically evoked motor responses observed in opossums are representative of the motor component of somatosensory cortex found in eutherian mammals.

Further, even after M1 is extirpated in primates, electrical stimulation of somatosensory cortex produces movements in skeletal musculature. Therefore, a motor component in the opossum's somatosensory-motor area is not sufficient grounds to consider it motor cortex. Finally, the cytoarchitecture and myeloarchitecture of the motor response area in Sm1 of Didelphids also suggests that this is a sensory area rather than a true motor cortex. This area has a notable granular layer (layer IV) and does not have the agranular appearance characteristic of true motor cortex (Walsh and Ebner, 1970; Frost *et al.*, 2000). The sensorimotor amalgam of opossum cortex is probably no more motor than the somatosensory cortex of, say, monkey. It is probably safe to suggest that in opossum cortex specifically, and primitive cortex generally, there is no true motor cortex. If this is the case, and if opossum provides a reasonable representation of early neocortical organization, then motor cortex must have arisen anew sometime later in evolution.

33.3.3 Emergence of a True Motor Cortex

In eutherian species, M1 (i.e., MsI) is situated rostral to S1 (i.e., SmI) and has a parallel somatotopic organization, though modern microstimulation studies (e.g., Gould *et al.*, 1986) have revealed that the motor map is organized in a fractionated mosaic distribution with respect to evoked joint movements, in contrast to the relatively precise topography of receptive field organization in somatosensory cortex. The neocortex of two extant marsupials, the North American opossum (*D. virginiana*) and the South American gray short-tailed opossum (*Monodelphis domestica*) have been studied extensively in order to determine if any evidence of a primordial motor cortex could be found rostral to S1. These more recent studies employed microelectrode stimulation and recording techniques, allowing much greater spatial resolution of somatosensory and motor maps than afforded by the surface stimulation and recording techniques used in the early 1960s. Examination of *Didelphis* cortex using microelectrode techniques revealed that movements can be evoked from several sites within, and some sites just rostral to S1 (Beck *et al.*, 1996). Stimulation most often resulted in movements of the tongue, though evoked movements of body parts, including several forelimb movements, were also seen. Together with the evidence that most CS fibers originate from S1 in *Didelphis*, and very few originate from areas rostral to S1, the results so far seem to favor the idea that *Didelphis* cortex contains S1, but not a true motor cortex.

The gray, short-tailed opossum (*M. domestica)* may possibly represent an even earlier stage of somatosensory-motor differentiation. In this species, movements can be evoked from a large area coextensive with the somatosensory representation, as was found for *Didelphis* (Frost *et al.*, 2000). However, unlike *Didelphis*, in which orofacial as well as proximal and distal forelimb movements were evoked, electrical stimulation in *Monodelphis* evoked movements restricted almost exclusively to the vibrissae, and to a lesser extent, jaw. In this study, three modes of stimulation were used:

1. intracortical microstimulation (750 kΩ electrode impedance) in layer V;
2. low-impedance depth stimulation (100–200 kΩ electrode impedance) in layer V; and
3. bipolar surface stimulation.

Results were qualitatively similar using the different techniques, though the excitable area increased progressively with methods 2 and 3 (Frost *et al.*, 2000). No motor representation of body parts below the level of the head was found in S1 or in more rostral areas.

These results suggest that evolutionary pressures for development of motor components in cerebral cortex may have begun long before the emergence of a true, differentiated motor cortex, possibly due to a need for more refined movements of the face (Frost *et al.*, 2000). This putative selective pressure may have resulted in an early substrate for mediating movements of vibrissae, long before sufficient CS pathways were in place to mediate direct control of the forelimbs and hindlimbs, and long before a separate motor representation rostral to S1 was established (Figure 1). In this regard, it is important to note that *Monodelphis* contains almost 20 times fewer CS neurons than *Didelphis*, and nearly 40 times fewer than rat (Nudo *et al.*, 1995). Thus, S1 of *Monodelphis* may represent a primordial condition in which a complete somatosensory map has been achieved and retained, but the motor components of S1 have not yet fully developed. In this primitive condition, the anatomical substrate required for cortical control of movements below the level of the face is not yet present.

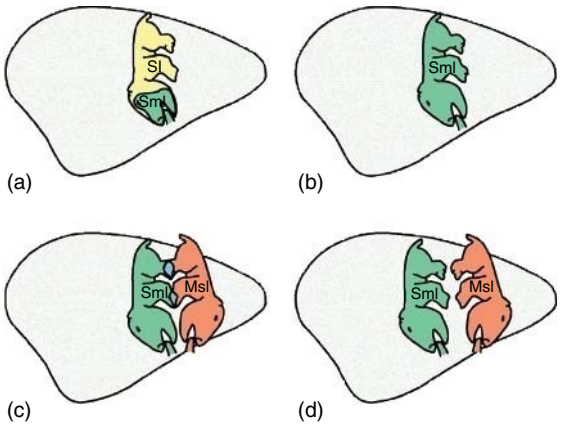

Figure 1 Hypothetical model of evolutionary divergence in sensorimotor cortex. a, Somatomotor representation in the earliest mammals, represented by *Monodelphis*. A complete somatosensory (SI) representation exists. An incomplete motor representation is congruent with the sensory representation of the face (SmI). b, A complete motor representation exists and overlaps the somatosensory representation (SmI). As noted by Lende, this sensorimotor amalgam is not unlike S1 of placental mammals. A reversed map characteristic of true motor cortex is absent. c, True motor cortex has emerged, as evident from a reversed motor representation (MsI) rostral to the somatosensory representation (SmI). MsI is largely segregated from SmI, though a partial overlap exists in the hindlimb and forelimb areas. d, In primates, MsI is completely segregated from SmI. Somatosensory cortex retains some motor properties, and motor cortex retains some somatosensory properties.

Based on these and other studies, it is thought that in opossums, motor functions at the level of the cerebral cortex, if they exist, are mediated by motor components of somatosensory cortex. Further, the motor map in S1 may have developed in stages, as it is restricted to the face, or to the face and upper extremity in *Monodelphis* and *Didelphis*, respectively. The cortical organization in monotremes, however, suggests that sensorimotor differentiation may not have followed a simple linear progression. For example, using surface stimulation techniques in the prototherian echidna (*Tachyglossus aculeatus*), Lende (1964) found a complete motor representation overlapping the somatosensory representation. Using surface stimulation techniques in the platypus (*Ornithorhynchus anatinus*), Bohringer and Rowe (1977) found a partial overlap of the motor and somatosensory representations. Electrical stimulation evoked movements of the bill and forelimb, but not the hindlimb. Thus, it is possible that the motor component of S1 is more complete in monotremes than in *Monodelphis* opossums.

Although motor components in S1 have been found in all mammals studied to date, the issue of whether a separate primordial motor cortex rostral to S1 exists in neurologically primitive mammals is not yet clear. In early surface stimulation studies in echidna (Abbie, 1938; Goldby, 1939) evoked movements were observed in an area rostral to S1. Surface stimulation studies in platypus also resulted in evoked movements rostral to S1, including representations of the forelimb. But the area immediately rostral to S1 in monotremes (rostral field or field R) contains neurons responsive to stimulation of deep receptors (Krubitzer *et al.*, 1995), similar to neurons in area 3a of eutherian mammals. Area 3a is considered to be a transition zone between area 3b of S1 and area 4 (M1). In eutherian mammals, movements can be evoked by microelectrode stimulation in area 3a using relatively low current levels. This raises the possibility that area 3a became differentiated from S1 prior to the emergence of a true motor cortex. Furthermore, based on electrophysiological and cytoarchitectonic criteria, a separate cortical area (the manipulation field, or field M) has been identified in both echidna and platypus (Krubitzer *et al.*, 1995). As field M shares some cytoarchitectonic similarities with M1, it is possible that this area is a homologue of M1 in eutherian mammals (Krubitzer *et al.*, 1995). However, at this point it is also possible that field M is a unique specialization in extant monotremes. Clearly, additional studies of its physiology and anatomy are needed to resolve this issue.

These findings of a possible M1 homologue rostral to S1 in monotremes suggest that the antecedents to a true motor cortex may have existed very early after the emergence of mammals. As evolutionary pressures for specialized motor functions (e.g., manual dexterity) grew, augmentations of the CST from a primitive to a more advanced pathway seem to have paralleled the origin of a true motor cortex.

It is not entirely clear when true motor cortex might have emerged. But it seems that somatosensory-motor differentiation occurred independently in several orders, and not just in primates. For example, a true motor cortex, as evidenced by a reversed motor representation rostral to the somatosensory representation and by movements evoked at relatively low microelectrode stimulating currents, is apparent in rats (Donoghue and Wise, 1982). Motor cortical fields have been localized to frontal cortex in other rodent species as well (Woolsey *et al.*, 1952; Hall and Lindholm, 1974). A separate and distinct topographic pattern comprising M1 in the rat forms a rough mirror image of the S1 representation, with the hindlimb caudomedial, the face rostrolateral, and the proximal and axial representations rostromedial (Neafsey *et al.*, 1986; Neafsey and Sievert, 1982). In rats, the separation of S1 and M1 is not complete, since some overlap has been demonstrated over most of the hindlimb representation and part of the forelimb representation (Hall and Lindholm, 1974; Donoghue and Wise, 1982; Sanderson *et al.*, 1983). This area of overlap has features similar to both S1 and M1 cortex. The overlapping hindlimb area receives a convergence of thalamic projections from the ventrolateral nucleus and the ventrobasal complex, whereas in nonoverlapping areas these projections are segregated (Donoghue *et al.*, 1979). Regardless of the partial overlap in rats, it appears that a true, separate motor area, distinct and separate from the S1 cortex exists in rodents, primates, and carnivores.

33.4 Evolution of the CST

The CST places the neocortex in direct neural contact with the spinal cord. The existence of direct synaptic connections between cortical neurons and spinal motoneurons in some eutherian mammals (including many primates) has been the structural basis for implicating the CST in a variety of motor functions, such as control of digital dexterity, conditioned movements, flexor activity, muscle tonus, and volitional movements (Lawrence and Kuypers, 1968; Beck and Chambers, 1970).

Since rather strong structure–function relationships for the CST can be advanced in primates, including humans, it has been tempting to presume that its function is the same in all mammals. However, it is now becoming apparent that because the morphology of the CST is so varied across mammals (in size, spinal course, manner of termination, etc.), the motor functions ascribed to the CST of primates probably cannot be extended *in toto* to other orders. Likewise, it cannot be assumed that the CST subserves only a motor function. It is also thought to be involved in descending control of afferent inputs, modulation of spinal reflexes, and trophic functions, to name a few (Lemon and Griffiths, 2005).

Furthermore, since its morphology is so varied, it often has been assumed that the CST arose more than once and possibly many times during the phylogeny of mammals (e.g., see Goldby, 1939; Noback and Shriver, 1969). More specifically, it has been suggested that the tract emerged independently within each mammalian order. In this view, any similarity in the tract across orders (such as its common origins in layer V of somatosensory-motor cortex) would be regarded as a consequence of parallel or convergent evolution and any similarity in function would be regarded as coincidental.

The present review attempts to reconstruct the morphological changes that have occurred in the CST during the course of mammalian evolution. Briefly, it is argued that the tract arose initially as collaterals of a phylogenetically older, corticobulbar tract (CBT), which itself includes part of the descending somatosensory system. Further, it is argued that these corticobulbar collaterals penetrated the cord after the major mammalian orders had diverged. Therefore, CSTs cannot, in principle, be traced to a single, common mammalian ancestor, but they can be traced to common corticofugal pathways that had their own origins early in mammalian evolution.

33.4.1 Variation in the Trajectory of CS Fibers in the Medullary Pyramids

Many pyramidal tract fibers terminate in the medulla before reaching the cord. However, many aspects of the pyramidal tract's morphology have been examined in a great number of species. Thus, to the extent that the CS fibers constitute a significant fraction of pyramidal fibers, a brief comparative analysis of the morphology of the pyramidal tract is not without interest.

It was first noted by Clarke in 1858 (Wiesendanger, 1981) and Spitzka in 1879 (Spitzka, 1879, 1886) that the pyramidal tract varies in size across mammals and that the anthropoid apes possess some of the largest pyramidal tracts. It is clear that absolute size of the tract, the number of fibers, the size of the largest fibers, and the average fiber size each increases in a phyletic series leading to humans. However, when body weight is held constant, all but one of these correlations collapses. The phyletic level remains significantly correlated only with the cross-sectional area of the pyramidal tract (Heffner and Masterton, 1983).

It is generally acknowledged that in most mammals, 90–95% of the CS fibers cross in the pyramidal decussation before entering the cord. But the exact location of the pyramidal decussation is not invariant. In the vast majority of mammals studied so far, the pyramidal decussation occurs just above the medulla–spinal junction. However, in at least one species of bat, and possibly in the anteater, the pyramidal fibers cross earlier, in the rostral medulla (Fuse, 1926; Broere, 1966); in at least two other species (pangolin, echidna), the pyramidal fibers seem to cross in the pons (Goldby, 1939; Chang, 1944); and in three other species (hyrax, mole, and hedgehog), no decussation has been observed at all (Linowiecki, 1914; Verhaart, 1967; Kunzle and Lotter, 1996). Finally, variation in the pyramidal decussation in humans has been frequently reported. Clearly, more information is needed on the consistencies and variation in this important aspect of pyramidal tract morphology.

33.4.2 Variation in the Trajectory of CS Fibers in the Spinal Cord

If the CST were homologous across mammalian orders, one might expect it to descend through the spinal cord in the same funicular pathway in each order. In contrast, CS fibers descend through the spinal cord in any of six different funiculi in mammalian species. These include the dorsolateral (sometimes called the lateral tract), dorsomedial (sometimes called the dorsal tract), and ventral funiculi (Armand, 1982). Further, CS fibers can travel in either side of the cord, i.e., contralateral or ipsilateral to the cells of origin in layer V of the cerebral cortex (Figure 2).

In an individual species, there is usually more than one CST. The principal tract (sometimes called the main tract) is usually defined as the one larger in area, containing more and larger fibers, and terminating in more caudal levels than the others. When the principal CSTs are arranged according to their funicular trajectories on a cladogram of mammalian

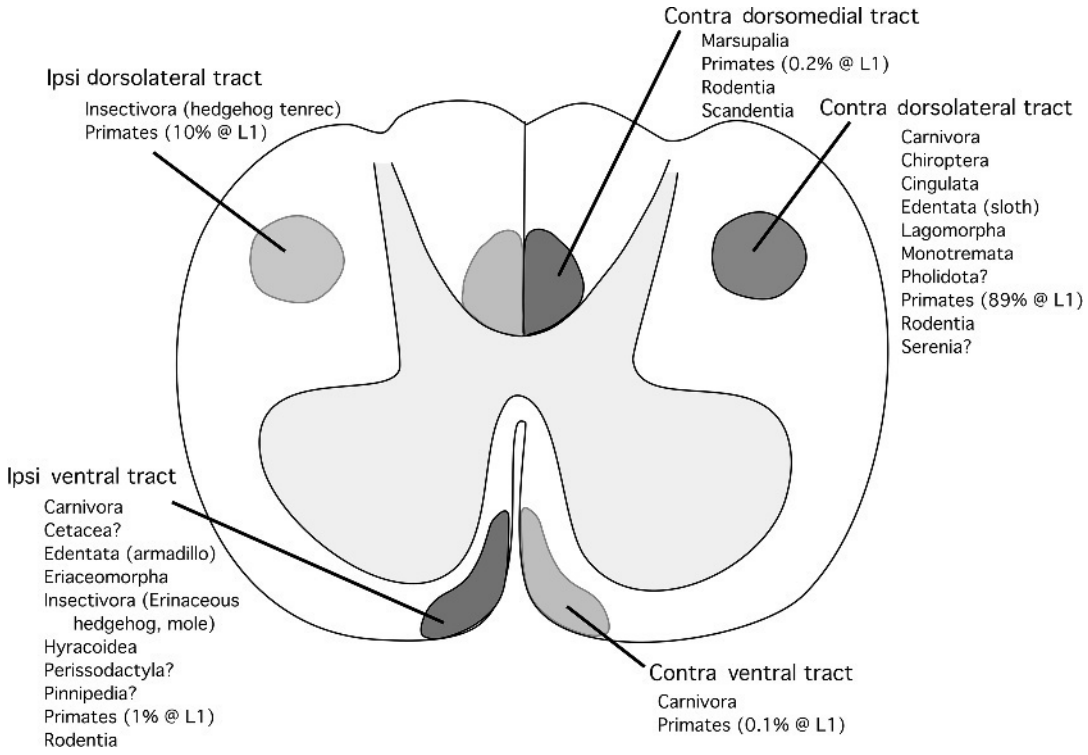

Figure 2 Location of CST in the spinal cord of mammals. All mammals studied to date possess CS neurons. The CST can travel in any one of six spinal cord funiculi: The dorsolateral, dorsomedial, or ventral funiculi, and either on the ipsilateral or contralateral side with respect to the cells of origin in layer V of cerebral cortex.

orders (based on other paleontological and comparative data), they do not appear to group into any other obvious subsets (Figure 3). This marked variation in the spinal location of the CST among orders of mammals has been the major point of reference for establishing the tract's early phylogeny. Based entirely on this variation in funicular trajectory among orders and the equally marked lack of variation within orders, it has been suggested that the CST may have penetrated the cord (and thus, arisen independently) within each mammalian order (Goldby, 1939; Noback and Shriver, 1966b).

Based upon the funicular trajectory of its principal tract then, the CST can be traced to a common ancestry within each mammalian order, but not to any higher-level taxa or more remote ancestry. Consequently, up to this point one may tentatively conclude that the majority of CS fibers entered the cord only after the various orders of mammals had diverged. This consistency within orders has not always been apparent because of changes in the taxonomy of the mammalian orders. For example, tree shrews, which possess a dorsomedial principal tract, were once classified as primates that possess a dorsolateral principal tract. Also, rabbits, now classified as lagomorphs, possess a dorsolateral principal tract, but were once classified as rodents, which possess a dorsomedial principal tract. It is of interest here that in the continuing debate concerning the taxonomy and evolutionary origin of tree shrews, the funicular location of the CST has been proposed as a criterion of classification. For more information, see review by Haines and Swindler (1972). This idea gains some further support by the observation that in some neurologically primitive mammals, relatively few CS fibers extend past upper cervical levels of the spinal cord. For example, in the short-tailed opossum, only 500 CS neurons have been identified (Nudo *et al.*, 1995); in the hedgehog tenrec, only 600 CS neurons have been identified (Kunzle and Rehkamper, 1992).

Until the late 1970s, the majority of studies examining the course and termination of the CST relied on either degeneration or autoradiographic techniques that have been superceded by more sensitive tract-tracing methods such as injection of wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) or more recently, biotinylated dextran amine (BDA). The older techniques were quite adequate for identifying the principal CST, and thus, the early findings have largely been replicated numerous times. However,

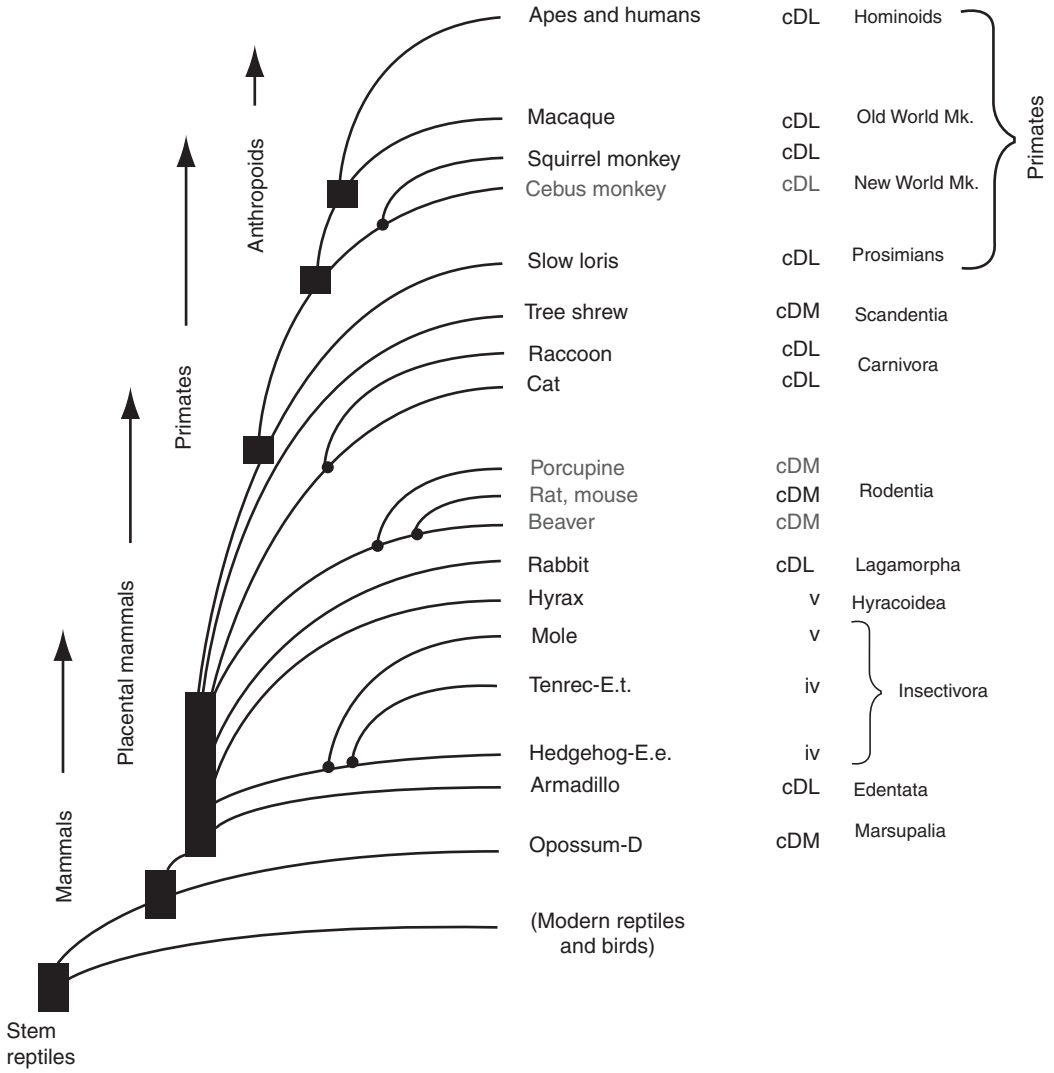

Figure 3 Principal CSTs in mammalian orders. Species are arranged according to progressively more recent common ancestry with apes and humans. Whereas the trajectory of the principal CSTs are the same within mammalian orders, they differ between orders. This has historically been the primary evidence to suggest that the CST emerged independently within each mammalian order. DL, dorsolateral funiculus; DM, dorsomedial funiculus; V, ventral funiculus; c, contralateral (to cell bodies of origin); I, ipsilateral (to cell bodies of origin).

as the more sensitive tracers have been employed, it has become evident that CS fibers travel in multiple funiculi in the same species. To date, while CS fibers have been examined using the modern, sensitive tract-tracers in only a few species (cat, macaque monkey, mouse, rat, hedgehog tenrec), at least some CS fibers travel in the dorsolateral funiculus (contralateral and ipsilateral), the ventral funiculus (ipsilateral), and the dorsomedial funiculus (contralateral) in each of these species (Table 2) (Satomi *et al.*, 1989; Kunzle and Lotter, 1996; Brosamle and Schwab, 1997; Lacroix *et al.*, 2004; Steward *et al.*, 2004). In cat, CS fibers have been described in all six spinal funiculi (Satomi *et al.*, 1989).

These recent results demonstrating common trajectories across orders of secondary CSTs suggest that at least some CS fibers in one or more spinal funiculi may be homologous. If so, the number of such fibers is likely to be very low indeed, based on results in *Monodelphis* opossum and the hedgehog tenrec. Thus, what we now call the principal CST very likely is a more recent specialization within each mammalian order that resulted from augmentation of the primordial CST. Because the bulk of the CST probably emerged after the radiation of mammalian orders, its funicular trajectory is probably of little value in determining its early origins in primordial mammals. However, the variation in the location of the principal CST in the various orders,

Table 2 Location of CS fibers in mammalian orders

		Funicular trajectory[a]		
Order	*Species (common name)*	*DM(c/i)*	*DL(c/i)*	*V(c/i)*
Artiodactyla	Goat		+	
Carnivora	Cat; raccoon	+/+	++/+	+/+
Chiroptera	Bat		++	
Edentata	Armadillo		++	
Hyracoidea	Hyrax			++
Insectivora	Mole, hedgehog	/	?/?	?/+
Lagamorpha	Rabbit		++	+
Marsupalia	Opossum, wallaby, potorou, kangaroo	++	+	
Monotremata	Echidna		++	
Pholidota	Pangolin		++	
Primates	Slow loris, bush baby, macaque, Cebus monkey, squirrel monkey	+/	++/+	+/+
Rodentia	Porcupine, guinea pig, gopher, hamster, beaver, mouse, woodchuck, coypu rat, common rat	++/+	+/+	/+
Scandentia	Tree shrew	++	+	+

[a]A fourth location of CS fibers, the intracommissural bundle, has been identified, though not with modern tract-tracing techniques. They will not be described further here.
++, Substantial number of fibers; +, relatively few fibers; ?, current data equivocal; DM, dorsomedial funiculus; DL, dorsolateral funiculus; V, ventral funiculus; c, contralateral; i, ipsilateral.

and the consistency within orders remains an intriguing clue that may yet provide a unique perspective with regard to the function of the CST.

33.4.3 Evolutionary Origins of CS Fibers: The Caudal Extension of the CBT

Although CS fibers appear to have entered the cord independently in each mammalian order, it is unlikely that they appeared, throughout their extent from cortex to cord, *de novo*. In seeking a more likely, more gradual origin, the most reasonable first guess is the CBT. This tract has a similar cortical origin and a similar trajectory within the brainstem as the CST. It is dissimilar only in its extent down the neuraxis. That is, regardless of order, both the CST and CBT descend in the internal capsule, cerebral peduncle, and pyramid before entering the cord (as the CST) or arborizing in the pons/medulla (as the CBT). Further, the CST and several of the CBTs originate in some of the same cortical areas (areas 4, 3, 1, and 2) (Wiesendanger and Wiesendanger, 1982).

Therefore, it is not unreasonable to suppose that CS fibers made their way into the spinal cord via the already established capsular, peduncular, and pyramidal tracts previously ending in the medulla, and it is not unlikely that they emerged first as collaterals of this phylogenetically older pathway to the medulla. This notion, shared by other investigators (Noback and Shriver, 1966a), has received support from the demonstration that many CS neurons send collaterals to dorsal column nuclei (Rustioni and Hayes, 1981). From an evolutionary viewpoint, however, the CS fibers are probably the collaterals of the older corticobulbar axons ending in the dorsal column nuclei, not vice versa.

33.4.4 Termination of CS Neurons

As would be expected from its multiple origins, the termination pattern of CS fibers (that is, where the CS fibers end within the spinal grey) varies greatly among mammalian orders. But perhaps unexpectedly, it also often varies within orders. This variation in termination pattern, along with the relationship to variations in function, have been discussed by Heffner and Masterton (1975) and will only be reviewed briefly here.

Two major points can be made concerning the termination of CS fibers. First, the caudal extent of the tract varies from cervical levels in goat and hedgehog to sacral (and even coccygeal) levels in primates, rodents, and some carnivores. Second, the fibers terminate in many spinal grey laminae. On one hand, regardless of order, all mammals are alike in that most CS fibers terminate at the base of the dorsal horn (lamina V–VI) at each spinal level. On the other hand, both among orders and among families within orders, the ventral (or anterior) limit of the CS terminations within the spinal cord grey varies widely. Although in many mammals, CS terminals reach no more ventral than lamina VI (e.g., goat, rabbit, marmoset, wallaby), in others (raccoon and most primates), they extend to both medial and lateral motoneurons in lamina IX.

Even though it appears that CS fibers penetrated the cord only after the orders diverged, certain trends in the termination patterns of the CST can be observed in a phyletic series across orders known to have successive (as opposed to sequential) origin (Heffner and Masterton, 1983). These trends are especially evident in a phyletic series of extant mammals having successive propinquity with humans. If CS termination parameters are plotted in such a series (with body weight constant), it is clear that with successive grades, first the CST reaches more caudal levels of the spinal cord, and second, at each cord level, the CST innervates more ventral laminae, eventually establishing direct contact with spinal motoneurons.

33.4.5 Cells of Origin of the CST

The search for the cortical cells originating the CST has a long history. Over the past century, as soon as a new neuroanatomical technique had been developed, it had been applied to this tract. Early in the history of its study, around 1830, Gall and Spatzheim recognized (by gross dissection) that the pyramidal fibers had their origins in cortex, but it was not until 1851 that Turck realized that many of these same fibers enter the spinal cord (Lassek, 1954; Clark and Dewhurst, 1974). When the Marchi technique for staining degenerating myelin was developed, electrical stimulation of the cortex guided investigators to the most probable sites for extirpation. For the next century, until the advent of modern neuronal tract-tracing techniques, this general procedure of electrical stimulation, extirpation, and the subsequent study of degenerated myelin, axons, or terminals (i.e., the Nauta–Gygax and Fink–Heimer techniques) yielded most of the knowledge concerning the origins of the CST.

However, the anterograde degeneration method can only reveal the general areas of cortex-originating pyramidal or CS axons. The exact cells of origin were still unknown by the turn of the century. At the time, it was suggested that large pyramidal cells (Betz cells) in the area gigantopyramidalis give rise to the CST. Although this idea has proved to be true, at the time it was erroneously thought that these were the only cells originating CS neurons. This incorrect notion was reinforced by retrograde degeneration after spinal hemisection (Holmes and May, 1909). Chromatolytic changes in Betz cells were observed but, once more, it was concluded erroneously that the CST originated exclusively from them.

Despite the powerful influence of Holmes and Page May's study over the next quarter century, some doubts were beginning to be cast on the notion that CS fibers arise strictly from Betz cells. By the 1930s, the anterograde degeneration method had revealed that the cortical origins of the CST were much more widespread than was previously thought (Kennard, 1935). The advent of precise electroanatomical techniques in the 1940s enabled investigators to locate pyramidal tract cells by electrically stimulating the tract on the ventral surface of the medulla and recording from antidromically stimulated cells in the cortex (Lance and Manning, 1954). Later, antidromically identified pyramidal tract neurons were injected with dye so that they could be visualized in cortical layer V of area 4 (Batuev and Lenkov, 1973).

However, the electroanatomical techniques hold one problem in common with the retrograde chromatolytic technique: large neurons are overrepresented. Nevertheless, it is clear that the CS area defined by either antidromic activation or by retrograde chromotolysis is coextensive with the area defined by anterograde degeneration techniques. But by the mid-1970s a detailed picture of the distribution of the cells originating CS axons had yet to be realized.

Precise information concerning the cells originating CS axons was obtained beginning in the mid-1970s based on the retrograde transport of horseradish peroxidase and other materials. This technique was first applied to the CST by Catsman-Berrevoets and Kuypers in 1975 (Catsman-Berrevoets and Kuypers, 1976). Based on retrograde tract-tracing studies over the past few decades, considerable anatomical information is now available regarding the morphology of neurons originating the CST in a wide variety of mammalian species (Nudo and Masterton, 1990a, 1990b; Nudo *et al.*, 1995). Several general features have emerged:

1. All CS neurons reside in cortical layer V. No exception to this sweeping generalization has yet been seen.
2. CS neurons are most concentrated in, but not confined to area 4.
3. Though less heavily concentrated, a very large number of CS neurons reside outside of area 4, especially in the somatosensory areas 3, 1, and 2 and in areas 5 and 6. This extent represents a far wider distribution than shown by earlier techniques, especially electrical stimulation, antidromic activation, or retrograde chromatolysis.
4. The giant Betz cells are neither the sole nor predominant originators of CS axons. In fact, Betz cells probably account for no more than about 3% of all pyramidal tract fibers. Pyramidal cells of many sizes contribute axons to the CST.

Aside from these sweeping generalizations, the morphology and distribution of CS neurons varies considerably. With regard to their distribution, there does appear to be some consistency across mammalian orders in that either two or three separate broad regions of neocortex originating CS fibers in all mammalian species studied. These regions are large but relatively well demarcated and are stable both in absolute and relative location on the cortical surface in all orders examined.

One broad region (region A in Nudo and Masterton, 1990a) contains nearly 90% of CS neurons. This region comprises primary somatosensory and motor cortex, as well as CS neurons in the dorsal premotor cortex, supplementary motor area, cingulate motor areas (at least in primates), and scattered cells in the parietal and frontal cortex. A second broad region (region B) comprises CS neurons in the second somatosensory area and related somatosensory areas of the parietal cortex. These two broad regions of CS neurons have been identified in every mammalian species studied to date, and thus can theoretically be traced to a common mammalian ancestor.

A third region of CS neurons is clustered in an area most likely corresponding to the primate ventral premotor cortex (region C). This group of cells appears to be uniquely present in all primate species studied to date, but in representatives of no other mammalian order. Another region (region C′) is present in all rodents studied as well as rabbit, but in no other mammalian species. This region, corresponding to the rostral forelimb area (RFA) of rats, will be discussed in a later section.

The total number of CS neurons varies considerably across mammalian species, with the lowest numbers in many insectivores and *Monodelphis* opossum, and the highest numbers in most primates and carnivores (Nudo *et al.*, 1995). As expected, body size and brain size are significant co-variates in the total number of CS neurons and in the amount of cortex devoted to the CST. However, even when body weight is held statistically constant, the number of CS neurons increases in the anthropoid ancestral lineage. Other characteristics of CS neurons also appear to have changed significantly in the anthropoid lineage, including an increase in average soma diameter and a decrease in volume density and concentration. Thus, with more recent common ancestry with humans, it appears that CS neurons became larger, more numerous, and less concentrated. This latter finding is interesting as it implies that the region of cortex-originating CS fibers added more non-CS neurons and more neuropil. Perhaps one of the unique attributes of primate motor cortex is the expansion of intracortical connectivity of regions originating CS neurons.

33.5 Specialization of Motor Areas in Primates

33.5.1 Criteria for Differentiation

It is generally accepted that no single feature is sufficient for characterizing an area as a distinct region. Features used to define cortical motor fields include its cytoarchitectonics, pattern of afferent and efferent connections, features of intrinsic connectivity, chemoarchitectonics, behavioral effects of ablation, and, particularly for motor cortical areas, the ability to elicit movements upon electrical stimulation.

A differentiated motor field has a unique cytoarchitecture, traditionally defined by stains for Nissl bodies and myelin. Additionally, areas have been examined and characterized based on cytochrome oxidase staining, acetylcholinesterase staining, neurofilament antibody staining, and receptor binding. Unique characteristics of cell types, laminar organization, cell density, fiber density, and various staining densities are all used for characterization. Extensive tract-tracing studies have been used to identify subareas of motor cortex based on differential afferent and efferent connections with the thalamus, basal ganglia, and other cortical areas, as well as their projections to the spinal cord. Intracortical microstimulation mapping procedures have been used to define somatotopic organization within each secondary motor area, with attention paid to minimal threshold requirements for initiation of movements, as well as the characterization of movements themselves. Finally, secondary motor areas have been characterized based on functional differences in ablation-behavior studies in nonhuman primates and functional imaging studies in humans.

33.5.2 Secondary Motor Areas in Primates

In addition to a primary motor area (M1 or area 4), there are several secondary motor areas recognized in the primate cortex (Kaas, 2004). These areas have been defined as having direct connections to both M1 and to the spinal cord. The premotor cortex, the supplementary motor area and the cingulate motor cortex have been identified in all primate species examined, including prosimian primates. Each of these secondary areas has been divided into subareas based on differences in cortical architecture that are related to hodological and functional differences. The lateral premotor area is divided into ventral and dorsal areas (PMv

and PMd, respectively), the supplementary motor area (SMA) into SMA-proper and pre-SMA, and the cingulate motor area has been subdivided into rostral (CMAr) and caudal (CMAc) divisions.

Neurologically more primitive primates represented by the prosimian bush baby (*Galago garnetti*) have a well-differentiated M1, with representation of the trunk, hindlimb, and face and a large forelimb representation, although there is little control of individual digit movements (Kanagasuntherum and Leong, 1966; see figures 1–5 in Wu *et al.*, 2000). In addition to M1, galagos possess most of the secondary motor areas that have been recognized in simian primates based on architectonic features, multiple somatotopic organization, and patterns of cortical and subcortical connections. These include the premotor areas, the supplementary motor areas, and the cingulate motor areas. These results suggest that as many as 10 motor fields emerged early in primate evolution (Wu *et al.*, 2000).

33.5.3 Is There a Primate Homologue to the Rodent RFA?

Intracortical microstimulation studies of sensorimotor cortex in the rat have shown a complete motor representation that is cytoarchitectonically defined as agranular cortex (Hall and Lindholm, 1974; Donoghue and Wise, 1982). The portion of this motor area in caudal portions of frontal cortex that is devoted to forelimb movements is referred to as the caudal forelimb area (CFA). In addition, a second motor representation of the forelimb has been identified in more rostral portions of the frontal cortex. This second forelimb representation, referred to as the RFA, is smaller than the CFA (Neafsey *et al.*, 1986). The RFA is separated from the CFA by a zone where intracortical microstimulation (ICMS) elicits vibrissa or neck muscle movements.

Because the presence of a secondary motor area in rats would appear to parallel the differentiation of motor areas in primates, suggestions have been made that the RFA is a homologue of one of the primate secondary motor areas. Based solely on the topographic location of CS neurons that originate in the RFA, Nudo and Masterton (1990a) concluded that secondary motor areas emerged independently in primates and rodents, and that there was no obvious homologue of RFA in primates.

However, it is important to consider additional details regarding the structure and function of the RFA in order to draw more firm conclusions. Tract-tracing studies of motor cortical connections in rat have shown differences in the thalamic, striatal, and cortical connections of CFA and RFA (Rouiller *et al.*, 1993). Comparison of these connections to the pattern of connections of primate motor areas suggests that RFA has some similarities to primate premotor areas. Roullier and colleagues found that the origins of thalamic inputs to the two areas are largely segregated, similar to the thalamic inputs to M1 and premotor areas in monkeys (Schell and Strick, 1984; Matelli *et al.*, 1989). Additionally, CFA has predominantly ipsilateral projections to the striatum, similar to M1 in primates (Leichnetz, 1986; Whitworth *et al.*, 1991). (The caudate and putamen are not differentiated in rodents. Thus, the collective term 'striatum' is used throughout this discussion for both rodents and primates.) RFA has diffuse bilateral corticostriatal projections equally dense to both hemispheres, similar to SMA and premotor cortex in primates (McGuire *et al.*, 1991).

In contrast, evidence suggests that lesions of RFA in rat result in more severe behavioral deficits than lesions of SMA in primates (Barth *et al.*, 1990; Passingham, 1993). Furthermore, the predominant layers of origin of intracortical connections between the RFA and CFA differ from those of M1 and PM/SMA in primates (Dum and Strick, 2005; Figure 4). In addition, the predominant thalamic connections of the RFA are with ventromedial thalamus, with few connections with the ventrolateral thalamus, rather than the predominantly ventrolateral and ventroanterior thalamic connections of primate PM and SMA (Figure 4).

Although corticofugal projections from CFA and RFA are similar, the RFA has interconnections with insular cortex similar to SMA and premotor cortex in primates. Additionally, the RFA does not appear to have cutaneous receptive fields, similar to supplementary motor cortex in primates (Neafsey *et al.*, 1986). Overall, based on its connections, CFA is more similar to the M1 forelimb area in primates and RFA in some ways appears to be more similar to nonprimary motor cortex in primates. It is not currently possible to decide whether RFA is a homologue of primate premotor cortex, supplementary motor areas, or a combination of secondary motor areas in primates (Rouiller *et al.*, 1993).

33.5.4 Further Differentiation of Primate Motor Areas

33.5.4.1 Nomenclature The nomenclature used for subdivisions of primate motor areas based on the study of macaques has varied across laboratories. The generalized current scheme includes M1 (or Brodmann's area 4); four subdivisions of the

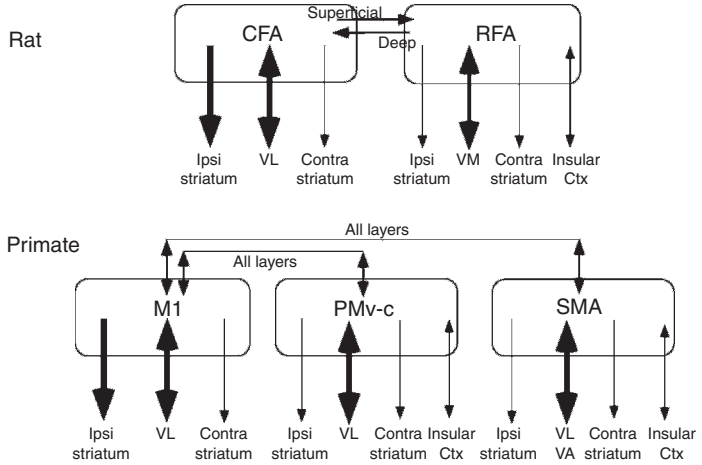

Figure 4 Comparison of secondary motor areas in rats and primates. Schematic diagram of the major (predominant) connections of the CFA and RFA in rat (top) compared to similar connections in M1, PMv-c, and SMA-proper in primates (bottom). CFA is similar to M1 based on its connections with ventrolateral thalamus (VL) and its strong projection to the ipsilateral lateral striatum (putamen in primates). RFA is similar to premotor areas of primates by its diffuse projections to ipsilateral and contralateral striatum (caudate and putamen in primates), and its interconnections with insular cortex. RFA differs from primate premotor cortex in its major connections with the thalamus (ventromedial thalamus in RFA vs. VL in PMd-c; VL and ventroanterior thalamus (VA) in primates) and its laminar differences in intracortial connectivity (deep layers of RFA project to CFA and superficial layers of CFA project to RFA, whereas all layers contribute projections between M1 and PMd or SMA).

lateral premotor cortex (area 6) (PMd-c, PMd-r, PMv-c, PMv-r, or F2, F7, F4, and F5, respectively); two premotor subdivisions on the mesial surface of the hemisphere (SMA and pre-SMA, or F3 and F6), and three subdivisions of the cingulate motor area within regions lining the cingulate sulcus (CMAr, CMAd, CMAv, or area 24c, area 6c, and area 23c). SMA has also been referred to as M2 (Figure 5).

33.5.4.2 Differentiation of lateral premotor cortex Studies suggest that a premotor area first appeared with the divergence of prosimian primates, as evidenced by lateral premotor representations coincident with distinct cytoarchitectonics rostral to the M1 representation. Two major subdivisions, PMd and PMv, have been identified in the prosimian Galago (Wu *et al.*, 2000). PMd has been further subdivided in Galago into rostral and caudal components (PMd-r and PMd-c, respectively; see figures 4 and 5 in Wu *et al.*, 2000). To the extent that Galagos represent a primordial state of primate motor cortex, it is likely that PMd-r, PMd-c, and PMv are homologues in all extant primates. Intracortical microstimulation studies have also identified PMv and PMd in New World and Old World monkeys (Gould *et al.*, 1986; Stepniewska *et al.*, 1993; Preuss *et al.*, 1996; Frost *et al.*, 2003; Hoshi and Tanji, 2004; see Figure 5). The PMd has been shown to consist of representations of both hindlimb and forelimb (He *et al.*, 1993; Ghosh and Gattera, 1995; Preuss *et al.*, 1996; Raos *et al.*, 2003), while PMv contains representations of the forelimb and orofacial muscles (Stepniewska *et al.*, 1993; Preuss *et al.*, 1996).

In addition to the areas noted above that have been identified in prosimian primates and New World monkeys, the PMv is further differentiated in Old World monkeys. A total of four subareas of lateral premotor cortex are identifiable in macaques: PMd-c, PMd-r, PMv-c, and PMv-r (or F2, F7, F4, and F5, respectively) based on cytoarchitectonics, intracortical microstimulation results, and connections (Rizzolatti and Fadiga, 1998; Rouiller *et al.*, 1998; Morel *et al.*, 2005).

Studies implicate PMv in the initiation and control of limb movements based on visual cues and other sensory information (Kurata and Tanji, 1986; Gentilucci *et al.*, 1988; Rizzolatti *et al.*, 1988; Mushiake *et al.*, 1991; Mushiake *et al.*, 1997), whereas PMd is involved with movement parameters (Fu *et al.*, 1993; Kurata, 1993; Crammond and Kalaska, 2000). Thus, PMv is important for the integration of visual information derived from extrapersonal three-dimensional space and involved in the spatial guidance of limb movements (Kakei *et al.*, 2001), whereas PMd is involved in the integration of internal body representation and target information for the preparation of motor actions (Kurata, 1994; Hoshi and Tanji, 2004).

Unlike macaque monkeys, there have been no delineations of subareas F4 and F5 (PMv-c and PMv-r) in PMv of prosimian primates or New World monkeys.

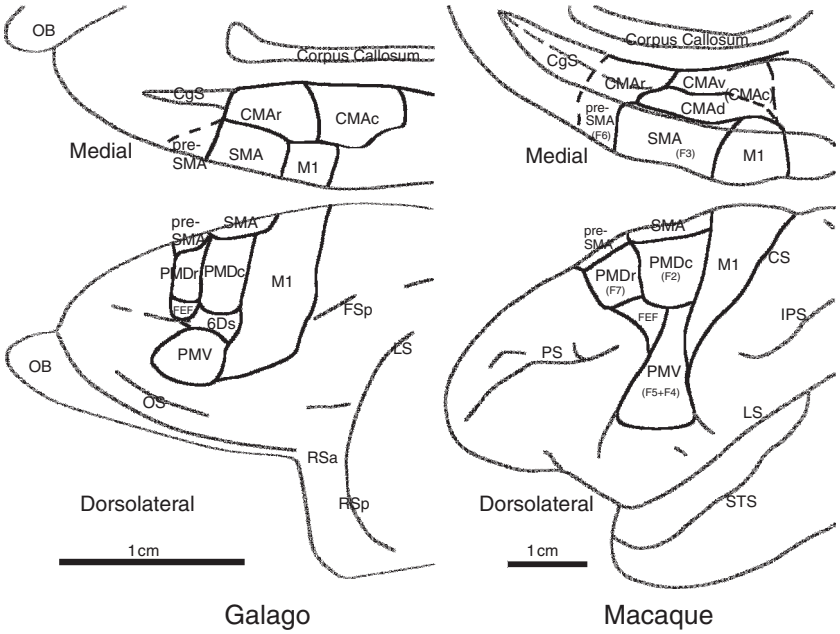

Figure 5 Multiple motor areas in galagos and macaques. Neurologically more primitive primates represented by the prosimian bush baby (*Galago garnetti*; left) have a well-differentiated M1, with representation of the trunk, hindlimb, face, and a large forelimb representation. Galagos possess most of the secondary motor areas that have been recognized in simian primates (macaque shown on right). These results suggest that as many as 10 motor fields emerged early in primate evolution.

In macaques, PMv is subdivided into caudal and rostral divisions based on cytoarchitectonic, connectional, histochemical, and physiological distinctions (Matelli *et al.*, 1985, 1991; Luppino *et al.*, 1999; Rizzolatti and Luppino, 2001; Rizzolatti *et al.*, 2002; Morel *et al.*, 2005).

In macaques, F4 appears to code goal-directed actions mediated by spatial locations (Rizzolatti *et al.*, 2002), and F5 has been shown to be involved in motor-action recognition (Umilta *et al.*, 2001) as well as in hand shaping in visuomotor transformations for grasping and manipulation (Fogassi *et al.*, 2001). Human area 44 has been shown to be involved in sensorimotor transformations for grasping and manipulation (Binkofski *et al.*, 1999) and is thought to be homologous to F5 in macaques (Rizzolatti *et al.*, 2002).

The special role of vision and visuomotor control in primates has led some to suggest that the remarkable increase in the size of the frontal lobes in this order, and perhaps the differentiation of frontal motor areas was driven by the increase in direct and indirect connections of the visual cortex with the frontal, especially motor cortex (Whishaw, 2003). The selective pressures for visually guided forelimb movements may have led to the specialized motor and sensorimotor areas in primates to integrate the somatosensory and visual frames of reference required for skilled movement.

33.5.4.3 Differentiation of the supplementary motor area Studies suggest that a supplementary motor area first appeared with prosimian primates, as evidenced by two distinct SMA motor representations corresponding to distinct cytoarchitecture located on the medial surface of the hemisphere in Galago. These two areas are referred to as SMA-proper and the pre-SMA, situated more rostrally (see figures 4 and 5 in Wu *et al.*, 2000). A supplementary motor area has also been identified in New World monkeys (Gould *et al.*, 1986).

Both SMA and pre-SMA (or areas F3 and F4) have been identified in macaques based on cytoarchitecture, intracortical microstimulation, and connections (Matelli *et al.*, 1991; Matsuzaka *et al.*, 1992; Luppino *et al.*, 1993; Rouiller *et al.*, 1999; Liu *et al.*, 2002; Morel *et al.*, 2005). Compared to SMA, pre-SMA has only sparse spinal projections, and high thresholds for evoking movement, and likely plays less of a direct role in the execution of movement (Luppino *et al.*, 1993; He *et al.*, 1995; Dum and Strick, 1996; Liu *et al.*, 2002). Pre-SMA is thought to have greater involvement in cognitive aspects of motor processing.

33.5.4.4 The cingulate motor areas Studies suggest that a cingulate motor area first appeared with prosimian primates, as evidenced by two cingulate motor representations and distinct cytoarchitecture in CMAr and CMAc in Galago (Wu *et al.*, 2000). A

third area that also has dense connections with M1 and the spinal cord in Galago was identified posteriorly in cingulate cortex and referred to as the cingulate somatomotor area (CSMA), although this may correspond to a supplementary sensory area (Wu *et al.*, 2000).

The primate cingulate cortex has traditionally been divided into rostral and caudal architectonic subdivisions (areas 24 and 23). More recently, in the macaque, the caudal CMA has been further differentiated into two distinct areas (CMAd and CMAv) based on distinct cytoarchitectonics and intracortical microstimulation studies identifying a third forelimb representation (Walsh and Ebner, 1970; Vogt *et al.*, 1987; Takada *et al.*, 2001; Hatanaka *et al.*, 2003).

Most medially, the rostral, dorsal, and ventral cingulate areas are buried in the cingulate sulcus (CMAr, CMAd, and CMAv, respectively). As with other secondary motor areas, the CMA areas send somatotopic projections directly to M1 and the spinal cord (Muakkassa and Strick, 1979; Dum and Strick, 1991, 1996; Luppino *et al.*, 1993; He *et al.*, 1995; Wang *et al.*, 2001). The somatotopy of CMA has been examined using intracortical microstimulation, demonstrating at least a forelimb representation in each of the subareas of CMA (Mitz and Wise, 1987; Luppino *et al.*, 1991, 1994; Takada *et al.*, 2001; Wang *et al.*, 2001; Hatanaka *et al.*, 2003). Tract-tracing studies in the macaque have shown that the CMAr and the caudal cingulate motor area (involving both CMAd and CMAv) are characterized by distinct patterns of intracortical and thalamocortical connections (Hatanaka, *et al.*, 2003). Functional studies examining cingulate motor areas suggest that CMAr plays a role in the cognitive control of voluntary movements, whereas the caudal CMA (CMAd and CMAv) is directly involved in the execution of voluntary movement (Devinsky *et al.*, 1995; Picard and Strick, 1996, 2001; Carter *et al.*, 1999; Tanji *et al.*, 2002).

33.6 Functional Significance of the Evolution of Motor Cortex

Because of the evolutionary changes that occurred in sensorimotor cortex and in the CST, especially in the human lineage, the functional contribution of the motor cortex and its descending outflow to the spinal cord is of natural interest. There is no question that evolutionary trends in motor control occurred in the human lineage resulting in increased dexterity of hand, especially of the digits. Investigators have long sought a morphological basis for the special motor skills of primates, including humans. It has been assumed that major changes must have taken place in the neural control of spinal cord motoneurons, since the peripheral anatomy of primates is remarkably similar (Napier, 1962).

In 1869, Spitzka suggested for the first time that the relative size of the pyramids might be related to the fine control of distal musculature. But even then, Spitzka realized that some species (e.g., seals and sea lions) possess large pyramidal tracts, but poor dexterity. In their review, Heffner and Masterton (1975) argue that among mammals, pyramidal tract morphology (size, number of fibers, fiber size) corresponds more closely to body size than to digital dexterity. However, the role of total number of fibers and fiber size should not be dismissed outright. Somewhat different results are obtained when correlations of pyramidal/CST parameters with digital dexterity are restricted to the human lineage (Heffner and Masterton, 1983). In this analysis, size of the pyramidal tract also becomes significant.

Also, CS morphology has for many years been suggested to be related to the specialized manual skills of primates. The giant Betz cells of M1 are likely to account for a significant proportion of corticomotoneuronal (CM) cells, and thus are likely to play a dominant role in skilled motor coordination. An important question though, is how closely CS soma size (as well as total number of CS neurons) is related to allometric scaling. When this issue was specifically examined, body weight accounts for over 30% of the variance in the number of CS neurons, and over 50% of the variance in their soma size (Nudo *et al.*, 1995). However, in primates, the number of CS neurons and their soma size deviate from the mammalian linear regression line. Primates have greater numbers and larger CS soma size than other mammals for their body weight. Strikingly, CS soma size and number of CS neurons track even more closely with neocortical surface area, which accounts for nearly 70% of the variance in number and over 80% of the variance in soma size in mammals. When this analysis is restricted to primates, neocortical surface area accounts for over 90% of the variance in CS soma size. (There was no significant difference between primates and nonprimates in the relationship between neocortical surface area and number of CS neurons.) Similar findings were found in a recent study of the allometric relationships of the size of Betz cells (Sherwood *et al.*, 2003). The authors suggests that Betz cells become larger in relation to body weight, brain weight, and encephalization quotient, but may not be related to digital dexterity, as others have suggested. Instead, the authors

propose that enlarged Betz cells may play a role in specialized locomotor behaviors in primates.

Before completely discounting the notion that CS soma size or Betz cell size is unrelated to specialized primate motor skills such as digital dexterity, one should consider that correlates of neocortical growth (brain weight, neocortical surface area, encephalization quotient) are not independent of the expansion and differentiation of motor and motor-related structures in primates. In fact, it is possible that morphologic alterations in motor structures (increased size of Betz cells and CS soma, differentiation of motor areas, increased intracortical circuitry, interconnected differentiated motor areas, increased corticofugal output, etc.) were major driving forces giving rise to a larger neocortex, at least in primates. Thus, number and size of CS neurons may still be important correlates of specialized motor skills such as digital dexterity, at least in the human lineage.

Mammals also differ widely in the pattern of CST terminations, and these differences appear to be related to digital dexterity. Heffner and Masterton (1975) showed that the mode of termination of CST fibers – both the extent of the fibers caudally in the cord and the ventral-most lamina in which they terminate – closely parallel the digital dexterity of a species. It is certainly reasonable to presume that animals with dexterous control of distal musculature might have direct cortical innervation of the (lateral) motoneurons that innervate this musculature. While this correlational study provides a rational hypothesis, the data were derived from older degeneration techniques that lack the sensitivity of more modern tract-tracing methods.

More recent neuroanatomical and electrophysiological data seem to corroborate this hypothesis. First, CST neurons that originate from a true motor cortex terminate in deeper laminae of the spinal cord (Ralston and Ralston, 1985). Most terminate in intermediate laminae in monkeys, somewhat more ventral to the termination of CS neurons originating in somatosensory cortex. However, a subset of CS neurons terminate in the ventral horn in the vicinity of motoneurons, and especially those motoneuron pools innervating muscles of the upper extremity. CS neurons originating in the primary motor cortex have the densest terminations to the deep spinal cord lamina where motoneurons innervating hand and finger muscles are located (Maier *et al.*, 2002).

There are also clear differences in the termination pattern of CS neurons among primate species. In some primates, a small subset of CS neurons terminates monosynaptically on motoneurons in the spinal cord. Such CM cells are present most notably in those primate species with the most highly developed digital skills. For example, squirrel monkeys accomplish grasping of objects with a prehensile, or power grip. A precision grip, in which the index finger is opposed to the thumb, is rarely if ever performed (Fragaszy, 1983). In this species, CM connections are relatively weak (Bortoff and Strick, 1993). Macaque monkeys display a precision grip and a number of other complex behaviors with the hand. This species possesses much more dense CM connections (Nakajima *et al.*, 2000). It has also been proposed that increased CM innervation is paralleled by decreased proprioceptive control of spinal cord motoneurons (Lemon and Griffiths, 2005).

Although rodents have surprisingly sophisticated motor control during grasping, and elaborate behavioral descriptions of complex movements of the digits have been reported (Walsh and Ebner, 1970; Iwaniuk and Whishaw, 2000), the termination of CS fibers differs from those in primates. CS neurons in rats tend to terminate in more dorsal laminae. Although there is a sparse termination in lamina IX, there is no evidence of monosynaptic connections between CS fibers and motoneurons (Yang and Lemon, 2003). Thus, control of the distal musculature may have evolved independently in various mammalian orders, and thus, neuroanatomical control mechanisms may differ among these species.

33.7 Summary and Conclusions

The evolutionary history of vertebrate motor systems is notable for its remarkable conservation of descending systems originating in upper levels of the neuraxis. All extant vertebrates apparently possess a similar subset of descending pathways that originated early in the evolution of the subphylum. Based on the available evidence from selected extant species, it would appear that the basic vertebrate plan was augmented by additional pathways, but antecedent pathways rarely became degenerate. The mammalian radiation was accompanied by the appearance of a new type of brain structure, a six-layered neocortex. In the earliest mammals, neocortex provided descending fibers to the medulla, and increasingly to the spinal cord. In multiple mammalian orders, a true motor cortex emerged, possibly through parallel or convergent evolution. Motor cortex became increasingly differentiated from the somatosensory cortex, and at least in primate species, terminated closer and closer to motoneuron pools, eventually providing fast, monosynaptic control of spinal cord motoneurons by the

cerebral cortex. At the same time, multiple motor areas became differentiated in the frontal cortex of primates, each with its own unique contribution to cortical motor control.

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34 The Evolution of Visual Cortex and Visual Systems

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Glossary

architecture The internal structure of a cortical area may contain unique histological features relative to adjacent cortical areas. There are several features that might distinguish an area. Two widely used histological markers result from (1) differences in meta bolic activity of neurons driven by inputs from the thalamus or (2) by the degree of myelination of intrinsic axons.

cortical layers Neocortex is commonly portrayed as having six layers, although these layers can be divided into additional sublayers depending on the cortical area. Layer 1 sits at the pial surface of cortex, whereas layer 6 is the dee pest layer.

extrastriate Refers to all of visual cortex outside of V1, as V1 is commonly referred to as striate cortex.

neuroanatomical tracing Because they are available in several colors, fluorescent tracers are parti cularly useful at retrogradely labeling multiple populations of neu rons. Injected extracellularly in small volumes (0.2 1.0μl) the tracer is taken in at synaptic sites of axon terminals over a relatively small region of cortex, spanning a half of a millimeter or less. Over a period of several days the fluorescent tracers are transported back along the axon to the cell body. In this way one is able to trace the origins of inputs to a particular cortical region.

34.1 Differences in Visual Cortex Complexity

Confronting the evolution of visual cortex is appealing in that it is perhaps the most dominant sensory system in humans, as evidenced by the large expanse of cortex devoted to visual processing. We have relatively big brains, so perhaps it is not surprising that we have a lot of visual cortex. Yet, compared to other sensory modalities, vision has a far greater representation in our cortex. Presumably our expanded visual system affords us a richer view of reflected light than animals with less cortex devoted to vision. Though our visual processing capabilities are due in part to a more elaborately constructed

retina, the visual thalamic relay stations and cortical areas in species with complex visual systems provide much more than a one-to-one correspondence of retinal ganglion cell output. The expanded size of our visual cortex can be used to infer an increased capacity for visual processing as size of mammalian cortex is often attributed to greater cognitive abilities. In line with such reasoning, comparisons of cortical sizes between several species of mammals are useful for determining a first approximation of the variability of cortically driven features. This is particularly informative when studying extinct species where sizes of brain endocasts can provide insights into cortical evolution (see How Can Fossils Tell us About the Evolution of the Neocortex?). But, the size of visual cortex in different mammals does not allow us to infer much about visual system complexity.

In addition to overall size, we know that mammalian cortex is subdivided into areas, or 'organs' of the brain (Brodmann, 1909). In the shrew, there is only one discernable visual area in cortex (Catania *et al.*, 1999). It is difficult to know what the shrew sees with that one area, but based on models of neuronal interconnectivity, the processing of visual features would be limited (Kaas, 2000; Mitchison, 1991; Ringo *et al.*, 1994). In mammals with greatly expanded visual cortex, such as carnivores and primates, as many as 19–40 visual areas have been proposed, based on anatomical and functional investigations (Felleman and Van Essen, 1991; Kaas, 1989b, 1997a; Sereno and Allman, 1991; Van Essen, 2004). The utility of having multiple visual areas is that each can be specialized for certain aspects of visual processing. By compartmentalizing there is a reduced need for intercommunication between all neurons, and thus fewer connections are required to process the information (Changizi and Shimojo, 2005; Kaas, 2000, 2002; Koulakov and Chklovskii, 2001; Mitchison, 1991; Ringo *et al.*, 1994). Areas can be further compartmentalized into processing modules, creating another level of complexity. Modular organization is more commonly reported in mammals that are considered to have moderate to high numbers of visual areas. Interestingly, while squirrels and tree shrews appear to have a similar, moderate numbers of visual areas, at least six (Kaas, 2002), they do not share the same level of modular organization (Bosking *et al.*, 1997; Van Hooser *et al.*, 2005a). Thus, one level of complexity, number of areas, does not necessarily lead to higher complexity of other levels, modularity.

We know that the numbers of cortical areas and degree of modularity within these areas varies across studied species (Kaas, 1989a, 2002). In the framework of cladistics, we can compare similarities in cortical organization across sister groups, and contrast with more distantly related species to determine what structures within the visual system represent the basic mammalian plan and from where features of more complex brains may have evolved (Kaas, 1995, 2002, 2004b; Striedter, 2005). However, sweeping comparisons are made difficult by the small number of examined species. Particularly lacking is a more detailed description of the number of visual areas, their functions, and modular organization. We have some evidence as to the number of cortical areas, their architecture and connection patterns in species from a handful of orders, but functional modular organization has been studied in detail in only a few species, namely monkeys, prosimians, cats, ferrets, tree shrews, and squirrels. Modular organization can and has been studied at the anatomical level in more species and this information, though less descriptive, is useful for making comparisons across several sister groups (Kaas, 2002). Another hindrance to species comparisons is that researchers who do study the organization of visual cortex in various mammals do not always agree with each other's interpretation of the evidence. Even in macaque monkeys, probably the most commonly studied animal for visual cortex organization, it has taken over 30 years to agree on the existence of the third visual area, V3 (Lyon and Kaas, 2002b; Van Essen, 2004; Zeki, 2003). Yet issues with V3 and the 20 or so other poorly defined areas remain, limiting our ability to make comparisons to other primates such as New World monkeys and even humans (Felleman and Van Essen, 1991; Kaas and Lyon, 2001; Rosa and Tweedale, 2005; Sereno and Tootell, 2005).

The goal of this article is to provide a reasonable interpretation of the available evidence on the organization of visual cortex and underlying structures in species from several mammalian orders (see Figure 1). Comparisons of the organizational schemes between species are made, highlighting several issues. How many visual areas were present in the earliest mammals? Have rodents diverged from the common mammalian plan? What are the organizational similarities and differences in species with moderate visual systems? How similar are the complex visual systems of cats and primates? Are any higher-order areas homologous? To address these questions, a focus is placed on a cladistic approach to species comparisons, but an emphasis is also made on similarities between distantly related species. The consolidation within this article of a wide body of comparative evidence on visual cortex organization should serve as a useful template for investigators probing cortical evolution.

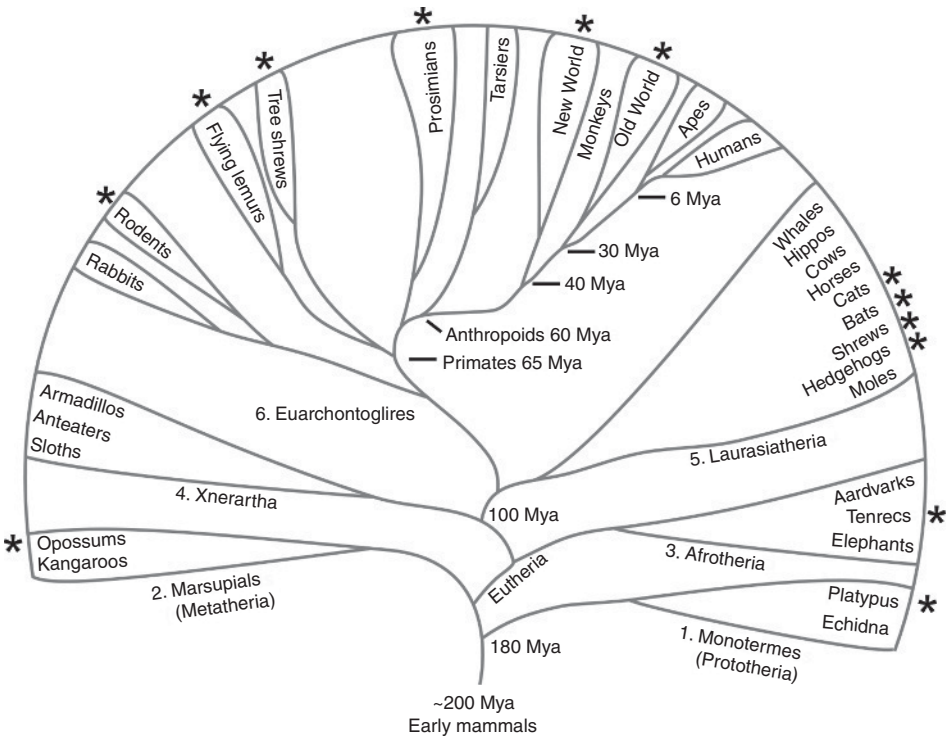

Figure 1 Phylogenetic relationships of the six mammalian superorders based on recent molecular studies (Murphy *et al.*, 2001). Early mammals diverged into prototherian monotremes, metatherian marsupials, and four superorders of eutherians. The organization of visual cortex in 13 taxa from five of the six superorders is covered in this article. * Species covered in text. Adapted from Kaas, J. H. 2005a. From mice to men: The evolution of the large, complex human brain. *J. Biosci.* 30, 155 165.

34.2 The Basic Mammalian Plan

34.2.1 Identification of V1 and V2

With perhaps only a couple of exceptions, mammals have at least two visual areas (Kaas, 1987; Kaas and Krubitzer, 1991; Krubitzer, 1998; Rosa and Krubitzer, 1999). An obvious exception would be mammals with vestigial eyes, such as subterranean species – the common mole (Catania and Kaas, 1995) and the naked mole rat (Catania and Remple, 2002) – where perhaps no visual cortex is present. Another exception can be found in the very small brains of the shrew where only a single visual area is present (Figure 2b; Catania *et al.*, 1999). Nevertheless, most mammals studied have at least two distinct visual areas. And, observed similarities in myeloarchitecture, subcortical inputs, and retinotopic organization across species have led to the generally accepted conclusion that the primary visual area (V1) and the secondary visual area (V2) are homologous across mammals (Kaas, 1987; Krubitzer, 1995; Krubitzer and Kahn, 2003).

V1 is typically found at the caudomedial extreme of neocortex and has several hallmarks of a primary sensory area that have been revealed through three basic techniques used for the study of cortical organization, namely architecture, connections, and retinotopic mapping (Kaas, 1987; Van Essen, 1979). The most conspicuous architectonic feature of V1 is the dense myelination of axons that can be revealed through a silver staining procedure (Gallyas, 1979). V1 also stains darkly for the metabolic enzyme cytochrome oxidase (CO; Wong-Riley and Carroll, 1984). Particularly when applied to flattened cortical preparations, these staining methods have proved very effective in identifying V1 in many species, from marsupials to monkeys (Kaas, 1987; Krubitzer, 1995), and have served as a useful landmark for further examination of the characteristics of V1.

Beyond the architecture, much of what we know about V1 in the majority of studied mammals comes through neuroanatomical tracing techniques and electrophysiological mapping of the representation of the contralateral visual hemifield. Through neuroanatomical tracing, we know that the primary retinal ascending pathway to V1 is relayed by the lateral geniculate nucleus (LGN) to the middle cortical layer, layer 4 (Jones, 1985; Steriade *et al.*, 1997). In most mammals, the LGN projects heavily to V1, and less so, if at all, to the remainder of visual cortex. Superficial layers 2 and 3 in V1 in turn project to layer 4 of V2. This basic cortical

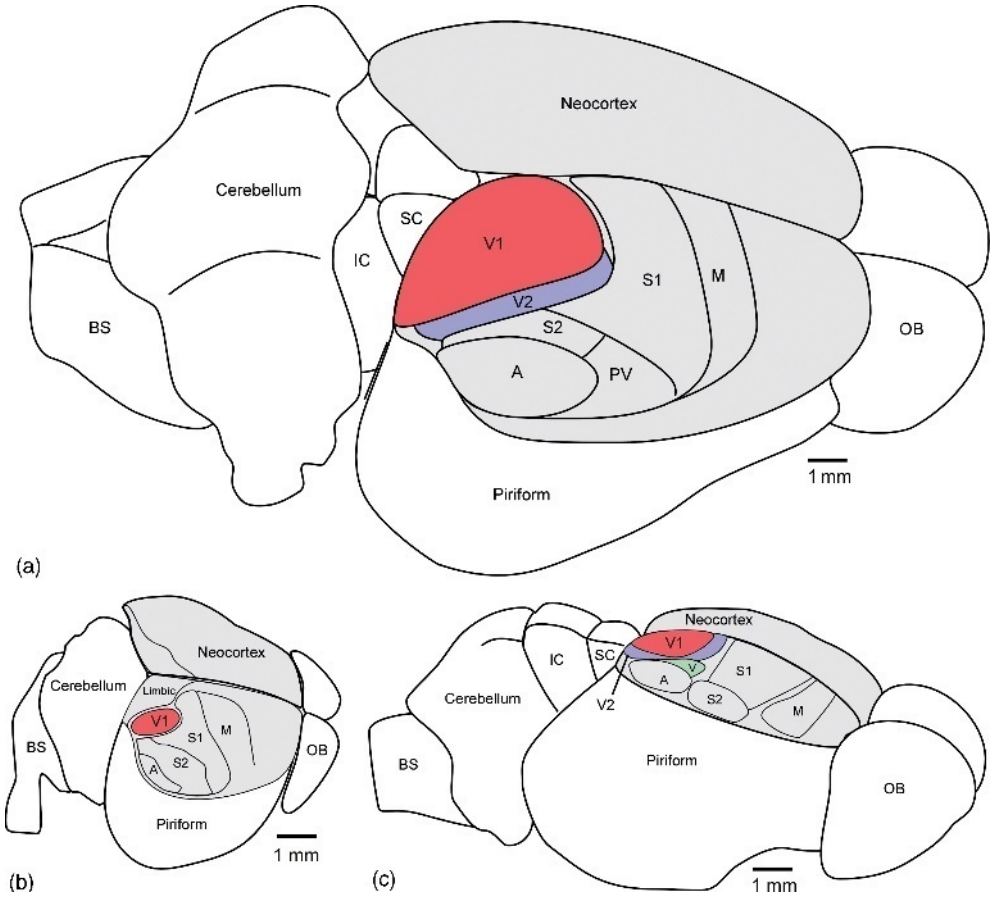

Figure 2 Visual cortex organization in small nonvisual mammals shown on whole-brain drawings. Based on similarities of several features, including brain sizes, insectivores (hedgehogs and shrews; (a and b) and tenrecs (member of the Afrotheria superorder; (c)) may have a cortical organization similar to the earliest mammals. Neocortex is relatively small and dominated by somatosensory areas 1 (S1) and 2 (S2), with a third area, parietal ventral (PV), found in the larger insectivore, the hedgehog. In insectivores, at most, only two visual areas are present, the primary (V1) and secondary (V2), with only a single area present in the smallest insectivores, the shrews. A third, visually responsive region (V) has been reported in the tenrec. Auditory (A) and motor cortex (M) are also present. Phylogenetically older piriform cortex is quite large. Likewise, the superior and inferior colliculi (SC and IC), the cerebellum, olfactory bulbs (OB), and brainstems (BS) are also relatively large. a, Data from Catania, K. C., Collins, C. E., and Kaas, J. H. 2000. Organization of sensory cortex in the East African hedgehog (*Atelerix albiventris*). *J. Comp. Neurol.* 421, 256 274. b, Data from Catania, K. C., Lyon, D. C., Mock, O. B., and Kaas, J. H. 1999. Cortical organization in shrews: Evidence from five species. *J. Comp. Neurol.* 410, 55 72. c, Data from Krubitzer, L., Kunzle, H., and Kass, J. 1997. Organization of sensory cortex in a Madagascan insectivore, the tenrec (*Echinops telfairi*). *J. Comp. Neurol.* 379, 399 414; and Kaas, J. H. 2002. Convergences in the modular and areal organization of the forebrain of mammals: Implications for the reconstruction of forebrain evolution. *Brain Behav. Evol.* 59, 262 272.

projection pattern is the early part of a general 'hierarchical' progression of areas within the visual system (Rockland, 1997). We also know that a secondary pathway provides less direct retinal input to cortex, by transmitting through the superficial layers of the superior colliculus (SC) to the pulvinar (also referred to as the lateral posterior nucleus, LP) and then to superficial layers of cortex. In contrast to the LGN, the pulvinar not only projects heavily to V1, but also provides large numbers of inputs to all areas of visual cortex (Casanova, 2004; Kaas and Huerta, 1988; Stepniewska, 2003).

Another basic tool for the examination of visual cortex is mapping of the visual topography (Allman and Kaas, 1971b; Gattass and Gross, 1981; Hubel and Wiesel, 1965; Kaas, 1997b; Kaas *et al.*, 1970; Rosa, 1997; Tusa *et al.*, 1979). By presenting isolated spots of light to different regions of the retina, the so-called retinotopic mapping has revealed a first-order map of the contralateral visual hemifield that is coextensive with architectonically defined V1 (Rosa and Krubitzer, 1999). Though the progressive placement of a microelectrode at sites traversing across the surface of V1 to determine the receptive fields of local neurons is painstaking, what emerges is an inverted map with the upper visual quadrant represented ventrally and the lower quadrant dorsally (for examples, see Figures 3–5; also, see

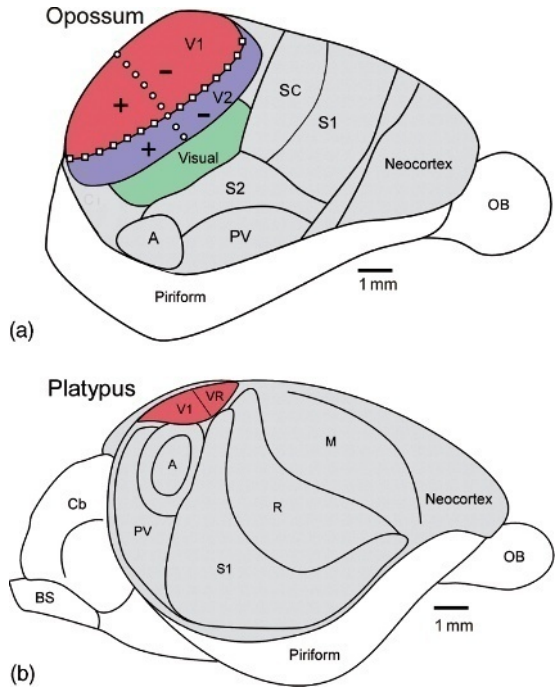

Figure 3 Visual cortex organization in nonplacental mammals, marsupials (a) and monotremes (b), shown on whole-brain drawings. a, The cortical organization of the opossum is typical of most small marsupials. Like the hedgehog, there are three large somatosensory areas, S1, S2, and PV, and a small auditory region as well as an additional caudal somatosensory area (SC) (A). However, unlike insectivores and tenrecs, visual cortex in opossum has expanded with comparatively large areas V1 and V2, and a large third region that contains visually responsive neurons. Areas V1 and V2 are both retinotopically organized (see schematic of visual hemifield in Figure 4a), with the representations of the upper (+) and lower ( ) visual fields located ventrally and dorsally, respectively. The + and visual field representations are bisected by the representation of the horizontal meridian (line of circles), while the representation of the vertical meridian (line of squares) separates areas V1 and V2, resulting in mirror-image representations of the visual field. b, In contrast to the marsupials, cortical organization in monotremes is dominated by three large somatosensory areas, S1, PV, and the rostral area (R), as shown in platypus. Motor cortex (M) is also quite large, and auditory cortex is comprised of at least two areas, a primary (A) and surrounding belt. Visual cortex is relatively small in monotremes. Though the evidence is limited, the caudal region of visual cortex may be homologous to V1 of other mammals, while the rostral visual area (VR) may represent a second visual area. a, Data from Beck *et al.* (1996), Rosa *et al.* (1999), and Kahn *et al.* (2000). b, Data from Krubitzer *et al.* (1995) and Krubitzer and Kahn (2003).

schematic in Figure 4a). The two visual quadrants are separated by the representation of the horizontal meridian (shown as a line of circles in several figures) which bisects V1. The representation of the vertical meridian (shown as a line of squares in several figures) is found at the rostral or anterior border of V1. Many more details of cortical organization are known from retinotopic mapping, such as variability in receptive field sizes, and in most species the magnified representation of central vision (see Allman and Kaas, 1971b; Rosa, 1997; Van Essen *et al.*, 1984). In addition to microelectrode mapping, the more recently developed techniques of intrinsic signal optical imaging and functional magnetic resonance imaging (fMRI) have been used to obtain retinotopic maps of large regions of cortex, making for easier comparisons across cortical areas (Sereno and Tootell, 2005). While retinotopic maps provide basic organizational information, neurophysiological studies describing the functional properties of V1 neurons have been reported for relatively few species, but they have provided for useful comparisons.

In most cases, the mammalian cortex also contains V2, located along the anterior border of V1. Characteristic of a nonprimary cortical area, V2 stains less darkly than V1 for CO and myelin (Krubitzer, 1995), receives feed-forward projections from V1 (Rockland, 1997), and receives its main subcortical retinal relays through the pulvinar, rather than the LGN (Jones, 1985; Steriade *et al.*, 1997). In addition, because V2 is substantially smaller than V1, it contains a compressed representation of the contralateral visual hemifield (see Rosa, 1997; Rosa and Krubitzer, 1999). The V2 retinotopic map can be distinguished from V1 by matching with CO and myeloarchitecture, but also because as the receptive fields for recording sites cross from V1 to V2 over the vertical meridian, the receptive field positions flip to form a rough mirror image of the representation in V1. The retinotopic organization of V2 has been revealed through microelectrode mapping and through connections with retinotopically defined regions of V1. Most evidence revealed through these techniques shows a similar organizational scheme for V2 in all mammals (see Rosa and Krubitzer, 1999).

34.2.2 Visual Cortex of Insectivores and Nonplacental Mammals

While most mammals have ample cortical space for more than two visual areas (Kaas, 1989b; Kaas and Krubitzer, 1991), certain constraints in the smallest mammals limit cortical representations to two or even only one visual area. Insectivores are notable for their small brain-to-body size ratio (Striedter, 2005), and in some species for small body size (Catania *et al.*, 1999). While the brain of a common laboratory rat may weigh as much as a few grams, the entire body weight of the smallest insectivore, the least shrew, is only 5 g. The extremely small size of the shrew brain is compounded further by the

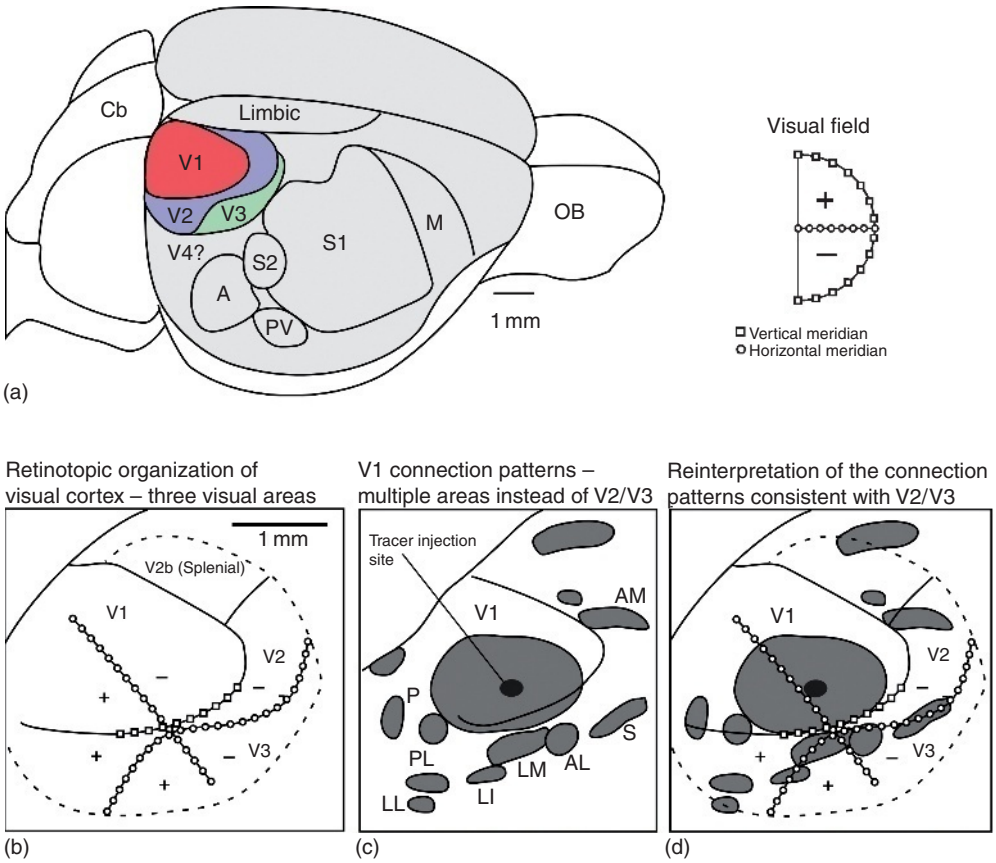

Figure 4 Visual cortex organization in a nonvisual rodent, the common mouse, shown on a drawing of the whole brain. a, Despite the somatosensory (S1, S2, and PV) dominance of cortical space, the mouse has at least three distinct cortical visual areas (V1, V2, and V3) and a fourth region (V4) that contains visually responsive neurons (Wagor *et al.*, 1980; Kaas and Krubitzer, 1991). b, While the visual areas are relatively small, V1, V2, and V3 are retinotopically organized (Wagor *et al.*, 1980; see schematic of the visual hemifield in panel a). c, In contrast, alternative schemes of the organization of visual cortex in nonvisual rodents propose as many as eight extrastriate areas in place of V2 and V3 (Olavarria and Montero, 1989). These results are based primarily on the multiple patches of neurons labeled from tracer injections in V1. In this example, a V1 injection was placed near the representation of the horizontal meridian. d, While several distinct clusters of labeled neurons are found in extrastriate cortex, these clusters are not inconsistent with the retinotopic organization of V2 and V3, and do not unequivocally support the proposal of eight extrastriate areas. For example, the majority of patches of labeled neurons can be found along the proposed V2/V3 border, which represents the horizontal meridian, and matches the retinotopic location of the injection site in V1. See Section 34.3 for abbreviations.

small size of neocortex relative to the rest of the brain, and further still by the majority of cortex representing somatosensory receptors, leaving little cortex representing vision (see Figure 2b; Catania *et al.*, 1999). With the larger shrews weighing upwards of 50g, these insectivores provide a close approximation to the size constraints presented to our earliest mammalian ancestors, which are estimated to have weighed ~30g (Allman, 1999). In addition to body and cortical size constraints, insectivores in general are considered to have retained many other structural features of the first mammals and to occupy similar habitats (see Kaas *et al.*, 1970). Because of these similarities to early mammals, the organization of visual cortex in shrews and other insectivores is likely to have diverged little from the original mammalian plan.

In five species of shrew, only a single visual area is present (Figure 2b; Catania *et al.*, 1999). It has the characteristic dark CO staining of mammalian V1, its neurons respond robustly to flashes of light presented to the eyes, and it receives projections from the LGN. Other insectivores, such as the European hedgehog (Figure 2a) and the tenrec of Madagascar (Figure 2c), do not fair much better than the shrew in terms of the amount of visual representation in cortex. However, there is a V2, present in these species. The hedgehog is much larger than the shrew, weighing about 1000g. Both V1 and V2 have been demonstrated through microelectrode mapping, with V2 forming a mirror representation of V1 (Kaas *et al.*, 1970). Indicative of a V1, hedgehog V1 stains very darkly for myelin and CO (Catania *et al.*, 2000; Kaas *et al.*, 1970; Krubitzer,

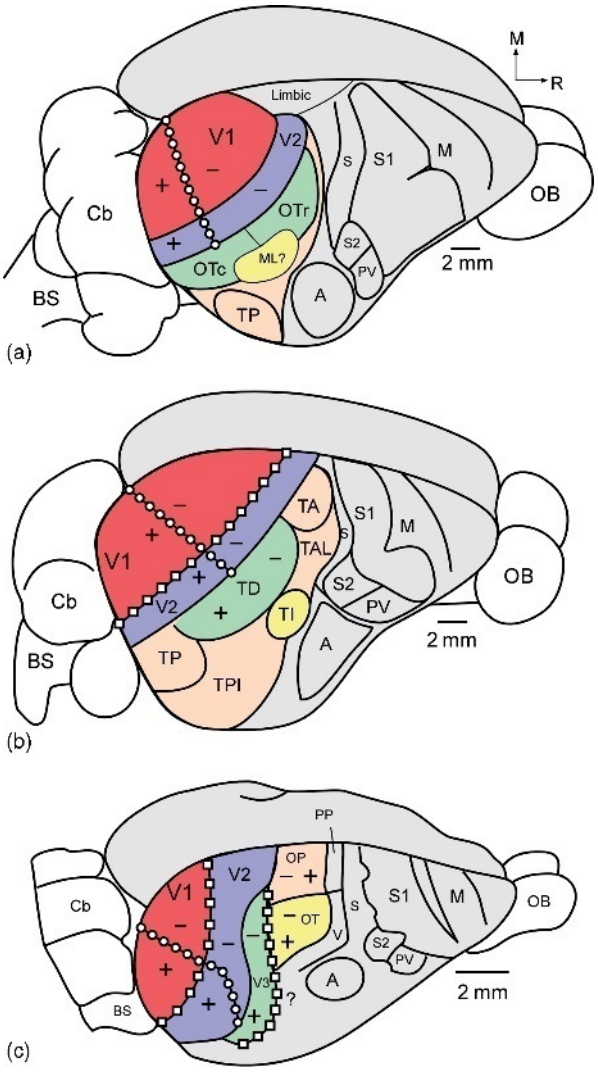

Figure 5 Visual cortex organization in species with moderately complex visual systems: squirrels (a), tree shrews (b), and flying foxes (c). At least half of cortex in these three highly visual species is devoted to vision (colored shading). In all three species, V1 and V2 are retinotopically organized and quite large, comprising nearly half of visual cortex. A V3-like region is found in all three species as well (green shading), with a fourth region (yellow shading) that in limited ways resembles area MT in primates (see Figures 12 14). Overall, several visual areas comprise the cortex. At least six visual areas have been defined in squirrels (Kaas *et al.*, 1989; Paolini and Sereno, 1998) and flying foxes (Rosa, 1999), and eight in tree shrews (Lyon *et al.*, 1998). Additional visual areas may be present in rostral cortex which has been shown to be visually responsive. See Section 34.4 for explanation of visual areas and abbreviations.

1995) and receives projections from the LGN (Hall and Ebner, 1970). Because many characteristics of the hedgehog resemble that of some of our earliest mammalian ancestors, it is postulated that V1 and V2 likely represent the prototypical mammalian plan for visual cortex (Kaas, 2005a; Kaas *et al.*, 1970), and this is supported by the evidence that most mammals, including marsupials, also have a V1 and a V2 (Rosa and Krubitzer, 1999). While the organization of the tenrec and hedgehog visual cortex is very similar, this similarity may be more of a reflection of the limitations imposed by small cortical size, as recent evidence shows that tenrecs should not be considered insectivores (Murphy *et al.*, 2001). In fact, tenrecs are now considered part of the African superorder of mammals, Afrotheria (see Figure 1), which includes golden moles, elephant shrews, aardvarks, and even elephants and sea cows.

Metatherian mammals, or marsupials, diverged from an older common ancestor than insectivores, but nevertheless, by most accounts, all studied marsupials have a V1 and V2 (Figure 3a). In several small marsupial species V1 is clearly delimited through CO or myeloarchitecture (Beck *et al.*, 1996; Kahn *et al.*, 2000; Martinich *et al.*, 2000; Rosa *et al.*, 1999). In addition, an orderly retinotopic map is evident (Rosa *et al.*, 1999; Kahn *et al.*, 2000). The case for a marsupial V2 initially was less certain. For example, in an earlier study on the mouse opossum (*Marmosa elegans*) tracer injections in V1 resulted in several patches of labeled neurons in cortex adjacent to the anterior V1 border (Bravo *et al.*, 1990). In a manner similar to conclusions made in some rodent studies (see Section 34.3.1) it was proposed that each patch represented a separate visual area, rather than a single V2. However, subsequent experiments on V1 connectivity in other small marsupials showed connectivity with V2 that is more consistent with the concept of a single visual area, V2 (Beck *et al.*, 1996; Kahn *et al.*, 2000). The existence of V2 was confirmed through detailed retinotopic mapping (Rosa *et al.*, 1999).

Prototherian mammals, or monotremes, were the first to diverge from the mammalian line, having split perhaps as many as 180 Mya (Figure 1; Murphy *et al.*, 2001). In the cortex of monotremes, at least two visual areas have been described (Figure 3b; Krubitzer, 1998; Manger, 2005). However, there is some question as to whether they can be considered homologous to V1 and V2 of metatherian and eutherian mammals. As a result, rather than V1 and V2, they have been tentatively termed the caudal and rostral visual areas, or Vc and Vr. Krubitzer *et al.* (1995) described a region of dark CO and myeloarchitecture that is coextensive with both visual areas. While Vr is somewhat lighter in appearance, there is no sharp architectonic division between the two areas as between V1 and V2 in other mammals. Both regions contain fairly complete retinotopic maps, but Vc can be distinguished from Vr in that it responds more vigorously to visual

stimulation (Krubitzer, 1998). Because of the slightly darker architectonic features, more vigorous response properties, and the fact that the caudalmost area occupies cortical territory similar to that of mammalian V1, it is possible that Vc is homologous to V1. However, there is some evidence that visual cortex in monotremes receives ascending inputs from a secondary visual thalamic nucleus, rather than the LGN. Thus, while monotreme cortex contains at least two visual areas, they only partly resemble visual areas in other mammals. Largely because so few studies have been done on the visual system in monotremes, homologies to visual cortex in marsupials and eutherian mammals remain in doubt, and it is plausible that the basic mammalian plan for the visual system underwent several modifications after having split from the monotremes (see Krubitzer, 1998).

34.2.3 The Basic Mammalian Plan: One Area, Two, or Even Three?

From the cortical organization evidence in marsupials and insectivores, as well as the implication that most other eutherian mammals have a V1 and a V2, there emerge two approaches for reconstructing the cortical organization of the earliest mammals. These approaches are somewhat in conflict and result in different conclusions. On the one hand, shrews and other insectivores retain several anatomical features of the earliest mammals and would be under similar constraints brought on by a small cortex. This overwhelming similarity has led to the conclusion that insectivore cortical organization reflects the basic mammalian plan. On the other hand, shrews have one less visual area than other, larger insectivores (see Figure 2), and the argument that all other mammalian sister groups, including marsupials, have a V1 and a V2 is used to support the view that the common ancestor to marsupials and eutherians was likely to have a V1 and V2 as well. So, do we devise the basic mammalian plan based on similarities between shrews and early mammals, or common features across all mammalian sister groups? Or can we use both criteria?

While the proposal that similarities in body and brain sizes, other morphological characteristics, and habitat make insectivores an ideal candidate for the species most resembling early mammals is fairly supportable, it clashes with earlier conclusions derived from comparisons across all mammalian sister groups. In particular, though all studied mammals (other than shrews, and possibly monotremes) possess at least a V1 and V2, many insectivores tend to have fewer visual areas than most other mammalian orders, including marsupials, which have at least three (Figure 3a). The issue now becomes whether any of the three or more visual areas are homologous or evolved independently in separate orders. While some have made the argument for homologies of at least three visual areas – V1, V2, and V3 (Rosa, 1999; Rosa and Manger, 2005) – others conclude that V3 is not homologous (Kaas, 2002).

Let us assume that only two areas are homologous across eutherian mammals. Even so, is the hedgehog, with V1 and V2, more representative of the basic mammalian plan than the shrew, which only has a V1? The shrew having only a V1 can be explained through evolutionary digression to adapt to an extremely small body and brain size, where there just is not enough room for a second visual area. However, it is important to consider here that all species studied that do have a V2 are significantly larger than shrews, and these species are also significantly larger than the estimated size of the first mammals. Shrews, on the other hand, are about the same size as the earliest known mammals. Thus, it is not implausible that shrew cortical organization is most representative of the first eutherian mammals.

If we postulate that only a V1 was present in the earliest mammals, then this has implications for the evolution of subsequent visual areas – V2 and every other extrastriate area could have evolved independently in some or several mammalian orders. Whether these areas are homologous would depend on whether a pre-existing mechanism allowing for the emergence of multiple visual areas in a similar fashion was in place in the earliest mammals (see Striedter, 2005). However, such a viewpoint would represent an extreme. What we can say for certain is that shrews and larger insectivores possess a visual cortex that is minimal in design, possessing only one or two visual areas and occupying a relatively small portion of cortex compared to the somatosensory system. As the visual system has expanded in other orders that place a greater emphasis on visual processing, it is generally agreed that V1 and V2 have retained enough of their basic features to remain homologous across species.

34.3 Rodent Visual Systems: Simple or Complex?

34.3.1 Have Small Rodents Diverged from the Common Mammalian Plan?

If we conclude that the basic mammalian plan for visual cortex calls for, at most, two areas, V1 and

V2, and we know that most mammals have at least three visual areas and probably more, then it follows that cortical evolution has resulted in the addition of visual areas. There are several theories as to how cortical areas have increased in number (see Captured in the Net of Space and Time: Understanding Cortical Field Evolution; Allman, 1999; Allman and Kaas, 1971a; Kaas, 1989a; Krubitzer, 1995; Krubitzer and Kaas, 2005; Krubitzer and Kahn, 2003; Northcutt and Kaas, 1995; Rosa, 1999, 2002; Rosa and Krubitzer, 1999; Rosa and Tweedale, 2005; Striedter, 2005). Rodents provide a good example for both slightly and more moderately expanded cortex, as has been shown in mouse and squirrel, respectively (see Figures 4 and 5a). And, because rodents are the most abundant of the mammalian orders, insights into their organization are important for the study of cortical evolution. Despite using similar techniques, efforts over the past 30 years to determine the organization of visual cortex in rodents have led to two very different conclusions as to the organization of extrastriate cortex (see Sereno and Allman, 1991; Rosa and Krubitzer, 1999). Discrepancies in extrastriate organizational schemes begin as early as V2. As detailed by Rosa and Krubitzer (1999), opposing opinions relate to whether subsequent extrastriate areas have been added to a pre-existing V2 or whether pre-existing extrastriate cortex in an early rodent ancestor, instead of a V2, was already subdivided into several small areas. As we have seen in species that branched from earlier common ancestors – marsupials, tenrecs, and insectivores – only a single area V2 is present, if at all (Figures 2 and 3). Thus, multiple areas in place of V2 in rodents would represent a divergent path in mammalian visual cortex evolution.

The controversial conclusions stem from studies in mouse and rat. Building upon an earlier microelectrode mapping of rat cortex anterior to V1 (Montero *et al.*, 1973b), the patchy extrastriate connection patterns of V1 in mouse and rat were interpreted as support for several distinct visual areas (see Figure 2c; Olavarria and Montero, 1989), rather than a single V2 (Figures 2a and 2b), as postulated by others (Malach, 1989; Rumberger *et al.*, 2001; Wagor *et al.*, 1980). A subsequent study used two to three distinguishable tracer injections placed in different retinotopic locations of V1 in single animals (Montero, 1993) to further establish the retinotopic organizations of the multiple regions. The emergence of multiple distinguishable tracers has proved to be a useful tool for estimates of extrastriate cortex, as will be demonstrated more fully in subsequent sections. Retinotopic maps by others reported similar organizational schemes in rat and hamster (Espinoza *et al.*, 1992; Espinoza and Thomas, 1983). In addition, supporting evidence was derived from callosal connections used to approximate the vertical meridian borders of several of the proposed areas (Olavarria and Montero, 1981, 1989; Thomas and Espinoza, 1987). For example, Thomas and Espinoza (1987) proposed seven extrastriate visual areas, four of which border V1. The largest of these proposed areas, LM, contains a retinotopic map similar to that of V2. Other schemes have postulated as many as nine areas along the V1 borders (see Sereno and Allman, 1991). While it is not unreasonable to consider LM a V2 homologue (Rosa and Krubitzer, 1999), its truncated size leaves several areas adjacent to V1, a pattern not typically reported in other mammalian orders (Kaas and Krubitzer, 1991).

Despite several corroborating experiments, other results contradict the interpretation of multiple areas in place of V2. For example, an early anatomical study using lesions in V1 to look for degenerated neurons in extrastriate cortex, revealed only a single patch of connected neurons in extrastriate cortex nearest V1 (Montero *et al.*, 1973a). In addition, microelectrode mapping studies in the hamster and mouse supported a retinotopic organization more consistent with a single V2 along the lateral border of V1 (see Figures 4b and 4d; Tiao and Blakemore, 1976; Wagor *et al.*, 1980). Interestingly, these results are similar to those reported for lagomorphs (see Sereno and Allman, 1991), the closest relatives to rodents (Figure 1; Murphy *et al.*, 2001). More recently, intrinsic signal optical imaging in mouse visual cortex also yielded results consistent with a single V2 lateral to the V1 border (Kalatsky and Stryker, 2003).

Likely contributing to the differences in data on the retinotopic organization in small rodents are inherent logistical problems in obtaining a clean, detailed retinotopic map in a cramped cortical space while trying to stabilize the very small eyes (Rosa and Krubitzer, 1999; however, see Wagor *et al.*, 1980). In addition, a heavy reliance on callosal connectivity patterns to reveal multiple areas could be misleading in that callosal connections in species with a well-defined V2 extending the entire lateral border of V1, as found in primates, reveals a similar pattern as that seen in rat (Cusick *et al.*, 1984). Thus, callosal patterns are often irregularly distributed within single areas and give the impression of multiple areas if one assumes their location marks the vertical meridian. For these reasons, the use of callosal input patterns as a primary means of

delimiting borders between visual areas is questionable. In addition to the evidence in small rodents, results from brains of more visually dependent squirrels (see the discussion below), argue against multiple areas immediately outside of V1 in rodents, and point to a single V2 (Figure 5a), consistent with the common mammalian plan (Kaas and Krubitzer, 1991).

Before moving on, it should be noted that many rodent studies designate a separate V2, V2b, sometimes referred to as area 18b (Wagor *et al.*, 1980), that lies medial to V1. While the lateral area 18a (Wagor *et al.*, 1980), or LM (Espinoza and Thomas, 1983), is considered to be the homologue to V2 in other mammals, Rosa *et al.* (1999) contend that the medial region is similar to the splenial visual area of limbic cortex found in other mammals, including primates (Rosa *et al.*, 1997), and is not part of mammalian V2.

34.3.2 How Many Visual Areas in Rodent Cortex?

Most of the upwards of 12 extrastriate areas described in rodents are all located along the V1 border (Sereno and Allman, 1991), three along the medial border and six along the lateral border. But, as discussed above, alternative evidence concludes that the region immediately lateral to V1 comprises a single V2 (Wagor *et al.*, 1980; Kaas and Krubitzer, 1991), and the medial region may be more consistent with limbic visual cortex found in other mammals (Rosa and Krubitzer, 1999). Moving beyond the V2 controversy, we know that the rodent has additional cortex devoted to vision, and has presumably expanded from the basic visual plan found in insectivores. So, how many visual areas do rodents have?

If we jump ahead to results from more modern techniques, we find two retinotopically organized extrastriate areas outside of V2 in a mouse. Kalatsky and Stryker (2003) optimized visual display signals to avoid confounding biorhythms. In this manner, they were able to maximize intrinsic neuronal activity measured through optical imaging (movies of the activation of visual cortex as stimuli sweep across the visual field are available in their online supplemental material – see 'Relevant Website'). In all, four extrastriate areas were described – V2, V3, V4, and V5. V2 represents a condensed mirror image of the representation found in V1, while V3 mirrors V2, and likewise V4 mirrors V3. Area V5 is found medially in a similar location to V2b described above, and is probably the visual region of the limbic cortex (see Rosa and Krubitzer, 1999). The optical imaging measurements are consistent with earlier microelectrode maps in the mouse, where V1, V2, a V3 and an additional lateral region were reported (Figures 4a and 4b; Wagor *et al.*, 1980).

34.3.3 The Complexity of Squirrel Visual Cortex

Although mice have an expanded visual cortex compared to insectivores, they rely largely on somatosensation through vibrissa on the snout, and through their sense of smell. The importance of the somatosensory system is reflected by a cortex dominated by the barrel fields representing the vibrissa. This leaves little cortical space for visual areas, and subsequently as few as two extrastriate cortical areas outside of V2 have been reported (Figure 4a; Wagor *et al.*, 1980; Kalatsky and Stryker, 2003), as described in the previous section. In contrast, squirrels, which are diurnal, have retinas comprised primarily of cones, 90–95% (Jacobs *et al.*, 1980), and have an extremely high density of ganglion cells projecting to the LGN and SC (Johnson *et al.*, 1998; Major *et al.*, 2003) allowing for higher visual acuity. This increase in visual input is reflected in the greater expanse of cortex devoted to vision (Kaas, 2002), and an increased number of extrastriate visual areas (Figure 5a; Kaas *et al.*, 1989; Paolini and Sereno, 1998). Thus, the somatosensory subfield for the barrel field is one-third the size in squirrels than in rats, whereas striate cortex and extrastriate cortex are four and eight times larger, respectively, in squirrels (Paolini and Sereno, 1998).

An early study identified three retinotopically organized visual areas 17, 18, and 19 (Hall *et al.*, 1971), similar to V1, V2, and V3 found in other mammals. Like other rodents, V1 is easily distinguished through myelin staining (Kaas *et al.*, 1989; Paolini and Sereno, 1998). However, an advantage of studying squirrels over smaller, less visual rodents is that V2 is also distinguishable as a uniform darkly stained myelin region along the lateral border of V1. Consistent with the common mammalian plan, squirrel V1 receives topographic projections from the LGN (Kaas *et al.*, 1972a), whereas the main visual thalamic input to extrastriate cortex comes through the relatively large pulvinar (see Figure 6a; Robson and Hall, 1977). V2 and V3 in squirrels are also relatively large compared to the descriptions for mice (Figures 4a and 5b). Connection patterns from tracer injections placed in V1 and V2 of squirrels revealed several features of these large regions (Figure 7a; Kaas *et al.*, 1989). Similar to small rodent studies described above, patchy

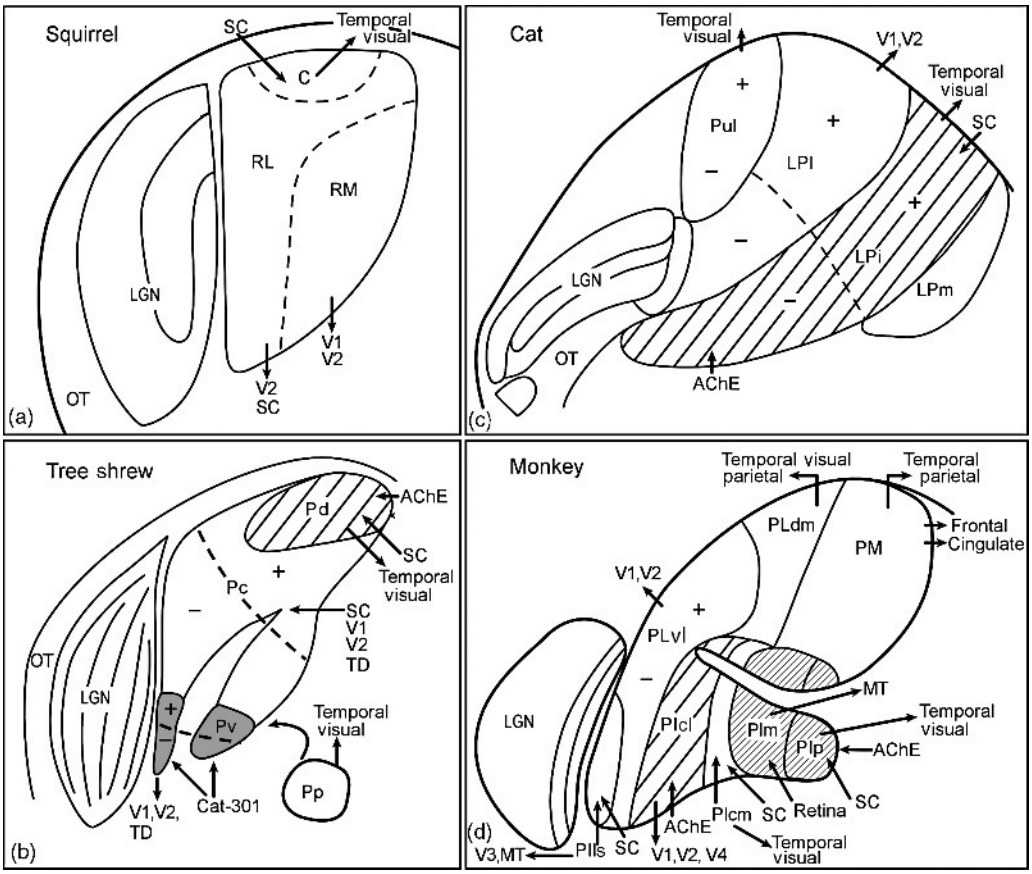

Figure 6 Organization and cortical connections of the pulvinar nucleus in four highly visual species. A thalamic nucleus relaying inputs from the superior colliculus (SC), the pulvinar receives feedback from and provides inputs to all of visual cortex. In highly visual species the pulvinar can be subdivided into distinct nuclei, each with a distinct pattern of connections to extrastriate visual cortex. a, There is evidence for three subdivisions in the pulvinar of squirrels, the rostrolateral (RL), rostromedial (RM), and caudal (C) divisions (Robson and Hall, 1977). RL receives projections from SC and projects to V2, whereas RM does not get SC projections but projects to both V1 and V2. The C subdivision is only found in the caudal extent of pulvinar, and is distinguished by SC inputs and connections with temporal visual cortex. b, Tree shrew pulvinar has four subdivisions distinguished through differences in architecture (Lyon *et al*., 2003a), input from SC (Luppino *et al*., 1988), and projections to visual cortex (Lyon *et al*., 2003b). The central (Pc) is the largest subdivision. It receives inputs from SC and has retinotopic projections to V1, V2, and TD. The ventral subdivision (Pv) also provides retinotopic inputs to V1, V2, and TD, and can be distinguished through immunoreactivity to Cat-301 (gray shading). A dashed line in both Pc and Pv separates portions representing the upper (+) and lower ( ) visual field representations in these subdivisions. The dorsal subdivision (Pd) with high levels of acetylcholinesterase (AChE; thatching) receives projections from SC and projects in turn to temporal visual areas. A posterior subdivision (Pp) is located in the posterior-most extent of the pulvinar and projects exclusively to temporal visual areas. c, The cat pulvinar is formed by the 'pulvinar' (Pul) and three subdivisions of the lateral posterior nucleus including lateral (LPl), intermediate (LPi) and medial (LPm; Casanova, 2004; Lyon *et al*., 2003b). Inputs from SC and projections to distinct cortical visual areas are shown (based on Lyon *et al*., 2003b; Casanova, 2004). For more details see Section 34.5.1. d, Monkey pulvinar has been split into at least eight subdivisions (Cusick *et al*., 1993; Stepniewska, 2003). The inferior pulvinar is comprised of five subdivisions, the posterior (PIp), medial (PIm), central medial (PIcm), central lateral (PIcl) and lateral shell (PIls). The lateral pulvinar has been split into at least two subdivisions, the ventral lateral (PLvl) and dorsal medial (PLdm), while conservative estimates treat the medial subdivision as a single region (PM). Inputs from SC and projections to distinct sets of cortical areas are shown (Lyon *et al*., 2005; Shipp, 2001; Stepniewska *et al*., 2000; Stepniewska, 2003). In all panels, the dorsal lateral geniculate nucleus (LGN) and the optic tract (OT) are shown for reference. Modified from Lyon, D. C., Jain, N., and Kaas, J. H. 2003b. The visual pulvinar in tree shrews. II: Projections of four nuclei to areas of visual cortex. *J. Comp. Neurol*. 467, 607 627.

connections in V2 resulted from V1 injections. Furthermore, injections in V2 resulted in intrinsic long-range connections of up to 6 mm. In addition, callosal connections revealed a patchy pattern, much like that reported for rats (Gould, 1984). In conjunction with the myelin pattern and retinotopic maps, these patterns are taken to reflect modular organization within V2, rather

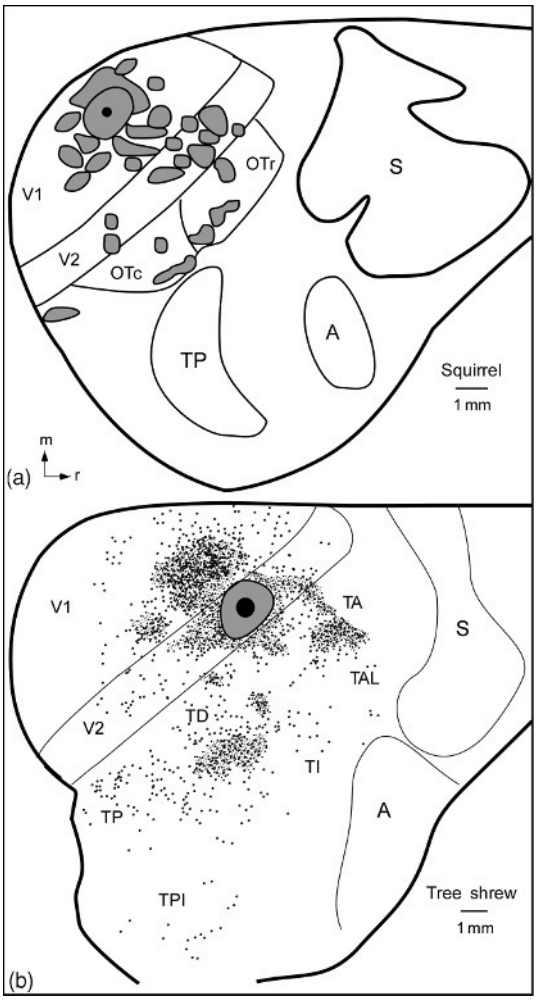

Figure 7 Connection patterns of early visual cortex are useful in identifying multiple extrastriate areas. a, An injection in V1 of squirrels results in a patchy pattern of label both intrinsically within V1 and in extrastriate cortex. In extrastriate regions, a wider displacement of these patches is attributed to separate cortical areas, OTc and OTr. b, In tree shrews, following a tracer injection in V2 the displacement of patches of labeled neurons in extrastriate cortex is more distinct, revealing several visual areas, TA, TD, TP, TPI. In both instances, these connection patterns can be used as guides for further exploration of extrastriate visual cortex, such as, targeting extrastriate areas for microelectrode recordings and further tracer injections. See Section 34.4 for abbreviations and more details. a, Based on Kaas, J. H., Krubitzer, L. A., and Johanson, K. L. 1989. Cortical connections of areas 17 (V-I) and 18 (V-II) of squirrels. *J. Comp. Neurol.* 281, 426 446. b, Modified from Lyon, D. C., Jain, N., and Kaas, J. H. 1998. Cortical connections of striate and extrastriate visual areas in tree shrews. *J. Comp. Neurol.* 401, 109 128, with permission from John Wiley & Sons.

than an indication of several distinct visual areas bordering V2.

Following V1 and V2 injections, patchy connections were also found in V3, termed the occipital–temporal zone (OT; Kaas *et al.*, 1989). However, because of widely spaced patchy connectivity it was proposed that V3 could be broken up into 2–3 areas labeled as rostral, middle, and caudal divisions of the occipital temporal cortex (OTr, OTm, and OTc; Kaas and Krubitzer, 1991; Kaas, 2002). Still, the connection patterns are somewhat consistent with a single area V3 (OT), if one considers multiple patches as a sign of modular organization as for V2, but in some cases the distance between patches in V3 is much larger than that found in V2 (Kaas *et al.*, 1989). In addition, earlier retinotopic maps of V3 were very limited (Hall *et al.*, 1971), leaving this region open to other interpretations. More conservative estimates split OT into two regions, OTr and OTc (Figures 5a and 7a; Kaas *et al.*, 1989; Kaas, 2002). Additional studies exploring the retinotopic organization with multiple tracer injections and retinotopic mapping would help determine whether OT is a single retinotopically organized region or actually comprised of 2–3 separate areas. As we shall see in the next section, the squirrel pattern of connectivity is similar to multiple divisions of V3 described for the highly visual tree shrew (Figures 5 and 7; Kaas and Krubitzer, 1991; Kaas, 2002).

The expanded temporal lobe of the squirrel provides additional space for two more large visual regions between OT and anterior cortex devoted to audition and somatosensation. These regions were identified as visual in nature through their connection patterns with V2. It is worth noting that tracing techniques, such as this, have proved to be a valuable technique for providing a first approximation of the number of extrastriate areas (see Kaas, 2004c). These visual areas have been termed the temporal posterior (TP) and temporal intermediate (TI). TP extends laterally from OTc, along the base of sensory cortex. TP stains very darkly for myelin and is sparsely interconnected with V2 (Kaas *et al.*, 1989). TI stains lightly for myelin and can be divided into at least two distinct regions based on interconnectivity with V2. Thus, outside of V2 and OT, the squirrel has at least three more extrastriate areas.

Based on response properties of neurons found in the OT visual region, Paolini and Sereno (1998) have designated two areas, the middle lateral (ML) and lateral (L). ML and L occupy territories similar to OTr and OTc. Further investigation revealed that ML and L are comprised of neurons selective for speed and the direction of moving stimuli (Paolini and Sereno, 1998).

The functional characteristics of neurons have been sparingly discussed up to this point. So, what

does it mean to find an area specialized for a visual feature? V1 neurons of rats, as well as squirrels, prefer particular aspects of features of a visual stimulus – orientation, direction, and length (Girman *et al.*, 1999; Ohki *et al.*, 2005; Van Hooser *et al.*, 2005b). However, V1 receptive field sizes are typically very small and thus will only respond to a stimulus presented in a very small portion of the visual field, but converging inputs to subsequent extrastriate areas enable neurons to respond to a larger region of the receptive field. In addition, particular features of a stimulus activate neurons in different extrastriate cortical areas to different degrees such that some areas may contain neurons that respond more vigorously to the motion of the object while being less influenced by the shape or color of the object, features which are important to neurons in other visual areas. While there are no reports of functionally specialized extrastriate areas in rats, a large body of evidence has detailed functionally selective areas in mammals with more complex visual systems, such as cats and primates (see Section 34.5).

With an extrastriate visual cortex eight times larger than the rat, and a conservative estimate of at least five extrastriate areas beyond V2, it seems clear that squirrel visual cortex is more complex than the rat or mouse, where likely only two more areas can be found beyond V2 (see previous section; Figure 4). This observation conflicts with a recent proposal that species within a particular order typically have the same level of cortical complexity (Manger, 2005). In support of similar complexity between rat and squirrel, one could argue that the multiple subdivisions proposed for V2 in rat and mouse increase the number of extrastriate cortex tremendously. Yet, this intricate pattern is not found in squirrels. Furthermore, if we give the rat nine visual areas along the V1 border, this would represent an organization more complex than visual cortex found adjacent to V1 in any other mammalian order, including primates.

34.4 The Moderate Visual Systems

In the species discussed thus far, we have seen as few as one visual area in the shrew, two in monotremes, hedgehogs, and tenrecs, two or three in some small marsupials, and perhaps as many as four in small rodents. While these species rely heavily on modalities other than vision, the number of areas jumps to at least seven in the more visually dependent squirrel. As we will see in this section, the number of visual areas is similar to that proposed for megachiropteran bats (flying foxes) and tree shrews, species that have been or still are considered close relatives of primates. These species, particularly tree shrews, are thought to have retained many of the features found in the common ancestor that led to primates, so an understanding of their cortical organization is essential for understanding the early evolution of the primate brain.

34.4.1 Visual Systems of the Tree Shrew and Flying Fox

Until recently, the superorder Archonta was used to group primates together with their three closest relatives – the gliding lemur, tree shrew, and flying fox. Two species of gliding lemur make up the order Dermoptera. While not actually a lemur, and despite having a rather large wing-like web of skin encircling its entire body, the gliding lemur resembles prosimian primates. In fact, recent DNA analysis indicates that gliding lemurs are indeed closely related to primates (Murphy *et al.*, 2001). The tree shrew, a squirrel look-alike, despite its name is more closely related to primates than shrews and other insectivores. Early classification based on brain similarities placed tree shrews within the primate order, though they have since been moved to their own order, Scandentia (see Martin, 1990). DNA evidence also supports a very close relationship between tree shrew and primates (Murphy *et al.*, 2001). The flying fox is the largest of the fruit-eating bats (Megachiroptera). While some unique anatomical similarities to primates led to the proposal that the flying fox should be classified separately from smaller, microchiropteran bats, and considered close relatives of primates (Pettigrew, 1986; Pettigrew *et al.*, 1989), more recent genetic evidence maintains bat monophyly (Van Den Bussche *et al.*, 1998) and has removed megabats from Archonta, claiming a closer relationship to cats than to either rodents or primates (Kaas, 2004b; Murphy *et al.*, 2001).

Despite the recent distancing of flying fox from tree shrew, neuroanatomical and physiological studies indicate some similarities in visual system organization (Figure 5). Both species are diurnal and boast retinas packed densely with ganglion output cells (Pettigrew, 1986; Kaas and Preuss, 1993) allowing for high visual acuity (Petry *et al.*, 1984). In thalamus there is a well-laminated LGN, which provides the main relay of retinal inputs to V1. Tree shrews have six architectonically distinct layers (Figure 6b), which segregate input from each eye and contain functionally distinct classes of neurons

(see Conley *et al.*, 1984; Jain *et al.*, 1994; Kretz *et al.*, 1986; Lyon *et al.*, 2003b; Wong-Riley and Norton, 1988). It has been suggested that the LGNs of the flying lemur and flying fox each have as many as six layers as well (see Kaas and Preuss, 1993), though only three layers are architectonically distinguishable in the flying fox (Ichida *et al.*, 2000; Manger and Rosa, 2005) and only Nissl stained sections, less than ideal for determining LGN layers, have been examined for the flying lemur (Kaas and Preuss, 1993). Thus, the similarity between the LGN of these species is uncertain. Furthermore, though these species show a relatively high number of geniculate layers compared to other species such as rodents, it has been argued that this feature is easy to evolve and has appeared several times through convergent evolution in distantly related species (Striedter, 2005).

As for most mammalian species, flying foxes and tree shrews have areas V1 and V2 that are clearly defined (Figure 5). Like the squirrel, these areas are fairly large, contain a retinotopic map of the contralateral hemifield (Kaas *et al.*, 1972b; Rosa *et al.*, 1993, 1994), and are easily distinguished through CO or myelin staining (Lyon *et al.*, 1998; Rosa *et al.*, 1994). While the LGN projects primarily to V1 in tree shrew, it provides a topographic but diffuse input to V2 (Lyon *et al.*, 2003b). A similar connection pattern appears to be present in the flying fox as well (Manger and Rosa, 2005). More detailed analysis of the tree shrew has also revealed several levels of modularity within V1 and V2 (Bosking *et al.*, 1997; Lund *et al.*, 1985; Lyon *et al.*, 1998), and these are described in Section 34.4.2.

Anterior to V2 in the flying fox, detailed microelectrode recordings revealed several fairly complete retinotopic maps (Figure 5c; Rosa, 1999). A V3 was identified adjacent to V2, as a narrower and shorter band of cortex representing a compressed mirror image of the retinotopic map of V2. Anterior to V3, two more retinotopically organized areas, the occipital temporal (OT) and occipital parietal (OP), were also identified. Anterior to OT was a narrow strip of cortex that contained visually responsive neurons. Because these neurons had large receptive field sizes, no retinotopic order was apparent. Anterior to OP, in the posterior parietal cortex (PP), just posterior to somatosensory areas S1 and S, neurons were responsive to both vision and touch. Uncharted cortex (?) ventral to OT and posterior to auditory cortex could also be part of visual cortex, as is the case for this region of cortex in squirrels (see previous section) and tree shrew (see below). In all, at least seven visual areas and visually responsive regions have been identified in the flying fox.

In tree shrews, injections of different, distinguishable tracers into different retinotopic locations of V1 and V2 (see Figure 7b) revealed several extrastriate visual areas lateral to V2 (Figure 5b; Kaas, 2002; Lyon *et al.*, 1998; Sesma *et al.*, 1984). In tree shrews, rather than a single V3 strip extending along the lateral border of V2, the existence of three separate areas has been proposed – the temporal anterior, dorsal, and posterior areas (TA, TD, and TP). The largest of the three areas, TD, is the most V3-like, in that it extends across much of the V2 border, and has retinotopic connections with areas V1 and V2. Areas TA and TP are found at the medial–anterior and lateral–posterior ends of TD. TA receives retinotopic inputs from V2, and interconnects with areas TD and TP. Likewise, TP receives crudely retinotopic projections from V2 and connects with TD and TA. The retinotopic pattern of connections with V2 and interconnections between areas TA, TD, and TP strongly support the proposal that a single, large V3 is not present in tree shrews (Lyon *et al.*, 1998). This conclusion may represent a divergence from the common mammalian plan wherein several species have an area resembling V3 (Rosa, 1999). Or, it may reflect the proposal that V3-like visual areas have evolved separately and are not homologous (Kaas, 2002). One suggestion is that a narrow strip more anterior in the temporal cortex, just adjacent to V2, could be homologous to a V3 (Rosa, 1999); however, retinotopic microelectrode maps of this region remain to be done. Alternatively, perhaps TD is homologous to V3, in that it is not unlike the proportions of V3 in the flying fox (Rosa, 1999) and compared to V2 it contains a condensed retinotopic map (Lyon *et al.*, 1998).

At least three more visual areas have been identified in cortex more anterior to TA, TD, and TP (Lyon *et al.*, 1998, 2003b). The temporal inferior area (TI) situated anterior–lateral to TD can be distinguished architectonically through staining for myelin and connections with TP. Interestingly, tree shrew TI is in a similar location to squirrel TP (see Figure 5) which also has some visual connections and stains darkly for myelin. Another extrastriate area in the tree shrew, TPI, lies lateral and inferior to TP with which, like TI, it is primarily connected. Lateral to TA, there is the temporal anterior lateral area (TAL) which receives input from both visual and somatosensory thalamus (Lyon *et al.*, 2003b) and is connected with visual and somatosensory cortex (Lyon *et al.*, 1998; Remple *et al.*, in press).

These bimodal anatomical inputs resemble the bimodal neuronal properties reported for OP of the flying fox (Rosa, 1999). In all, at least eight visual areas and regions have been identified in the tree shrew.

Both the tree shrew and flying fox also have an enlarged and subdivided pulvinar (Figure 6b; Luppino *et al.*, 1988; Lyon *et al.*, 2003a, 2003b; Manger and Rosa, 2005). This increase in pulvinar size is typically seen in species with a greater expanse of extrastriate visual cortex, including squirrels (Figure 6a; Robson and Hall, 1977). In addition, distinct subdivisions of the pulvinar project differently to regions of visual cortex, and evidence of these connection patterns is used as support for the existence of multiple extrastriate areas. In the flying fox, three architectonic subdivisions are discernible through CO staining, the lateral, intermediate, and medial (Pl, Pi, and Pm, respectively) (Manger and Rosa, 2005). Tracer injections across several areas in visual cortex, show that Pl, which is located adjacent to the LGN, projects strongest to V2, whereas subsequent subdivisions project more strongly to visual areas V3, OT, and OP, which are located progressively anterior in cortex (Manger and Rosa, 2005). In tree shrews, four pulvinar subdivisions have been identified through different connections to extrastriate cortex and through distinct architecture (Figure 6b; Lyon *et al.*, 2003a, 2003b). Adjacent to the LGN, is the largest, central subdivision, Pc, which projects topographically to V2 as well as to adjacent cortex in area TD. The ventral subdivision, Pv, stains darkly for the antibody to Cat-301, a marker for large diameter neurons, and projects topographically to V2 and TD, as well as to TA. The dorsal subdivision, Pd, stains darkly for acetylcholinesterase (AChe) and projects to posterior extrastriate areas TP and TPI. A posterior subdivision, Pp, projects exclusively to the most anterior extrastriate visual areas, TAL, TI, and also to TPI.

Despite the rather distant relationship between tree shrews and megachiropteran bats, these visually dependent species have a similar proportion of cortex devoted to vision, with a similar number of areas. Areas V1 and V2 are similar in size and retinotopic organization; there is a third visual area, V3 in the bat and TD in the tree shrew, that both have a compressed mirror image of the representation of the visual field in V2. Each species has two more visual areas that are retinotopically organized, OT and OP, in the bat, and TA and TP in the tree shrew. Additionally, a bimodal sensory zone, representing somatosensory and vision, is present in each species, OP in bat and TAL in tree shrew. While tree shrews are considered a good approximation of the primordial primate, the basic cortical organization seen in tree shrew has more in common with megabats, and even squirrels, than with extant primates. However, comparisons up to this point have only focused on the location, retinotopic organization, and connection patterns of visual areas. The modular organization within visual areas, both anatomical and functional, indicates that tree shrews are more primate-like than squirrel-like, as discussed below.

34.4.2 Building Levels of Complexity: Number of Areas and Functional Modularity in Tree Shrew and Squirrel Visual Cortex

While tree shrews are considered among the closest relatives to primates and interspecies comparisons can provide insights into the evolution of primate visual cortex, it is also useful to compare tree shrews to other species occupying a similar ecological niche (see Kaas, 2002). In the previous section, tree shrews were compared with the flying fox. These mammals shared many common organizational features of visual cortex. In addition, squirrels and tree shrews have many similarities in behavior, and in fact were considered squirrels by the local people in their native Southeast Asian habitat (see Martin, 1990). Both of these highly visual mammals have two types of cone photoreceptors in the retina allowing for some color vision (Jacobs *et al.*, 1980; Petry and Kelly, 1991), and have a high density of ganglion cell output (Kaas and Preuss, 1993). Furthermore, both species have a large and well-differentiated LGN (Figures 6a and 6b; Johnson *et al.*, 1998; Kaas, 2002), a large distinctive pulvinar (Figures 6a and 6b; Lyon *et al.*, 2003a), an unusually large SC (Kaas and Collins, 2001), and a moderate number of visual areas, seven or eight (Figures 5a and 5b). Finally, genetic studies have shown that tree shrews are more related to squirrels than bats (Figure 1; Murphy *et al.*, 2001).

An additional organizational component in mammals with expanded visual systems is that of anatomical and functional modules within early visual areas V1 and V2. At first glance, the organization of V1 and V2 between tree shrews and squirrels seems similar. In each species, the two areas can be identified through myelin staining and they have retinotopic maps that form mirror images. In addition, they show patchy intrinsic connection patterns. However, a closer examination reveals that the anatomical organization of visual cortex in tree shrews is much more elaborate than in squirrels (Bosking *et al.*, 1997; Fitzpatrick, 1996; Rockland and Lund, 1982; Rockland *et al.*, 1982;

Van Hooser *et al.*, 2005c). One distinguishing feature of tree shrews is the distinct ocular dominance layers of V1 (Hubel, 1975). These layers subdivide layer 4 and preserve the ocular segregation of the inputs from separate geniculate layers. While individual neurons in rodent visual cortex can receive relayed input primarily from one eye or the other, there is no evidence for structural preservation of ocular dominance. Ocular dominance is a prominent feature in ferrets, cats, and Old World monkeys, which take the shape of columns rather than layers. While ocular dominance is very distinctive in some highly visual species, it remains unclear whether a functional advantage can be attributed to these modules (Horton and Adams, 2005).

Anatomically, tree shrews and squirrels both exhibit long-range intrinsic connections (Figures 7a and 8a; Rockland and Lund, 1982; Kaas *et al.*, 1989). Yet, the pattern is patchy and more widespread in tree shrews (Figure 8a; Rockland and Lund, 1982; Rockland *et al.*, 1982; Lyon *et al.*, 1998; Van Hooser *et al.*, 2005c). Rockland and colleagues (1982) revealed several, fine 200μm wide bands of labeled cell bodies and axon terminations evenly distributed throughout a several millimeter wide region of V1 (up to 8mm) from a single tracer injection in V1. The size and periodicity of the bands resembled banding from 2-deoxyglucose (2-DG) uptake following full field visual stimulation of moving bars presented at a single orientation (Rockland *et al.*, 1982). In 2-DG experiments, radioactive glucose is introduced to the blood supply. Regions of the brain that are metabolically active will incorporate more of the 2-DG into the local neurons. Repetitively stimulating the visual field with bars of a single orientation will preferentially activate only those neurons that prefer that orientation. Thus, this experiment not only demonstrated that the tree shrew brain contains regularly distributed clusters of neurons preferring a similar orientation, it suggested that these regions are likely to be interconnected.

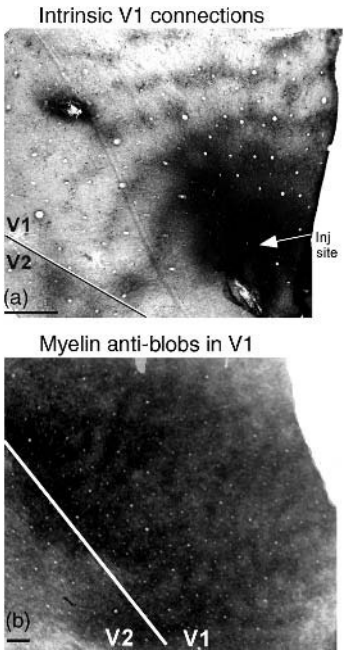

Figure 8 Anatomically defined modules in tree shrew V1. a, An injection of biotynilated dextran amine (BDA) reveals the intrinsic anterograde and retrograde connections of V1. Shown on a tangential section through cortical layer 3, a single injection resulted in a repeating pattern of regularly spaced clusters of labeled terminals and cell bodies within V1. b, Regularly spaced dense and light patches of myelin are also discernable across superficial cortical layers. Scale bar: 500μm. Reproduced from Lyon, D. C., Jain, N., and Kaas, J. H. 1998. Cortical connections of striate and extrastriate visual areas in tree shrews. *J. Comp. Neurol.* 401, 109 128, with permission from John Wiley & Sons.

Consistently with this prediction, recent studies using intrinsic signal optical imaging showed that the connections tend to link similar regions of cortex containing neurons that prefer similar orientations of a visual stimulus (Figure 9c; Bosking *et al.*, 1997). While presenting different oriented sinusoidal gratings to the tree shrew, intrinsic signals related to the neuronal activation were imaged optically and converted into a cortical map of orientation preference (Figures 9a and 9b). Similar maps are also a feature of primary visual cortex in ferret, cat, and all studied primates (see next section). When compared to patterns of connections from tracer injections placed into single orientation domains a high correlation between the location of axon terminals and similar preferred orientation was found (Bosking *et al.*, 1997). In contrast, recent work by Van Hooser *et al.* (2005a, 2005b) has shown that orientation domains are not present in the squirrel (Figure 10). In keeping with this observation, the intrinsic connectivity of squirrel V1 shows qualitatively only a limited patchy pattern (Figure 7a; Kaas *et al.*, 1989) and quantitatively this weak patchiness is not statistically significant (Van Hooser *et al.*, 2005c).

Other anatomical modules in tree shrew include the myelin dark 'anti-blobs' seen in V1 (Figure 8b; Lyon *et al.*, 1998) and the banding pattern of connections in V2 (Sesma *et al.*, 1984; Lyon *et al.*, 1998). While these features are not present in the squirrel, they are characteristics of primate V1 and V2. In tree shrew myelin patches are found in the superficial layers of V1. Such myelin patches have also been demonstrated in the superficial layers of primate V1, and they tend to occupy regions in-between CO blobs, hence the term 'anti-blob'. The functional significance of these myelin-dense patches remains to be determined, but axon

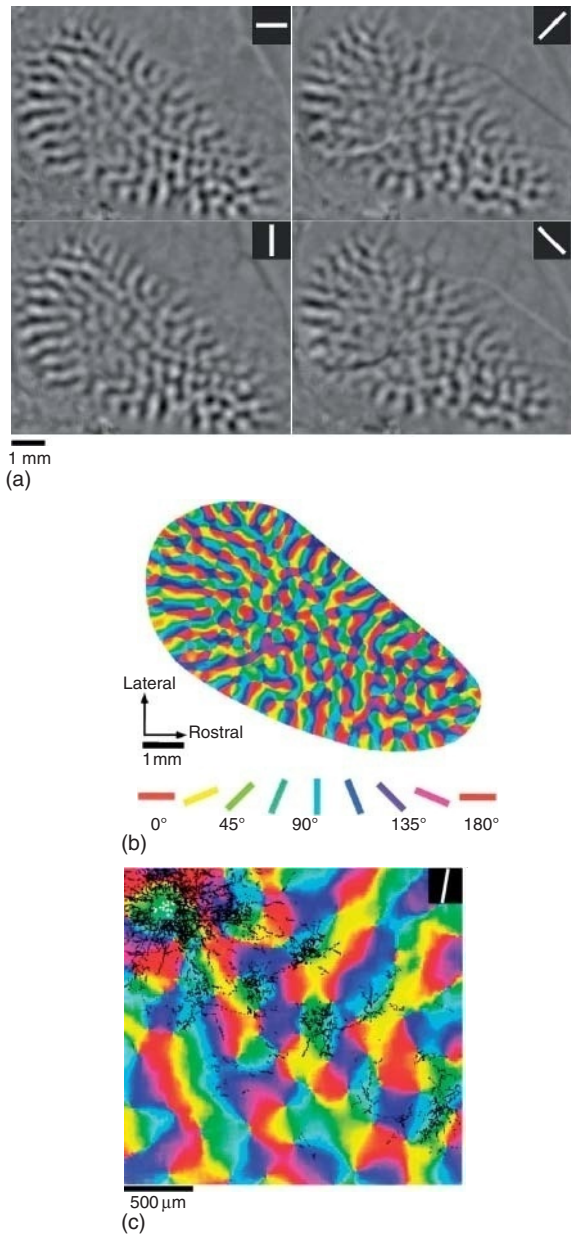

Figure 9 A regular pattern of functionally defined modules in tree shrew V1 can be revealed through intrinsic signal optical imaging. A major advantage of this technique is that it can simultaneously measure the responses of a large group of neurons over a wide area of cortex. a, Different populations of neurons are activated (black region) by a visual stimulus presented at one of four orientations (indicated in the upper right of each panel). Neurons preferring the same orientation are clustered together into modules, called orientation domains. b, A regular pattern of eight different orientation domains in V1 is apparent following stimulation with eight different orientated bars (below). The color-coding of the bars matches the color-coding of the domains. c, An anterograde neuronal tracer injected (white stipple) into a domain comprised of neurons preferring 45° (green) preferentially projects to like-domains. Reproduced from Bosking, W. H., Zhang, Y., Schofield, B., and Fitzpatrick, D. 1997. Orientation selectivity and the arrangement of horizontal connections in tree shrew striate cortex. *J. Neurosci.* 17, 2112 2127, copyright 1997 by the Society for Neuroscience, with permission.

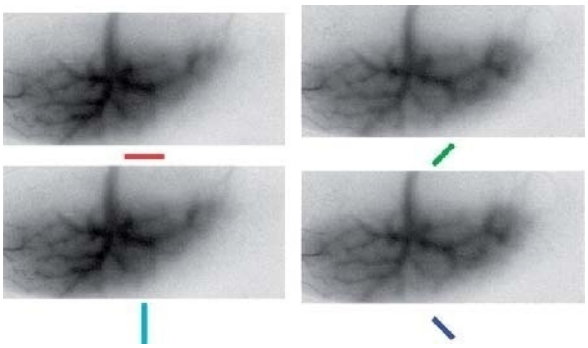

Figure 10 While intrinsic signal optical imaging of V1 in most highly visual mammals has revealed modular organization with respect to orientation processing (see Figure 9), orientation domains are not found in squirrels. These highly visual rodents, while having a comparable number of areas to tree shrews, exhibit less refinement of the internal structure of V1 and in this respect are similar to nonvisual rodents, such as mice and rats. Reproduced from Van Hooser, S. D., Heimel, J. A., Chung, S., Nelson, S. B., and Toth, L. J. 2005a. Orientation selectivity without orientation maps in visual cortex of a highly visual mammal. *J. Neurosci.* 25, 19-28, copyright 2005 by the Society for Neuroscience, with permission.

myelination is a key factor in increased conductivity speed so perhaps these clusters subserve some form of faster processing in V1.

The banding pattern in tree shrew V2 has been revealed through bidirectional tracers injected into V1 and V2. Terminals and cell bodies in V2 labeled by V1 tracer injections were arranged into several bands about 250–300 µm thick that extended from the V1/V2 border to the outer V2/TD border (Sesma *et al.*, 1984). Similar, but more constricted banding was observed following some individual V2 tracer injections, with the bands appearing up to 3 mm from the injection (Lyon *et al.*, 1998). These bands may be similar to bands found in primate V2 (see next section).

While tree shrews and squirrels have in common a moderate number of visual areas, the level of complexity in modular organization is very different. Functional and architectonic modular organization has not been demonstrated in V1 of squirrels, and the organization appears no different than V1 of mice and rats. In contrast, tree shrew V1 is highly modular and resembles many features found in V1 of carnivores and primates. Additionally, tree shrew V2 contains some of the modular anatomical features seen in primates. While the similarity in V1 and V2 between tree shrews and primates can be used to bolster their status as a close primate relative, the lack of modular organization in squirrels (Van Hooser *et al.*, 2005a, 2005b, 2005c) refutes the generally held belief that modularity and expanded visual systems come hand in hand. It may come as a

surprise that squirrels, with a relatively expansive visual system, lack modular organization, but, differences in modular organization in visual cortex between squirrels and tree shrews present an opportunity to explore the functional contributions of modular organization in otherwise similar complex systems.

34.5 Highly Complex Visual Systems in Carnivores and Primates

In contrast to the available data on the cortical organization of insectivores, rodents, bats, and tree shrews, the reported literature for cats and monkeys is vast, and includes detailed studies of functional characteristics of neurons while the animals are unanesthetized and performing visual tasks. The number of proposed visual areas in each taxa ranges from 15–19 in cats (Kaas and Krubitzer, 1991; Sereno and Allman, 1991; Payne, 1993) to 25 or more in monkeys (Kaas, 1997a; Van Essen, 2004; Rosa and Tweedale, 2005). While there is widespread agreement that monkeys and cats have a large number of visual areas, there has been less agreement as to each area's name, exact location, function, and even its very existence. In the first three sections here we will sample the available evidence to form a general overview of the organization of visual cortex in cats and ferrets, prosimian primates, and monkeys. In the subsequent sections, a more detailed description of certain cortical areas defined in primates – V1, V2, V3, and middle temporal (MT) – will provide the basis for comparisons with similar areas described in carnivores and other mammals.

34.5.1 Cat and Ferret Visual System

The cat as a model system for the study of visual cortex came into prominence through the early experiments of Hubel and Wiesel (1962, 1965, 1998). This seminal work was instrumental in developing an understanding of the receptive field properties of individual neurons in early cortical areas, identified as areas 17, 18, and 19 (but referred to here as areas V1, V2, and V3; see Figure 11). These three areas were considered to represent serial steps in a hierarchical progression where cells found early in the system were characterized as 'simple' and led to 'complex' and 'hypercomplex' cells at subsequent stages in visual cortex (see Payne and Peters, 2002). Though this description is oversimplified and there is a lot of mixing of simple and complex cells at early and later stages in the hierarchy, this organizational scheme set the stage for how mammalian visual cortex is viewed today, namely that visual processing becomes increasingly complex as it progresses across subsequent areas in visual cortex (see Bullier, 2004; Gross, 1997; Salin and Bullier, 1995).

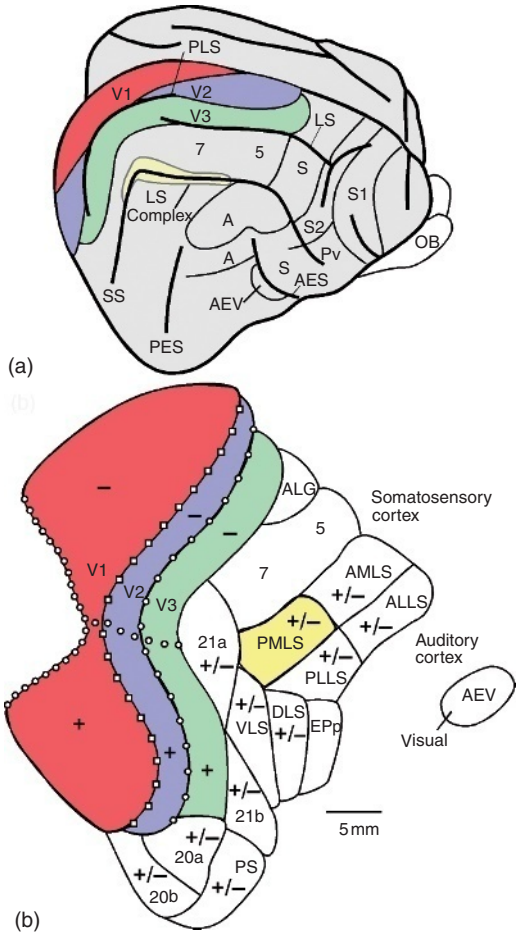

Figure 11 The organization of visual cortex in cats. a, Prominent visual areas, V1, V2, V3, and the LS complex (lateral suprasylvian) are shown relative to sulci, other visually responsive areas (5, 7, and anterior ectosylvian visual area (AEV)), and several nonvisual areas on the surface of an intact cortical hemisphere. Visually responsive areas occupy approximately half of the entire cortical surface. Anterior ectosylvian sulcus (AES); lateral sulcus (LS); posterior ectosylvian sulcus (PES); posterior lateral sulcus (PLS); suprasylvian sulcus (SS). b, A flattened cortical view of the relative sizes and locations of cat visual areas. Of the 19 visual areas shown, many are retinotopically organized (see schematic in Figure 4), though several of the higher-order areas contain incomplete representations of the contralateral visual field (see Kaas and Krubitzer, 1991). V1 (red) and V2 (blue) are considered homologous to early cortical areas found in most mammals. However, whether V3 (green) and PMLS (yellow) can be considered homologous to visual areas in other mammals, in particular areas V3 and MT in primates, remains a matter of debate (see Sections 34.5.5 and 34.5.6). See Section 34.5.1 for further description of cat visual areas and abbreviations. a, Based on Kaas and Krubitzer (1991) and Sereno and Allman (1991). b, Based on Sereno and Allman (1991) and Payne (1993).

As we have seen throughout this article, the earliest cortical stage in the visual system of mammals is V1, as it is the main recipient of the geniculate relay of retinal information. In addition to V1, cat V2 also receives direct projections from the LGN. While V2 in most mammals receives input from the LGN, the projections are less dense than those in cats, especially in other highly visual mammals, such as tree shrews (see Lyon *et al*., 2003b) and primates (Benevento and Yoshida, 1981; Bullier and Kennedy, 1983) – and somewhat less pronounced in the flying fox (Manger and Rosa, 2005). In cats, a large proportion of geniculate projections, stemming primarily from the Y-type ganglion cells (see Section 34.5.4.1), go directly to V2 (Stone *et al*., 1979). In this sense, V2 can be considered primary-like, an issue considered in great detail by Payne and Peters (2002). The specific projections of the Y cells directly to V2, provides a nonduplicative primary role for the region. As a result, cell properties in V2 reflect the fast conducting, transient response properties of the Y-cell inputs, whereas many of the V1 cells reflect the slower latency and sustained firing properties of the X-cell inputs. The lack of distinction between primary–secondary is also reflected in the myeloarchitecture of both cat and ferret cortex where, particularly in flattened preparations, there is no major differences between V1 and V2 at the border (Matsubara and Boyd, 2002; Olavarria and Van Sluyters, 1985; unpublished observations). Furthermore, CO blobs, a hallmark of primate V1 (Carroll and Wong-Riley, 1984; Horton and Hubel, 1981), are distributed evenly throughout V1 and V2 in the cat (Matsubara and Boyd, 2002).

Despite certain histological similarities between V1 and V2 in cats, differences in retinotopic organization can be used to reliably delineate the two areas. Through retinotopic microelectrode recordings pioneered by Hubel and Wiesel (1965), a stepwise progression from V1 to V2 revealed that these areas were split by the representation of the vertical meridian, whereas V2 and V3 were separated by the representation of the horizontal meridian. Subsequent studies exploring the retinotopic organization and connectional patterns of cat visual cortex revealed the size and extent of areas V1–V3 (see Figure 11; Tusa *et al*., 1978, 1979). As seen in tree shrew, bat, and squirrel visual cortex, nearly the entire laterorostral extent of V1 is bordered by V2. However, unlike these other species, cat V3 is substantially larger as it is nearly coextensive with the outer border of V2. As for other mammals, the lower visual field is represented dorsally, whereas the upper visual field is represented ventrally. One prominent feature of the retinotopy in these early areas in cats, which is even more pronounced in monkeys, is the greater representation through cortical magnification of the central visual field (see figure 1 in Payne, 1993).

In comparison to the mammals covered earlier in this article, cat cortex is quite expansive, and subsequently in this highly visual species there is room for a larger number of visual areas. Due to the overwhelming number of studies on the retinotopy of these areas and their interconnections, the focus here will be on presenting an overview of the relative locations of each area, while subsequent sections will highlight some of the characteristics of a few of these areas (for extensive reviews of extrastriate cortex in cat, see Payne, 1993; Payne and Peters, 2002; Rosenquist, 1985; Sereno and Allman, 1991; Tusa *et al*., 1981). Figure 11b, adapted and modified from Sereno and Allman (1991) and Payne (1993) shows 19 total visual areas on an unfolded, or flattened, cortical sheet. Beyond V3, as for the moderately complex visual systems in other species, the areas are substantially smaller, and contain complete or nearly complete representations of the contralateral visual hemifield. The relative locations of these areas to the two main sulci, the lateral and suprasylvian, are shown in Figure 11a. Naming of many of the extrastriate visual areas is based on their relative locations along or near one of these sulci. For example, dorsally, just adjacent to the peripheral lower field representation of V3, lies an area along the anterior lateral gyrus, area ALG (Symonds and Rosenquist, 1984). Other areas have been named as an extension of the alternative numbering scheme adapted from Brodmann (1909) that refers to areas V1–V3 as areas 17, 18 and 19 (see Payne and Peters, 2002). These areas, 20 and 21, are found adjacent to the middle and ventral parts of area 19 (V3), and have been split into two areas each – 20a, 20b, 21a, and 21b (Rosenquist, 1985; Tusa and Palmer, 1980). However, the liberal subdividing of these regions results in areas with only incomplete representations of the contralateral visual hemifield (see Kaas and Krubitzer, 1991). Just anterior to the two ventral areas, 20a and 20b, is the posterior suprasylvian area, PS, which also contains an incomplete retinotopic map in that it primarily represents the lower field (Updyke, 1986). While ALG, is among the smallest extrastriate areas described in cats, areas 5 and 7, which are also named based on similarities to architectonically defined regions of Brodmann (1909), are among the largest (after V2 and V3). Anterior to the dorsal portion of V3, in a similar region of posterior parietal cortex as visual areas reported in the flying fox (OP) and tree shrew (TAL; see Section 34.4.1), areas

5 and 7 have only crude retinotopic organizations and contain bimodal neurons responding to visual and somatosensory stimulation (see Manger *et al.*, 2002b). Ventrally in the lateral suprasylvian sulcus is the aptly named area VLS, situated somewhat between areas 21a and 21b. On the opposite, dorsal bank of the suprasylvian sulcus, VLS is bordered by area DLS. While there is some evidence for retinotopic organization of VLS and DLS, area EPp, immediately anterior to DLS, is not retinotopically organized (see Payne, 1993).

Just medial to VLS and DLS, but still along the lateral suprasylvian sulcus there is a large complex of visual areas that roughly corresponds in location to the Clare–Bishop area, a region first identified as visually responsive over 50 years ago (Clare and Bishop, 1954). This complex has been split into as few as two areas (see Sherk, 1986a, 1986b; Shipp and Grant, 1991) and as many as four regions as portrayed in Figure 11 (see Rosenquist, 1985). The four areas are named PMLS, AMLS, PLLS, and ALLS, based on their posterior (P) or anterior (A) locations on the medial (M) and lateral (L) banks of the lateral suprasylvian (LS) sulcus. This region of extrastriate cortex, particularly PMLS, has been studied in detail and comparisons have been made to the direction selective MT, or V5, of primates (see Grant and Hilgetag, 2005; Payne, 1993; also see Section 34.5.6). Lastly, the anterior ectosylvian visual area (AEV), located several millimeters anterior to the nearest visual area is unique in that it sits surrounded by cortical areas responsive to modalities other than vision (see Sereno and Allman, 1991). Perhaps because of its unusual isolation, it is helpful to include visual as part of its name.

The cat also contains a rather large visual pulvinar complex in the thalamus (Figure 6c), as we have seen for other visual species. The large size of the cat pulvinar, or lateral posterior-pulvinar complex, reflects the expansiveness of extrastriate visual cortex with which it is extensively interconnected. The LP-pulvinar can be divided into subdivisions of the lateral posterior region including lateral (LPl), intermediate (LPi) and medial (LPm), and the pulvinar-proper (e.g., Hutchins and Updyke, 1989), based on connection patterns with visual cortex and differences in architecture (see Lyon *et al.*, 2003b; Casanova, 2004). The lateral subdivision of the lateral posterior region, LPl, is the only subdivision interconnected with early visual areas V1 and V2. It also connects with the complex of areas in and around the PMLS region, and areas 19, 20, and 21. The medial division, LPm (sometimes considered the inferior subdivision, LPi), also connects with areas 19–21, the PMLS region, as well as posterior parietal areas 5 and 7, and AEV. Likewise the pulvinar-proper connects to the same areas as LPm, but does not connect with AEV. LPi has been distinguished architectonically from LPm as staining darkly for both substance P (Hutsler and Chalupa, 1991) – a marker associated with SC inputs – and AChE (Graybiel and Berson, 1980). This dark staining for AChE is similar to the dorsal subdivision of the tree shrew pulvinar (Figure 6b; see Lyon *et al.*, 2003a).

Other than the multiple areas forming the PMLS complex, the majority of the extrastriate areas in cat, have been identified in their mustelan cousin, the ferret, in a series of retinotopic mapping experiments by Manger and colleagues (Innocenti *et al.*, 2002; Manger *et al.*, 2002a, 2002b, 2004). However, it has been suggested that areas similar to those found in the cat PMLS complex are also present in ferrets (Manger, 2005), so the two species appear to share the same number of cortical visual areas. Because there is a rather substantial overall difference in brain size between the two species, it is perhaps surprising that the number of extrastriate visual areas is comparable. Manger (2005) has used these observations as support for the idea that, within orders, there is little interspecies variability in cortical organization. However, we have already seen substantial differences between squirrels and smaller, less visual rodents (see Section 34.3). Yet, rodent species vary significantly in their emphasis on vision, and it can be argued that the entire carnivore order is highly visual, as visual cortex is also large in other carnivore species such as the mink (see McConnell and LeVay, 1986). Perhaps, this is what drives the similarity in visual cortex organization in carnivores. Another issue to consider is that V1 and V2 in both cats and ferrets is highly modular as revealed through connectivity patterns and optical imaging (Gilbert and Wiesel, 1983; Kisvarday *et al.*, 1997; Weliky *et al.*, 1996; White *et al.*, 1999). Yet, one of the more striking anatomical modules found in cats and primates, the CO blobs in V1, are not present in ferret V1. Perhaps at this level, cats and ferrets differ in complexity as differences in V1 modular organization exist between tree shrews and squirrels as well (see Section 34.4.2).

34.5.2 Prosimian Visual System

Prosimian primates – lemurs and lorises – having split over 50 Mya from other primates are considered to have retained many early primate features (see Fleagle, 1988). Thus, understanding prosimian cortical organization provides insights into the organization that may have been present in the earliest primates. Only a few prosimian species have been

studied, with the galago or bush baby (*Otolemur garnetti*) examined in the most detail. As we have seen for the mammals covered previously in this review, V1 is easily distinguishable as a large, densely myelinated region in the caudal-most portion of the prosimian cortex. As in cats, staining for the metabolic enzyme, CO, reveals regularly distributed blobs throughout V1 (Casagrande and Kaas, 1994). Earlier reports found a lack of CO blobs in certain prosimian species (see Sereno and Allman, 1991). Because CO blobs are present in all studied monkeys and apes, but not in every prosimian species, this left open the possibility that blobs evolved independently in monkeys and prosimians (see Preuss and Kaas, 1996). However, the absence of CO blobs in earlier preparations may have been due to technical difficulties, as subsequent studies demonstrated that CO blobs are a common feature in prosimian V1 (Preuss and Kaas, 1996).

In galagos, retinotopic maps of V1 show an orderly representation of the contralateral visual hemifield, with a larger portion of cortex representing central vision (Rosa *et al.*, 1997). In addition, galago V1 exhibits intrinsic patchy connectivity (Cusick and Kaas, 1988b; Preuss *et al.*, 1993; Lyon and Kaas, 2002c) and contains an orderly orientation preference map (Xu *et al.*, 2005). Immediately adjacent to V1 lies V2 (see Figure 12), which can be identified through myeloarchitecture as well (Krubitzer and Kaas, 1990; Collins *et al.*, 2001; Lyon and Kaas, 2002c). Galago V2 resembles V2 in other species as it represents a compressed, rough mirror image of the representation of the visual field in V1 (Rosa *et al.*, 1997), and receives feedforward inputs from and sends feedback to V1 (Collins *et al.*, 2001; Symonds and Kaas, 1978; Tigges *et al.*, 1973).

A second extrastriate cortical area common to all primates is the MT area (see Kaas and Lyon, 2001). First described in New World monkeys (Allman and Kaas, 1971b), homologous area was subsequently identified in prosimians using similar criteria (Allman *et al.*, 1973; Tigges *et al.*, 1973; Symonds and Kaas, 1978). Whether a homologous area is present in other mammals is a matter of debate (see Section 34.5.6; Payne, 1993; Kaas, 2002; Rosa and Tweedale, 2005).

In prosimians, as many as 12 more extrastriate visual areas (Figure 12) are discernable through feedback connections to V1 (Cusick and Kaas, 1988b; Krubitzer and Kaas, 1993; Lyon and Kaas, 2002a; Preuss *et al.*, 1993), V2 (Collins *et al.*, 2001), and MT (Krubitzer and Kaas, 1990). One of these 12 extrastriate areas, V3, had not been identified in prosimians (Allman *et al.*, 1979; Beck and Kaas, 1998a; Collins *et al.*, 2001; Rosa *et al.*, 1997) until recently, when several tracer injections placed in estimated retinotopic locations of V1 proved instrumental in dividing extrastriate visual cortex of the galago (Lyon and Kaas, 2002a). As a result, for example, the dorsal medial area, DM, originally identified through microelectrode mapping of retinotopy (Allman *et al.*, 1979, Rosa *et al.*, 1997), has

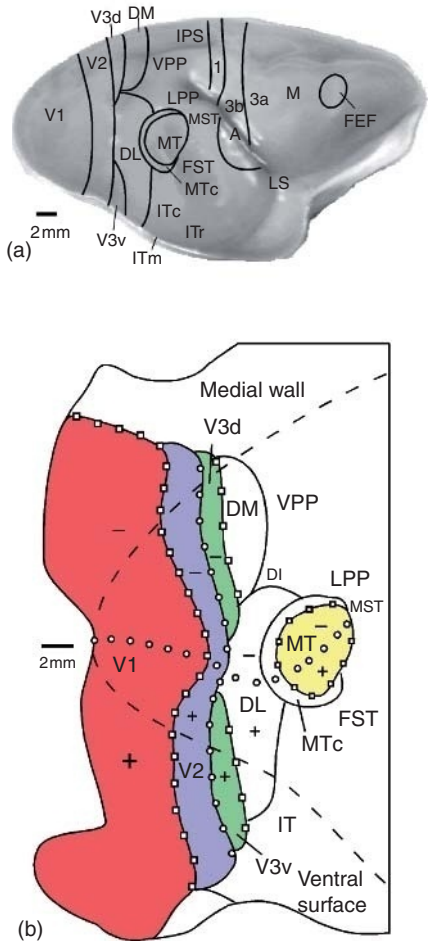

Figure 12 The organization of visual cortex in prosimian primates. a, The locations of several visual and nonvisual areas are shown on a digital photograph of the cortical surface of an intact galago brain. The intraparietal (IPS) and lateral (LS) sulci are clearly visible, while the superior temporal sulcus is present as a slight dimple ventral to MT. b, A flattened cortical view of the relative sizes and locations of galago visual areas. V1 (red) and V2 (blue) are likely homologous to similar areas present in other mammals, while primate areas V3 (green) and MT (yellow) may be unique to the primate order. The homology of primate V3 and MT to areas present in nonprimates is a matter of debate (see Sections 34.5.5 and 34.5.6). Nevertheless, the existence of V3 and MT, as well as several other visual areas (DL, DM, DI, MTc, MST, IT) in prosimian primates indicates that these areas were present early in primate evolution. For abbreviations and detailed descriptions of galago visual areas see Section 34.5.2. a and b, Modified from Lyon, D. C. and Kaas, J. H. 2002a. Connectional evidence for dorsal and ventral V3, and other extrastriate areas in the prosimian primate, *Galago garnetti*. *Brain Behav. Evol.* 59, 114 129.

been slightly displaced by V3 from its original location adjacent to the anterior border of dorsal V2 (see Figure 12; Lyon and Kaas, 2002a). Anterior to DM and dorsal to MT, at least two more areas have been identified the lateral and ventral areas of posterior parietal cortex, LPP and VPP (Beck and Kaas, 1998a; Lyon and Kaas, 2002a). Ventral to DM, along the anterior border of the ventral two-thirds of V3 is the dorsal lateral visual area, DL (Cusick and Kaas, 1988b), a possible homologue of macaque monkey V4 (see Stepniewska *et al.*, 2005). Some studies suggest that prosimian DL can be split into caudal and rostral subdivisions as proposed for both New and Old World monkeys (Cusick and Kaas, 1988a; Lyon and Kaas, 2002c; Stepniewska *et al.*, 2005). Anterior to the ventral half of DL, at least three areas in inferior temporal cortex (IT) can be distinguished based on clusters of labeled cells in different relative locations – the caudal (ITc), medial (ITm), and rostral (ITr) areas, as described for New World monkeys (Weller and Kaas, 1987). Surrounding MT, three more areas, the MT crescent (MTc), the area of the fundus of the superior temporal sulcus (FST), and the medial superior temporal area (MST) are also distinguishable.

Some of the available evidence suggests that galagos and perhaps prosimians in general represent a scaled-down version of the organization of monkey visual cortex (see Kaas, 2004a, 2004b). For example, monkey V1 is larger and contains a greater representation of central vision (Van Essen *et al.*, 1984; Gattass *et al.*, 1987; Rosa *et al.*, 1997). Furthermore, while both monkeys and prosimians have CO blobs in V1, the monkey – but not galago – V2 also contains distinct cytoarchitectonic bands (see Sections 34.5.3 and 34.5.4; however, see Preuss and Kaas, 1996). In addition, the pulvinar nucleus, which is a distributor of visual information to all of visual cortex, is larger in monkeys than in prosimians and can be divided into several subcompartments based on architectonic criteria and cortical projection patterns (Beck and Kaas, 1998b; Stepniewska, 2003). Despite these differences, the number and locations of visual areas described for prosimians is very similar to those described in monkeys (see Figures 12 and 13; Kaas, 1997a, 2004a, 2004b; Kaas and Lyon, 2001; Van Essen, 2004; Rosa and Tweedale, 2005).

34.5.3 New World and Old World Monkey Visual Systems

Several species of New World monkeys have been examined from the very small 300g marmoset monkey and moderately sized 1kg squirrel monkey, to the large, macaque-sized cebus monkey. Various species of the macaque monkey genus have been the Old World monkey of choice. Weighing as much as 15kg and boasting a brain size as large as 10 times that of the tiny marmoset (80g vs. 8g; see Rosa and Tweedale, 2005), macaques are used as the primary model for comparisons to the human visual system (Brewer *et al.*, 2002; Fize *et al.*, 2003; Orban *et al.*, 2004; Sereno and Tootell, 2005; Tootell *et al.*, 2003; Van Essen, 2004). Emphasis on the macaque monkey as a human model is due in part to historical factors and the ready availability of this species (Rosa and Tweedale, 2005). Yet, despite the size differences and the 10 million years of evolution that separate the New World and Old World monkey lineages, several studies indicate that the organization of their visual cortex is quite similar (Casagrande and Kaas, 1994; Lyon and Kaas, 2002b, 2002c; Lyon *et al.*, 2002; Rosa and Tweedale, 2005). This is particularly true for the early, caudal visual areas that have been studied in more detail in both New and Old World species (see Kaas and Lyon, 2001; Kaas, 2004a). Therefore, this section will review studies on visual cortex organization in both New and Old World monkeys.

Monkey visual areas V1 and V2 are well established as the two largest visual areas in primate cortex (see Figures 13 and 14). V1 and V2 are found at the caudal end of cortex and are likely homologous to V1 and V2 of all mammals (see Sections 34.2 and 34.5.4). Of all the remaining extrastriate visual areas proposed to exist in monkeys, area MT (see Section 34.5.6) is the only one that is unanimously accepted (see Felleman and Van Essen, 1991; Kaas and Lyon, 2001). MT, also referred to as V5, was first described as a densely myelinated retinotopically organized region located several millimeters anterior to V2 in the middle temporal lobe of New World owl monkeys (Allman and Kaas, 1971b) and as a region on the posterior bank of the superior temporal sulcus (STS) in macaque monkeys that received direct projections from V1 (Zeki, 1971). Subsequently, MT of macaques was also shown to be retinotopically organized (Gattass and Gross, 1981; Van Essen *et al.*, 1981). Additional work demonstrated that MT is likely to be a common primate feature (see Kaas and Lyon, 2001; Kaas, 2004a). Whether primate MT evolved from an area common to nonprimates is a matter of debate (Kaas, 2002; Rosa and Tweedale, 2005; see Section 34.5.6.1). Studies on the functional properties of MT show that it plays a major role in the processing of binocular

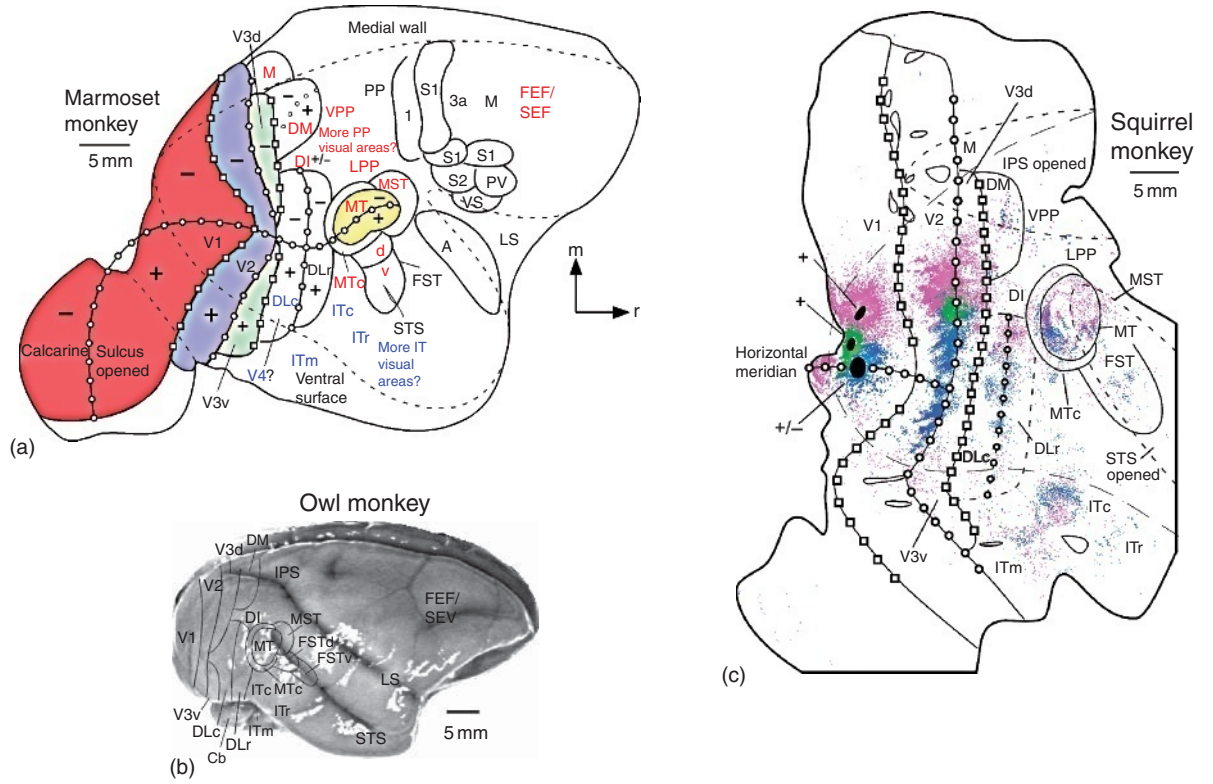

Figure 13 The organization of visual cortex in New World monkeys. a, The locations and sizes of visual areas in New World monkeys are shown on a flattened sheet of an entire cortical hemisphere from a marmoset monkey. Like other highly visual species V1 (red shading) and V2 (blue shading) are quite large, and retinotopically organized (see schematic in Figure 4a). V3 (green shading) is present as two distinct dorsal (d) and ventral (v) divisions, each representing a compressed mirror image of the retinotopic organization in dorsal and ventral portions of V2. Several more retinotopic areas (DLc, DLr, DI, DM, and MT) are present in extrastriate cortex (indicated by +/). Areas lettered in red, fed predominantly through feed-forward projections from direction selective area MT, are thought to comprise the dorsal stream of visual processing related to motion and spatial processing, whereas areas lettered in blue, fed predominantly by V4 (Dlc/DLr), are related to the processing of color and form (see Section 34.5.3). SEF, supplementary eye field; FEF, frontal eye field. b, The positions of several areas relative to the sulcus pattern on the cortical surface are shown on a digital photograph of an owl monkey brain. Intraparietal sulcus (IPS), lateral sulcus (ILS), superior temporal sulcus (STS). c, The pattern of retrogradely labeled neurons following injections (black ovals) of three tracers into different retinotopic locations of V1 in a single case is shown on a flattened reconstruction of the caudal half of squirrel monkey cortex. Colored dots represent individually labeled neurons from the corresponding tracer injection. An injection of fast blue (blue) was made on the representation of the horizontal meridian (line of circles, see schematic in Figure 4a), thus labeled neurons in extrastriate cortex were found near known locations of the horizontal meridian. For example, the horizontal meridian is located at the V2/V3 and DLc/DLr borders, as well as the caudal border of MT. Injections of diamidino yellow (green) and cholera toxin subunit-b (CTB; purple) were placed near the horizontal meridian, but slightly medial, in cortex representing progressively more peripheral vision. As a result, the labeled cells in extrastriate cortex from each of the two injections were located progressively more medial along the horizontal meridians at the V2/V3 and DLc/DLr borders. Interestingly compared to the smaller marmoset monkey V3 which is split into dorsal and ventral divisions (a), V3 in squirrel monkeys is a continuous strip. Additional retrogradely labeled neurons were found in several other cortical areas, indicating that a wide region of visual cortex provides feedback to V1. For abbreviations and detailed description of the organization of New World monkey visual areas see Section 34.5.3. a, Modified from Lyon, D. C. and Kaas, J. H. 2001. Connectional and architectonic evidence for dorsal and ventral V3, and dorsomedial area in marmoset monkeys. *J. Neurosci.* 21, 249 261. b, Modified from Lyon, D. C., Xu, X., Casagrande, V. A., Stefansic, J. D., Shima, D., and Kaas, J. H. 2002. Optical imaging reveals retinotopic organization of dorsal V3 in New World owl monkeys. *Proc. Natl. Acad. Sci. USA* 99, 15735 15742; Based on data from Lyon, D. C. and Kaas, J. H. 2002c. Evidence from V1 connections for both dorsal and ventral subdivisions of V3 in three species of New World monkeys. *J. Comp. Neurol.* 449, 281 297. c, Modified from Lyon, D. C. and Kaas, J. H. 2002c. Evidence from V1 connections for both dorsal and ventral subdivisions of V3 in three species of New World monkeys. *J. Comp. Neurol.* 449, 281 297, with permission from John Wiley and Sons.

disparity and the direction and speed of visual stimuli (Albright, 1984; Born and Bradley, 2005; Felleman and Kaas, 1984; Maunsell and Van Essen, 1983b), and is likely a primary source of input to the motion and spatial processing areas in the dorsal stream of visual processing (Maunsell and Van Essen, 1983a; Shipp and Zeki, 1995; Ungerleider and Desimone, 1986).

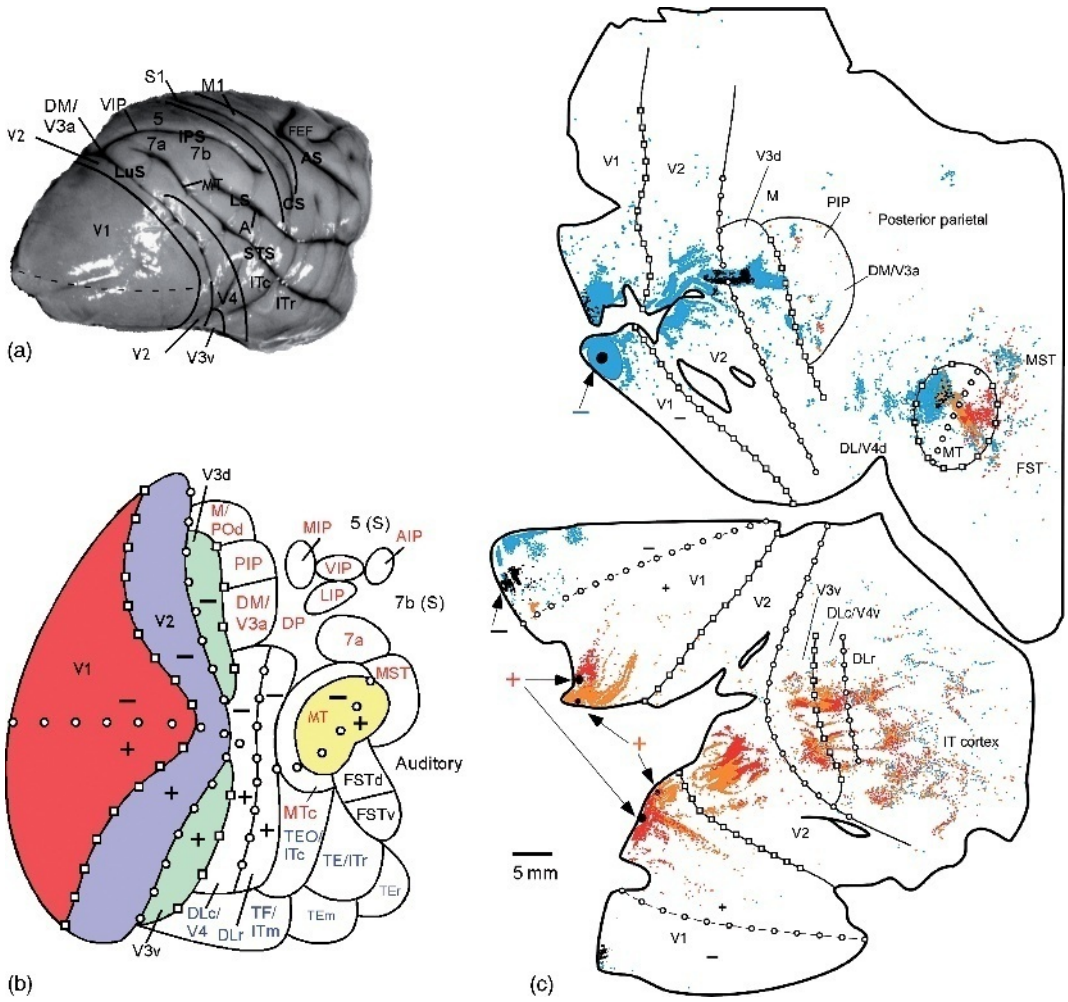

Figure 14 The organization of visual cortex in Old World macaque monkeys. a, The positions of several areas relative to the sulcus pattern on the cortical surface are shown on a digital photograph of a macaque monkey brain. The majority of V2, all of V3 and V3a, are buried within the lunate sulcus (LuS). Area MT and its satellites are buried within the dorsal extent of the superior temporal sulcus (STS), whereas much of the higher-order ventral stream areas in inferotemporal cortex (IT) are located within the ventral extent of the STS. Higher-order dorsal stream areas such as the ventral intraparietal area (VIP) are buried within the intraparietal sulcus (IPS). AS, arcuate sulcus; CS, central sulcus; FEF, frontal eye field. b, The relative locations and sizes of visual areas in macaque monkeys are shown on a flattened representation of the caudal half of cortex. Many visual areas reported for the macaque are likely homologous to those proposed for New World monkeys (see Figure 13), especially in the caudal extent of visual cortex, including areas V1, V2, V3, V4 (DLc/DLr), MT, MTc, MST, FSTd/v, DM (V3a), POd (M), DP (DI). However, several more areas have been described in the higher-order stations of the dorsal and ventral processing streams. It is uncertain whether these areas represent an expansion of visual cortex in Old World monkeys because (1) similar areas have been described in larger New World cebus monkeys (see Rosa and Tweedale, 2005) and (2) few studies have looked at the detailed organization of higher-order visual cortex in the more commonly studied smaller New World monkey species. TE, temporal cortex; TEm, middle subdivision of TE; TEr, rostral subdivision of TE; TEO, temporal occipital cortex. c, The pattern of retrogradely labeled neurons following injections (black ovals) of four tracers into different retinotopic locations of V1 in a single case is shown on a flattened reconstruction of the caudal half of macaque cortex. Colored dots represent individually labeled neurons from the corresponding tracer injection. Two injections were placed in the upper visual field representation in ventral V1, one (orange) nearer the representation of the vertical meridian (line of squares) found at the V1/V2 border, and the other nearer the representation of the horizontal meridian (line of circles; see schematic in Figure 4a). In adjacent ventral cortex, a series of mirror reversals of the retinotopic locations of the V1 injections was found in the pattern of retrogradely labeled cells in extrastriate areas V2, V3, DLc (V4), and DLr. A similar pattern of labeled cells resulted in dorsal visual cortex following two tracer injections in the lower visual field representation of dorsal V1. Retinotopic patterns of retrogradely labeled cells from the dorsal and ventral V1 injections were found in MT as well. A crude retinotopic pattern of connections with MST was also present. Labeled neurons in areas V3a and PIP were sparse, yet indicated a crude retinotopy. Few cells, if any, were labeled in PP, indicating that higher-order dorsal stream areas do not provide feedback to V1. In contrast, numerous cells were labeled in IT cortex (nonretinotopically), indicating that, like New World monkeys, higher-order ventral stream areas provide feedback to V1. For abbreviations and detailed description of the organization of macaque monkey visual areas see Sections 34.5.2 and 34.5.3. c, Modified from Lyon, D. C. and Kaas, J. H. 2002b. Evidence for a modified V3 with dorsal and ventral halves in macaque monkeys. *Neuron* 33, 453 461, with permission from Elsevier.

Probably the greatest factor contributing to the establishment of V1, V2, and MT as valid visual areas is that these are the only areas that can be reliably identified through architectonic criteria, such as stains for myelin and CO (see Figures 15 and 16). The numbers, locations, and sizes of the myriad of remaining, proposed extrastriate visual areas in monkey cortex remain uncertain. In some cases it is simply a difference in area size and terminology (DL vs. V4; see Stepniewska *et al.*, 2005), while in other cases it is a reported difference in connectivity and function (dorsal V3 vs. ventral V3; see Section 34.5.5). As a result, several different organizational schemes of monkey visual cortex have been proposed (see Kaas, 1997a; Rosa, 1997; Van Essen, 2004). Methods used to identify areas play a large role in the observed variability. For example, fine-scale methods such as microelectrode mapping have been used to demonstrate several retinotopically organized areas in the third tier of visual cortex (Allman and Kaas, 1975; Rosa and Schmid, 1995); conversely a single area, V3, is revealed through techniques capable of measuring activity of larger regions of cortex simultaneously, such as intrinsic signal optical imaging (Lyon *et al.*, 2002; Xu *et al.*, 2004) and fMRI (Brewer *et al.*, 2002; Fize *et al.*, 2003). While others have provided comparisons of the many proposals of monkey visual cortex organization (Kaas, 1997a; Rosa, 1997; Van Essen, 2004), the goal of this section is to present a composite representation (see Figures 13 and 14) of the various versions to allow for straightforward comparisons of New and Old World monkeys, and to provide a scheme that can be readily compared to those presented for other mammals.

Other than areas V1, V2, and MT, a conservative estimate of the number of proposed visual areas in monkeys is slightly greater than 20 (see Figures 13a and 14b). Areas early in the cortical hierarchy located more caudally have been identified through similar methodologies in both New and Old World monkeys. Retrograde tracing studies that focused on the feedback connectivity patterns of these areas to V1 have served as a useful means for comparison across species, revealing many similarities in organization (see Figures 13c and 14c). On the other hand, cortical areas located more distantly from V1, for example in PP, have been studied more extensively in macaque monkeys (see Andersen, 1995; Andersen *et al.*, 1985b; Colby and Duhamel, 1991; Lewis and Van Essen, 2000a, 2000b), making comparisons with New World monkeys more difficult.

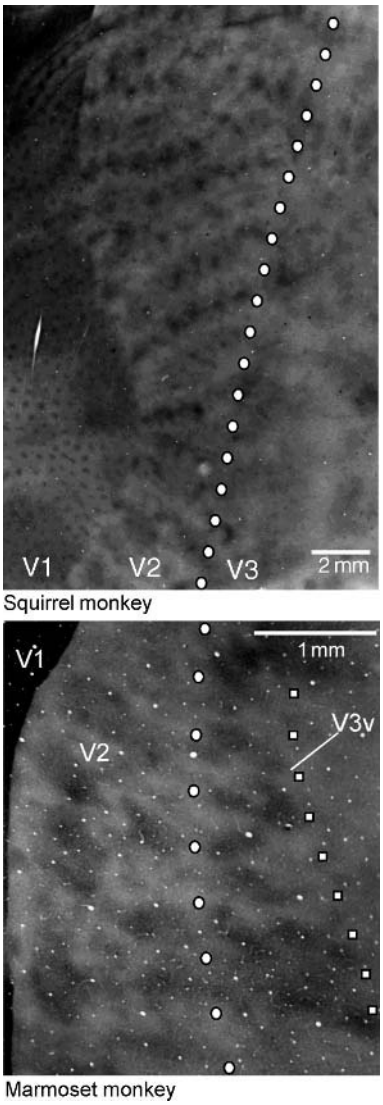

Figure 15 When processed for the presence of cytochrome oxidase (CO), cortex manually flattened and cut tangential to the cortical surface shows a regular pattern of dark (high concentration of CO) and light (low concentration of CO) modules in early visual areas of monkeys. As shown in squirrel monkey cortex (top panel), in V1, the dark CO patches form small, discrete blobs, whereas in V2 the blobs are strung together to form stripes, or bands. The V2 bands are characterized as either 'thick' or 'thin', and have been linked to the dorsal and ventral visual processing streams, respectively (see Section 34.5.4.1). Though less distinct than the bands in V2, dark CO bands are often visible in V3 (bottom panel). Whether these bands are associated to either of the visual processing streams remains to be determined, but it has been shown that band-like patches of labeled neurons become labeled in V3 following tracer injections into dorsal stream areas DM and MT (Lyon and Kaas, 2001). Squirrel monkey, reproduced from Lyon, D. C. and Kaas, J. H. 2002c. Evidence from V1 connections for both dorsal and ventral subdivisions of V3 in three species of New World monkeys. *J. Comp. Neurol.* 449, 281 297, with permission from John Wiley & Sons. Marmoset monkey, reproduced from Lyon, D. C. and Kaas, J. H. 2001. Connectional and architectonic evidence for dorsal and ventral V3, and dorsomedial area in marmoset monkeys. *J. Neurosci.* 21, 249 261, copyright 2001 by the Society for Neuroscience, with permission.

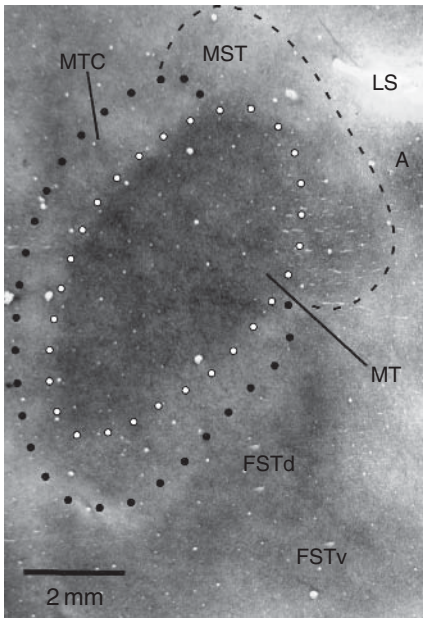

Figure 16 In flattened cortical preparations, dense myelination easily distinguishes primate area MT from surrounding cortical areas. Furthermore, in this section through the superficial layers of cortex dark and light patches within MT indicate a modular organization of dense and light myelination. In addition to differences in myelination, several forms of modular organization within MT have been revealed through a variety of techniques (see Sections 34.5.3 and 34.5.6 for more details of MT and surrounding cortical areas). Reproduced from Lyon, D. C. and Kaas, J. H. 2001. Connectional and architectonic evidence for dorsal and ventral V3, and dorsomedial area in marmoset monkeys. *J. Neurosci.* 21, 249 261, copyright 2001 by the Society for Neuroscience, with permission.

In addition to the identification of discrete cortical areas, primate visual cortex can be segregated into the dorsal and ventral streams of visual processing (see Figures 13a and 14b). The identification of these processing streams derives from lesion studies in cats and primates, including humans, revealing a segregation of function into separate cortical pathways (see Ungerleider and Mishkin, 1982; Ungerleider and Pasternak, 2004). Motion and spatial information are processed in a stream of neighboring visual areas found more dorsally within occipital and parietal cortex, the dorsal or parietal stream. Conversely, color and form processing is most pronounced in neighboring ventral regions within occipital and temporal cortex, the ventral or temporal stream. The higher-order areas comprising the two streams are fed in varying degrees by early caudal visual areas, V1, V2, V3, V4 and MT (Van Essen and De Yoe, 1995).

V3, considered a provider of inputs to both streams (see Gegenfurtner *et al.*, 1997), is located immediately adjacent to V2 as a narrower strip of cortex that was first identified through its connectivity with V1 (Cragg, 1969; Zeki, 1969). While a V3-like area has been identified in several other mammalian species as discussed throughout this article and elsewhere (see Rosa, 1999), the organization and even the very existence of primate V3 remains controversial (Kaas and Lyon, 2001; see Section 34.5.5). Monkey V3 has a retinotopic map representing a condensed mirror image of the visual field representation in V2 as revealed through microelectrode mapping (Gattass *et al.*, 1988), intrinsic signal optical imaging (Lyon *et al.*, 2002; Xu *et al.*, 2004), fMRI (Brewer *et al.*, 2002; Fize *et al.*, 2003), and connections with primary visual cortex (Lyon and Kaas, 2001, 2002b, 2002c). Importantly, V3 can be identified in flat mounted sections through myelo- and cytochrome architecture (Figure 15; Lyon and Kaas, 2001, 2002c; Sincich *et al.*, 2003; Xu *et al.*, 2004). In this regard, V3 joins the company of the three well-established areas – V1, V2, and MT. Though less reliably demonstrated, the CO architecture in V3 reveals modular light and dark bands. These bands are like those found in V2, yet thicker and less differentiated (Lyon and Kaas, 2001; Xu *et al.*, 2004). Connection patterns with well-established areas such as MT reveal a band-like pattern of modular organization in V3 as well (Lyon and Kaas, 2001).

Often associated with the ventral stream, V4, or DL, lies adjacent to V3. This region of cortex is less distinguishable through staining procedures, but has been identified principally through microelectrode mapping and connection patterns with well-established cortical areas (Gattass *et al.*, 1988; Piñon *et al.*, 1998; Stepniewska *et al.*, 2005). Issues as to the dorsal and ventral extents of DL/V4 remain unresolved. Some researchers have the area extending well onto the ventral cortical surface, adjacent to the entire extent of ventral V3 and even extending as far as the ventral extreme of V2 (Gattass *et al.*, 1988), whereas others have proposed a truncated version based on a change in connectivity patterns for tracers injected more ventrally (Stepniewska *et al.*, 2005). The ventral DL/V4 border illustrated in Figures 13 and 14 is intermediate to the proposals contrasted above. This border is derived from studies on the connection patterns from tracer injections placed in different retinotopic locations in V1 of New and Old World monkeys, including the peripheral representation of the upper visual quadrant, which is likely to be found near the ventral-most regions in areas V3 and V4 (Lyon and Kaas, 2002b, 2002c). As MT is often viewed as a distributor of feedforward inputs to dorsal stream

areas, V4 is considered to be a main distributor to ventral stream areas (see Figure 13a; Shipp and Zeki, 1995; Van Essen and De Yoe, 1995).

The dorsointermediate, dorsomedial, and medial areas (DI, DM, and M, respectively), are consecutively located dorsal to DL/V4. In the organizational scheme presented here, the dorsal-most area M lies adjacent to the dorsal-most portion of V2. Area M, first described in New World owl monkeys (Allman and Kaas, 1971a), may be a homologue of the dorsal parietal occipital area (POd) or the posterior intraparietal area (PIP) described in macaques (Colby *et al.*, 1988; Felleman and Van Essen, 1991). Ventral to M, areas DM and DI are displaced from the outer border of V2 by dorsal V3. Earlier descriptions based on studies of New World monkeys placed these areas immediately adjacent to V2 (Allman and Kaas, 1975; Rosa and Schmid, 1995); however, subsequent connectional and architectonic evidence has shown that these areas, particularly DM, are in the location of V3a as described in macaques (Lyon and Kaas, 2001, 2002b, 2002c; Van Essen and Zeki, 1978). Whether DI in macaques lies ventral to DM/V3a, or V3a can be split into two regions, the ventral of which may correspond to DI, is uncertain. Connections with V1 show separate clusters of cells in the dorsal and ventral halves of V3a, termed PIP or M dorsally and V3a (Figure 14c) or DM ventrally. However, it may be that the ventral portion corresponds to DI and the dorsal portion to DM/V3a. This interpretation is more consistent with results found in similar experiments of New World monkeys where connections between V1 and M were not found (Lyon and Kaas, 2002c).

In the vicinity of the dorsal extent of the STS (the exact location relative to the STS depends on the size of the monkey; see Figures 13b and 14a), MT is easily identified through its myelo- and cytoarchitecture (Figure 16). Particularly in flattened cortex preparations, MT serves as a reliable point of comparison for the examination of architecture, connection patterns, and microelectrode mapping of visual areas in the immediate vicinity. Sometimes referred to as the 'MT-satellites' several areas surrounding MT have been identified (Allman and Kaas, 1974; Desimone and Ungerleider, 1986; Kaas, 2004a; Krubitzer and Kaas, 1990; Rosa, 1997; Rosa and Elston, 1998). The MTc surrounds much of the dorsal, posterior, and ventral borders of MT (Allman and Kaas, 1974; Rosa and Elston, 1998) and can be differentiated from MT as a retinotopically organized arc of moderately myelinated cortex and through its unique pattern of connections with the ventral division of area FST (Kaas and Morel, 1993; Lyon and Kaas, 2001; Rosa and Elston, 1998). FST, first identified in macaques (Desimone and Ungerleider, 1986), extends from the ventral border of MT and MTc and can be split into separate dorsal and ventral areas, FSTd and FSTv (Krubitzer and Kaas, 1990; Kaas and Morel, 1993). The MST area is located adjacent to the representation of the peripheral visual field in MT, along its medial anterior border (Maunsell and Van Essen, 1983a; Desimone and Ungerleider, 1986; Ungerleider and Desimone, 1986; Rosa and Elston, 1998) and receives inputs from functionally distinct modules in MT (Berezovskii and Born, 2000).

Based on connectivity patterns (Felleman and Van Essen, 1991) and the evidence for roles in the processing of increasingly complex moving stimuli, such as spiral motion (Duffy and Wurtz, 1995), the MT satellites can be considered the second stages of the dorsal stream after MT. Dorsal to MT, areas in PP represent a third stage in the dorsal stream as they receive the bulk of their input from the MT satellites. In New World monkeys, based on feedback projections to V1, DM and the SC (Collins *et al.*, 2005; Krubitzer and Kaas, 1993; Lyon and Kaas, 2001, 2002c), only two areas within PP have been identified, LPP, located just dorsal to MT, and VPP, dorsal to LPP and just anterior to DM. Initial macaque monkey studies identified similar regions, area 7a (Andersen *et al.*, 1985a, 1985b; Motter and Mountcastle, 1981) just dorsal to MT, and the ventral area of the intraparietal sulcus, VIP (Maunsell and Van Essen, 1983a). Subsequent studies have proposed several more visual areas in PP. While none of these areas shows any precise retinotopic organization, contributing to the uncertainty, connection studies placing injections directly into different regions of the PP and functional mapping studies have led to the identification of at least four areas located medial, ventral, lateral, and anterior in the intraparietal (IP) sulcus – areas MIP, VIP, LIP and AIP (Figure 14b; see Colby and Duhamel, 1991; Andersen, 1995; Lewis and Van Essen, 2000a).

Despite uncertainty as to the exact number of areas in PP, this region seems greatly expanded compared to the amount of PP processing vision in the cat (see Payne, 1993). Neurons in this region of monkey cortex are highly selective for complex spatial perception (Siegel and Read, 1997) and are heavily involved in deciding where next to move

the eyes (Duhamel *et al.*, 1992) – cortex involved in eye movements is less prominent in cats (see Payne, 1993). Accordingly, these areas, as well as MT and some of its satellites, are heavily interconnected with eye movement fields in prefrontal cortex (FEF; Schall *et al.*, 1995). In addition, neurons in some areas are multimodal, responding to both visual and somatosensory stimuli (Duhamel, 2002), while neurons in AIP play a visual role in the guidance for the grasping of objects (Fogassi *et al.*, 1996; Jeannerod *et al.*, 1995) – a feature unlikely to be of as much use to carnivores.

Areas comprising the ventral stream are located ventral to MT in the IT, and have little interconnections with areas in PP (Baizer *et al.*, 1991). Like the areas in PP, retinotopic organization of areas in IT is coarse at best (Boussaoud *et al.*, 1991; Desimone and Gross, 1979). Even so, the IT region can be subdivided into at least four areas based on connectivity patterns and functional differences (see Figure 14b; Baizer *et al.*, 1991; Buffalo *et al.*, 2005; Distler *et al.*, 1993; Weller and Kaas, 1987). V4 at the head of the ventral stream is fed through V2 projections originating in CO light interbands and CO dark thin bands (Felleman *et al.*, 1997; Shipp and Zeki, 1995; Van Essen and De Yoe, 1995; Xiao *et al.*, 1999). In turn, V4 projects to adjacent area TEO/ITc (where TEO = temporal occipital cortex; Nakamura *et al.*, 1993). These areas can also be identified through direct feedback connections to V1 (Figure 14c; Lyon and Kaas, 2002b, 2002c; Rockland and Van Hoesen, 1994). The segregated V4 inputs are thought to give rise to the specialized object and color processing of cells in the ventral stream. TEO, for example, has been implicated in color selectivity (Tootell *et al.*, 2004) and the perception of objects (Brincat and Connor, 2006), while areas further anterior in the hierarchy are selective for complex objects such as faces (Perrett *et al.*, 1982, 1984).

34.5.4 V1 and V2 in Cats and Primates

As we have seen earlier in the article, V1 and V2 are present in most mammals and are considered to have been retained from an early common ancestor (see Section 34.2). And, as described for other highly visual mammals, such as tree shrews (see Section 34.4.2), cats, ferrets, prosimians, and monkeys have patchy, long-range, intrinsic connections within V1 and V2 (Rockland and Lund, 1982; Gilbert and Wiesel, 1983; Casagrande and Kaas, 1994). Also, as seen for tree shrews, correlation of the connection patterns to functional maps of orientation preference show that like-orientation domains are preferentially connected (Kisvarday *et al.*, 1997; Malach *et al.*, 1993; Schmidt and Lowel, 2002). While these features are found in tree shrews and ferrets, as well as primates and cats, V1 and V2 of the complex visual systems of cats and primates have independently evolved additional features of modular organization. Most notable is the regular distribution of CO blobs in V1 (Casagrande and Kaas, 1994; Horton and Hubel, 1981; Matsubara and Boyd, 2002; Murphy *et al.*, 1995).

While several features have been attributed to CO blobs, such as the processing of color in primates (Livingstone and Hubel, 1988, 1984), these interpretations remain controversial (see Sincich and Horton, 2005). The simplest explanation for blobs is that they arise from increased activity brought about by direct thalamic connections (Livingstone and Hubel, 1982), as CO is a marker for higher metabolic activity (Wong-Riley, 1979) and thalamic afferents provide strong driving inputs (Reid and Alonso, 1996). Blobs, which are most prominent in superficial layer 3, coincide with the termination zones of K cell geniculate afferents (Casagrande, 1994). The lighter interblob regions lack geniculate afferents. Consistent with the interpretation of thalamic afferents resulting in increased CO activity, layer 4C, the main geniculate input zone, receives dense nonpatchy geniculate afferents from M and P cells and as a result stains uniformly dark for CO. Patchy geniculate afferents in cat have also been correlated with the blobs (Matsubara and Boyd, 2002).

34.5.4.1 Early parallel processing From the early work in cats and primates, the concept emerged that three ganglion cell types (X, Y, and W) give rise to parallel processing streams relayed through the LGN – X, Y, and W geniculate cells in cats, and the P, M, and K geniculate cells in primates – to V1 (see The Evolution of Parallel Pathways in the Brains of Primates; Stone *et al.*, 1979; Casagrande and Xu, 2004). In primates, the parallel channels remain segregated in their projections to V1 and this was thought to play a crucial role in the emergence of the dorsal and ventral processing streams in higher-order cortex (Livingstone and Hubel, 1988; Ungerleider and Mishkin, 1982; Ungerleider and Pasternak, 2004; Zeki and Shipp, 1988; see Section 34.5.3). M cells can be characterized by features that lead to motion and spatial perception – fast conducting axons, high temporal resolution, and sensitivity to low-contrast stimuli – characteristic of visual areas

in the dorsal stream. In contrast, P cells exhibit features that could lead to the perception of form and color (high spatial resolution and chromatic contrast), characteristics of the ventral stream. In cats, there is less segregation of the X and Y channels in V1 (Casagrande and Xu, 2004; Humphrey *et al.*, 1985; Payne and Peters, 2002). Nevertheless, there is support for dorsal and ventral streams in visual cortex of cats and ferrets (Lomber *et al.*, 1996a, 1996b; Manger *et al.*, 2002b, 2004; Payne, 1993).

While the parallel geniculate inputs to V1 in primates remain much more segregated than the geniculostriate projections of cats, there is clear evidence that mixing of the streams occurs at subsequent processing stages within V1 of primates (Callaway, 1998, 2005; Casagrande and Kaas, 1994; Merigan and Maunsell, 1993; Sincich and Horton, 2005). Thus, it is unclear whether the segregation of geniculostriate projections is relevant to subsequent outputs to the dorsal and ventral streams. This convergence within V1 is consistent with receptive fields becoming more complex as information flows through the visual hierarchy, but it leads to a complicated picture of the emergence of the dorsal and ventral streams (see Van Essen and De Yoe, 1995). Nevertheless, most evidence points towards some segregation of the functional streams – dorsal stream areas receive an M dominated relay, whereas the ventral stream receives a relay containing P and M signals. However, recent findings have shown a convergence of M and P geniculostriate projections that are in turn relayed directly to dorsal stream area MT, indicating that M and P signals are mixed even within the dorsal stream (Nassi *et al.*, 2006).

A major evolutionary modification present in V2 of monkeys, compared to cats, tree shrews, and prosimians is the three architectonically distinct bands, or stripes, that can be revealed through CO architecture (Krubitzer and Kaas, 1990; Livingstone and Hubel, 1982; Tootell *et al.*, 1985). These compartments have distinct connection patterns and functional selectivity of their neurons (see Sincich and Horton, 2005) that feed higher-order areas in the dorsal and ventral streams (see Section 34.5.3; Shipp and Zeki, 1995; Van Essen and De Yoe, 1995). The thick CO bands are reported to receive input from the M dominated stream in V1, whereas interbands and thin CO bands receive inputs from a mixture of M and P sources. The thick bands are thought to feed subsequent dorsal stream areas, whereas interbands and thin bands feed areas in the ventral stream. While the CO staining is a fairly reliable marker there is some question as to its functional relevance. While band location can be correlated with inputs from V1 and projections to areas V4 and MT, the dark CO bands in V2 may actually result from thalamic afferents arriving from the pulvinar nucleus (Levitt *et al.*, 1995).

34.5.5 The Controversy over Monkey V3

Is V3 a feature common to most mammalian visual systems? As we have seen for V2, the size, the relative position, feedforward projections from and feedback projections to V1, and a compressed mirror reversal of the retinotopic organization of V1 are the main reasons for considering V2 as part of the common mammalian plan. This is true even for cat and ferret V2, where, through divergent evolution, V2 has become more primary-like. Using the same criteria, a comparison across species shows that an area exists in a similar location, adjacent to the anterior border of V2, represents a compressed mirror image of the retinotopic organization of V2, and receives feedforward projections from V1 and V2, while providing feedback to these areas. While these criteria point toward a homology of V3 across mammals, there are some obstacles to overcome. While many of these obstacles have been addressed in earlier sections (34.2.3 and 34.4.1), a remaining obstacle in accepting V3 as a homologous region across most mammals, is that even in the most visually complex order, the primates, there is a debate over the existence of V3 (see the discussion below; Kaas, 2005b; Kaas and Lyon, 2001; Rosa and Manger, 2005).

Since the early retinotopic mapping experiments by Allman and Kaas (1975), the existence of a V3 in primates has been questioned (see Kaas and Lyon, 2001; Lyon and Kaas, 2002c). One issue, deriving from work on New World monkeys, is whether several visual areas, in place of a V3, can be found immediately adjacent to V2 (Allman and Kaas, 1975; Rosa and Schmid, 1995; Lyon and Kaas, 2001, 2002c; Lyon *et al.*, 2002; Rosa *et al.*, 2005). A second issue, derived from studies on Old World monkeys, is whether there are functional and connectional differences between the dorsal and ventral halves of V3 (Van Essen, *et al.*, 1986; Lyon and Kaas, 2002b), resulting in the identification of two separate areas.

In Old World monkeys the dorsal and ventral halves of V3, V3d and V3v, contain the representation of the lower and upper visual fields, respectively (Gattass *et al.*, 1988). A reported difference in the neuronal properties and projection patterns from

V1 led to a split of V3 into two separate areas (Burkhalter *et al.*, 1986; Felleman and Van Essen, 1987; Van Essen *et al.*, 1986). V3d became V3, representing only the lower visual quadrant, whereas V3v became the ventral posterior area, VP, representing only the upper visual quadrant. The functional properties of neurons may not be relevant to the homology of V3 in other species – as the function of V2 can differ between species. Nevertheless, it is unusual that functional properties such as chromatic sensitivity and direction selectivity would differ between the upper and lower visual fields of a single area. However, the functional characteristics of neurons in V3v have only been examined in one study (Burkhalter and Van Essen, 1986), so confirmation of the proposed differences in V3v and V3d neurons is needed. This is particularly important because several studies have yielded different characterizations of the response properties of neurons just within V3d (Baizer, 1982; Felleman and Van Essen, 1987; Gegenfurtner *et al.*, 1997; Zeki, 1978). Part of such differences in characterizations is due to different criteria used to define the characteristics of the neurons (see Gegenfurtner *et al.*, 1997), and part is likely due to the difficulty of accessing V3d in the macaque monkey, as it is completely buried within the lunate sulcus. It is also unusual for the connection patterns to differ between the two visual fields of a single area (see Kaas, 1996; Zeki, 2003). Accordingly, a re-examination of the connection patterns has revealed the missing connections. Whereas, initially, it was reported that V3d was interconnected with V1, but that V3v was not (Van Essen *et al.*, 1986), more recent work has revealed that both V3d and V3v are interconnected with V1 (Lyon and Kaas, 2002b). Thus, the controversy over V3 in Old World monkeys has become more settled (see Kaas, 2005b; Van Essen, 2004).

For New World monkeys, differences in interpretation over the size and location of V3 continues. The main issue in New World monkeys concerns the organization of cortex just anterior to the dorsal extent of V2. Early microelectrode mapping experiments in owl monkeys found the representation of the upper visual quadrant immediately adjacent to dorsal V2 (Allman and Kaas, 1975). As dorsal V2 represents the lower visual quadrant, the existence of an adjacent upper field representation argued against the idea of a V3 strip of cortex mirroring the V2 retinotopy. This new region was demonstrated to have a complete retinotopic map occupying cortex similar in size to area MT. Subsequent experiments in the smaller marmoset monkey also found an upper field representation immediately adjacent to dorsal V2, but the resulting DM contained an unconventional split representation of the horizontal meridian (Rosa and Schmid, 1995). More recently, however, connection patterns with V1 in four species of New World monkeys found no evidence for an upper field representation near the anterior border of V2 (Lyon and Kaas, 2001, 2002c). Instead, the observed connections from upper field V1 were displaced at least 1–2 mm anterior to the dorsal V2 boundary. Along with evidence from cytochrome and myeloarchitecture, dorsal V3 was identified as a strip of cortex displacing DM from dorsal V2. Counter to these connection patterns, however, a subsequent study placing injections anterior to dorsal V2 did show connections with the upper field representation in marmoset V1 (Rosa *et al.*, 2005). However, injection sites were centered nearly 2 mm from the outer V2 border. Thus, the upper field connections likely arose from the upper field location of DM.

In addition to the conflicting reports of the upper field connectivity of DM, it has been argued that the microelectrode maps of this dorsal region clearly demonstrate an upper field representation immediately adjacent to the dorsal V2 border (Rosa and Schmid, 1995; Rosa *et al.*, 2005; Rosa and Manger, 2005; Rosa and Tweedale, 2005). Yet, careful examination of the published data reveals some ambiguities, and there are additional results that are rather perplexing. First of all, the evidence for an upper field representation immediately adjacent to V2 (Rosa and Schmid, 1995) can be interpreted in another way, especially in light of more recent connection studies (Lyon and Kaas, 2001, 2002c). The receptive fields of recording sites considered as part of the upper field were centered very near the outer border of V2 representing the horizontal meridian, as a result much of the recorded receptive fields of individual neurons were found in the lower visual field as well the upper (Rosa and Schmid, 1995). As most of these recording sites were located near the V2 border they were taken as evidence for upper field representation immediately adjacent to V2. However, as the V2–V3 border represents the horizontal meridian it is not surprising that the receptive field would partially overlap with the upper field, and thus this evidence in itself cannot be used to argue against a V3.

While this issue is open to interpretation, there are other findings reported from the retinotopic maps of DM, such as an irregular progression from the central to peripheral visual field and the representation of two separate horizontal meridians (Rosa, 2002;

Rosa and Schmid, 1995), that lead one to question the reliability of the microelectrode technique as a means for such issues, especially in regions that have been difficult to define. Alternatively, techniques such as intrinsic signal optical imaging offer a fairly high spatial resolution but, unlike microelectrode mapping, allow the recording of neural activity from a large array of cortex simultaneously (see Figure 9). This procedure is helpful in reducing any ambiguities that may arise through the reconstruction of electrode tracks in several coronal or parasagitally cut sections. In owl monkeys, optical imaging of neural activity revealed only the representation of the lower visual field adjacent to V2 (Lyon *et al.*, 2002). The resulting retinotopic map revealed a slightly compressed mirror-image representation of the retinotopy in dorsal V2, consistent with a dorsal V3 (Figure 17). Also consistent with a dorsal V3, the V2–V3 border was marked by the representation of the horizontal meridian, while the outer V3 border was marked by the vertical meridian. These results are also consistent with the retinotopic connections of this region to V1 in owl monkeys, three other New World monkey species, and two species of Old World macaque monkeys (Lyon and Kaas, 2001, 2002b, 2002c). In addition, the observed retinotopy of dorsal V3 in owl monkeys is similar to the retinotopic organization revealed in macaque monkey V3 through microelectrode mapping (Gattass *et al.*, 1988) and fMRI (Brewer *et al.*, 2002; Fize *et al.*, 2003).

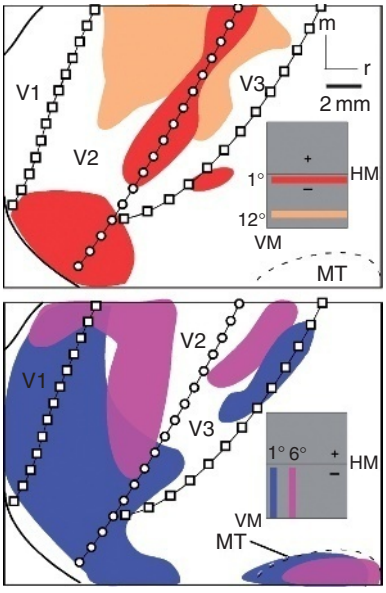

Figure 17 Reconstructions of the retinotopic organization of monkey V2 and V3. These summary diagrams are reconstructed from data in Lyon, D. C., Xu, X., Casagrande, V. A., Stefansic, J. D., Shima, D., and Kaas, J. H. 2002. Optical imaging reveals retinotopic organization of dorsal V3 in New World owl monkeys. *Proc. Natl. Acad. Sci. USA* 99, 15735 15742.

34.5.6 The Middle Temporal Area, MT

Primate area MT exhibits several features that make it the most prominent extrastriate area, outside of V2. As revealed through connection patterns, MT is strongly interconnected with V1, V2, and V3 (Born and Bradley, 2005; Lyon and Kaas, 2002b, 2002c; Shipp and Zeki, 1989a, 1989b), receiving inputs perhaps dominated by the M geniculate pathway (Merigan and Maunsell, 1993; Van Essen and De Yoe, 1995), but also inputs from the P pathway (see Nassi *et al.*, 2006). In addition, MT receives direct but sparse input from the K geniculate cells (Sincich *et al.*, 2004; Stepniewska *et al.*, 1999) and receives fairly dense disynaptic projections from the SC (Lyon *et al.*, 2005). In turn, MT is a primary provider of inputs to many of the areas in PP that form the dorsal stream of visual processing (Maunsell and Van Essen, 1983a; Ungerleider and Desimone, 1986; Shipp and Zeki, 1995). Another distinctive feature of MT is its dense and patchy myelination (Figure 16; Allman and Kaas, 1971b; Krubitzer and Kaas, 1990) and blob-like staining for the metabolic enzyme CO (Tootell *et al.*, 1985; Lyon and Kaas, 2001).

Functionally, MT contains a precise first-order representation of the contralateral visual hemifield (Allman and Kaas, 1971b; Rosa and Elston, 1998) similar to that found in V1 and a systematic representation of direction preference that is arranged in a columnar fashion (Albright *et al.*, 1984) that is in many ways similar to the orientation columns of V1 (see Albright and Desimone, 1987). Columns are also seen in MT architecture and connection patterns. V1 projections to MT terminate in a modular arrangement (Rockland, 1989) and MT cells projecting to other extrastriate areas, such as DM, FST, and MST, are also arranged in small, modular clusters (Kaas and Morel, 1993; Krubitzer and Kaas, 1993; Berezovskii and Born, 2000). The internal architecture of MT revealed through stains for CO (Tootell *et al.*, 1985) and myelin (Krubitzer and Kaas, 1990) contains intermittent dark and light regions comparable in size to MT functional columns. Interestingly, callosal projections from MT have been correlated with the myelin dark regions (Krubitzer and Kaas, 1990). Subsequent studies have indicated that similar functionally defined modules may be correlated to differences in MT architecture and patchy intrinsic connectivity (Born and Tootell, 1992; Malach *et al.*, 1997). More recently, Berezovskii and Born (2000) have

shown that MT regions responsive to local motion cues project preferentially to the dorsal half of MST, whereas MT regions more responsive to global motion cues project preferentially to the ventral half of MST and to FST.

34.5.6.1 Is MT found in other mammals? Whereas there is a similar V3-like area found in most mammalian species, it is even less certain whether there is an MT-like area across species. MT is a prominent region in primate visual cortex and thought to have emerged early in primate evolution. One theory suggests that tree shrew TD, rather than a V3-like area, is a potential MT homologue and that MT gradually drifted further anterior from the V2 border as cortex evolved in successive primate lines – prosimians, New World monkeys, and Old World monkeys (Northcutt and Kaas, 1995). Like MT, tree shrew TD shows an increase in myelination compared to surrounding regions and is heavily connected with V1. However, there are several extrastriate areas in primate that are connected with V1, including V3 which is also moderately myelinated (Lyon and Kaas, 2001). Additionally, there are several other extrastriate areas in the tree shrew that lie more anterior to V2 and could represent an MT-like area based on other criteria. The most prominent area, TI, is connected with V2 rather than V1, but it does stain conspicuously dark for myelin and lies very close to primary auditory cortex (Lyon *et al.*, 1998). The dark myelination of primate MT is probably its most distinctive feature, and like tree shrew TI, primate MT is found just posterior to auditory cortex (see Lyon and Kaas, 2001). What is sorely lacking is any evidence of functional similarities between MT and TD or TI, as in tree shrew there are no reports on the functional properties of neurons outside of V1.

In other species, areas in location similar to TD have also been proposed as MT homologues. A squirrel MT homologue, area ML-L, has been proposed on the basis of functional similarities to primate MT (Paolini and Sereno, 1998) and it is in a similar location to tree shrew TD. A region anterior to V2 in the flying fox has also been proposed as a homologue to MT based on its myelination, retinotopic organization, and input from V1 (see Kaas and Preuss, 1993). However, like the tree shrew, this proposed MT homologue has been considered a homologue to V3 in other interpretations (Rosa, 1999). While the data in support of an MT homologue is limited for these species, a significant body of evidence has implicated the cat PMLS complex as a homologue to MT and its surrounding satellites (see Grant and Hilgetag, 2005; Hilgetag and Grant, 2000; Payne, 1993). Indeed, PMLS and MT share many similarities. Both regions are located well anterior to V2 in temporal cortex and are comprised of neurons specialized for direction discrimination. In addition, they both have orderly retinotopic maps, stain darkly for myelin, receive feedforward projections from V1, V2, and V3, and provide feedforward projections from similar cortical layers to the surrounding motion processing areas. Furthermore, Matsubara and Boyd (2002) report similarities in the origin of the projections from V1 to the PMLS region in cat and MT in monkey, showing that cells located in CO blobs within the layer 4B project to these areas. Despite the great number of similarities, a cladistic approach makes it difficult to conclude that PMLS is an MT homologue because the evidence for an MT homologue in intervening species is so limited (Kaas, 2002). Nevertheless, MT-like regions have been proposed for species in sister groups to both cats (flying foxes) and primates (tree shrews and squirrels) leaving open the possibility that primate MT emerged from an area common to many mammals.

34.6 Conclusions

Despite detailed evidence reviewed here on the organization of visual cortex in several mammalian taxa, at this point we know very little about how differences in mammalian visual cortex organization evolved (see Streidter, 2005). As presented in this article, we can look at the organizational schemes of several different mammalian orders from the small nonvisual insectivores to the large and highly visual carnivores and primates. Still, because detailed evidence is lacking, we can say little about homologies between extrastriate visual areas. Should we care whether cat visual areas are homologous to the primate? Is an understanding of whether brain structures are homologous or homoplaseous critical to understanding how the brain functions? For, despite uncertainty of the homology between species, is it not unreasonable to use neuronal properties of, for example, cat PMLS to help us understand how monkey and human brains process motion? If area PMLS is homologous to MT, will that help us understand the brain any better? More likely, understanding the brain will help us determine whether areas are homologous.

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PRIMATE BRAIN EVOLUTION

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35 Primate Brain Evolution

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Glossary

anthropoids The primate group comprised of the New World monkeys (platyrrhines) and the Old World monkeys, apes, and humans (catarrhines).

Archonta The group of mammals consisting of primates and their closest relatives, currently thought to include tree shrews and flying lemurs.

catarrhines Members of the suborder Catarrhini, the primate group comprised of the Old World monkeys and the hominoids (apes and humans).

clade Any monophyletic group; that is, the set of species descended from a common ancestral species.

encephalization The disparity (usually expressed as a ratio) between the size of the brain in a particular species and the size that would be expected based on its body size.

haplorhines Members of the suborder Haplorhini, the primate group comprised of the tarsiers and anthropoids.

neomorphism An evolutionarily new feature, that is, a derived feature of a group of animals that has no homologue at the same level of organization in related mammals.

platyrrhines Members of the infraorder Platyrrhini, the New World monkeys.

prosimians Members of the primate group comprised of strepsirhines plus tarsiers; most workers think this is not a monophyletic group, so the term is not as widely used as a formal taxonomic term as it once was.

shared derived character Also known as synapomorphy. A character that evolved in the stem lineage of a group; typically, such a character is present in many descendents of the stem lineage and serves to distinguish that group from related groups.

stem lineage The segment of the evolutionary tree that connects the last common ancestor of a group of interest to the last ancestor it shares with its closest extant relatives. For example, stem primates are animals on the lineage, or on branches extending from the lineage, that connects the last ancestor of modern primates to the last ancestor primates share with other archontans (such as tree shrews).

strepsirhines Members of the suborder Strepsirhini, the branch of the primate tree consisting of the lemur group and the loris bush baby group.

taxon (pl. taxa) Any formal grouping of related organisms, for example, any particular species, genus, family, etc.

35.1 Introduction

Almost anyone with some exposure to the neurosciences can tell you the story of primate brain evolution: through evolutionary time, brains became bigger and more complex, and their bearers increasingly intelligent, a process that culminated in the appearance of *Homo sapiens*, the brainiest and most intelligent animal of them all. This conception of primate brain evolution is so deeply embedded in

the foundations of neuroscience that it is easy to lose sight of the fact that the idea actually has a history, that it was the intellectual product of particular scientists at particular time points in the development of ideas about primatology and about neurobiology.

Times change. Neuroscience, as a discipline, has of course enjoyed tremendous growth over the past several decades, fueled in part by the development of a succession of new techniques that make it possible to explore brain organization and function in finer and finer detail. Less generally appreciated, perhaps, is that evolutionary biology has also undergone profound developments, with the introduction of new methods for determining how species are related to each other and for reconstructing the history of evolutionary change. The phylogenetic scale is gone, replaced by the branching tree of life. Evolutionary biologists now no longer read the history of life as the story of the Ascent of Man: humans are regarded as one of numerous specialized end-points of evolution. Unquestionably, we are justified in taking a particular interest in our own species, but we misunderstand ourselves, and other species, if we conceive of evolution as being mainly about how to get to *H. sapiens*.

As a consequence of the fundamental methodological and conceptual changes in evolutionary biology, we have a much better understanding of the evolutionary history of primates and the relationship of primates to other mammals, and textbooks on primate evolution that were current in, say, 1975, seem about as quaintly dated today as textbooks of neurobiology or physiological psychology from the same era. These new ideas and approaches to evolutionary biology have begun to make their mark on the neurosciences, as witnessed, for example, by the publication of Georg Striedter's landmark textbook on vertebrate brain evolution (Striedter, 2005) and of the volumes in the present series, but there is a long way to go, as so much of the neuroscientific enterprise takes place without any explicit reference to evolution. My main purpose in writing this article is to further the integration of primate evolutionary biology and primate neuroscience. I begin by setting the comparative context for understanding primate brain evolution with an overview of primate taxonomy, past and present ideas about primate origins and adaptations, and current thinking about how primates are related to other mammalian groups. I then review selected issues in primate brain evolution, attempting to localize specializations of primate brain organization within the context of primate evolutionary history.

35.2 Primate Origins and Evolution

35.2.1 The Primates

Primatologists currently recognize the existence of over 200 extant species of primates (Purvis, 1995; Fleagle, 1999). Traditionally, the order Primates was divided into two main groups, termed prosimians and anthropoids (the latter also known as 'simians'), or more formally, suborder Prosimii and suborder Anthropoidea. The familiar anthropoids consist of a group native to the Old World – the Old World monkeys, plus the hominoid (ape-human) group – and a group native to the New World, the New World monkeys. Formally, the Old World group is known as the Catarrhini and the New World group as the Platyrrhini. The Old World monkey group is the most successful primate group in terms of diversity, comprising no fewer than 87 extant species distributed from South Africa to Japan. These include the familiar macaque monkeys, widely used as research subjects by neuroscientists. By contrast, there are only 14 extant species in the ape-human group, and most of these are the so-called 'lesser' apes, that is, the gibbons. The prosimians consist of the lemurs, found today only on the island of Madagascar, the loris–bush baby group, with species distributed across sub-Saharan Africa and south Asia, and the tarsiers, native to the islands of the western Pacific.

Although this conventional taxonomy groups tarsiers with the lemurs and lorises, there has long been a body of opinion holding that tarsiers are actually more closely related to the anthropoids (Figure 1a). This relationship implies a different classification, in which the two major groups of primates become suborder Strepsirhini (the lemur and loris–bush baby group) and suborder Haplorhini (tarsiers plus the anthropoids). The strepsirhine–haplorhine taxonomy appears to be the preferred taxonomy among primatologists at the present time, because the majority of primatologists accept that tarsiers are the closest relatives of the anthropoids, although this matter is not entirely settled (Ross and Kay, 2004b).

Certain useful generalizations about the biology and behavior of the major primate groups can be stated (Martin, 1990; Fleagle, 1999). Compared to anthropoids, the strepsirhines tend to have small bodies and relatively small brains (when brain size is adjusted for body size). Many of the strepsirhines are nocturnally active. The smaller, nocturnal strepsirhines – such as bush babies (galagos), lorises, and mouse lemurs – tend to be solitary and subsist on a diet of insects, small vertebrates, fruit, and flowers. Some of the larger lemurs on Madagascar, however,

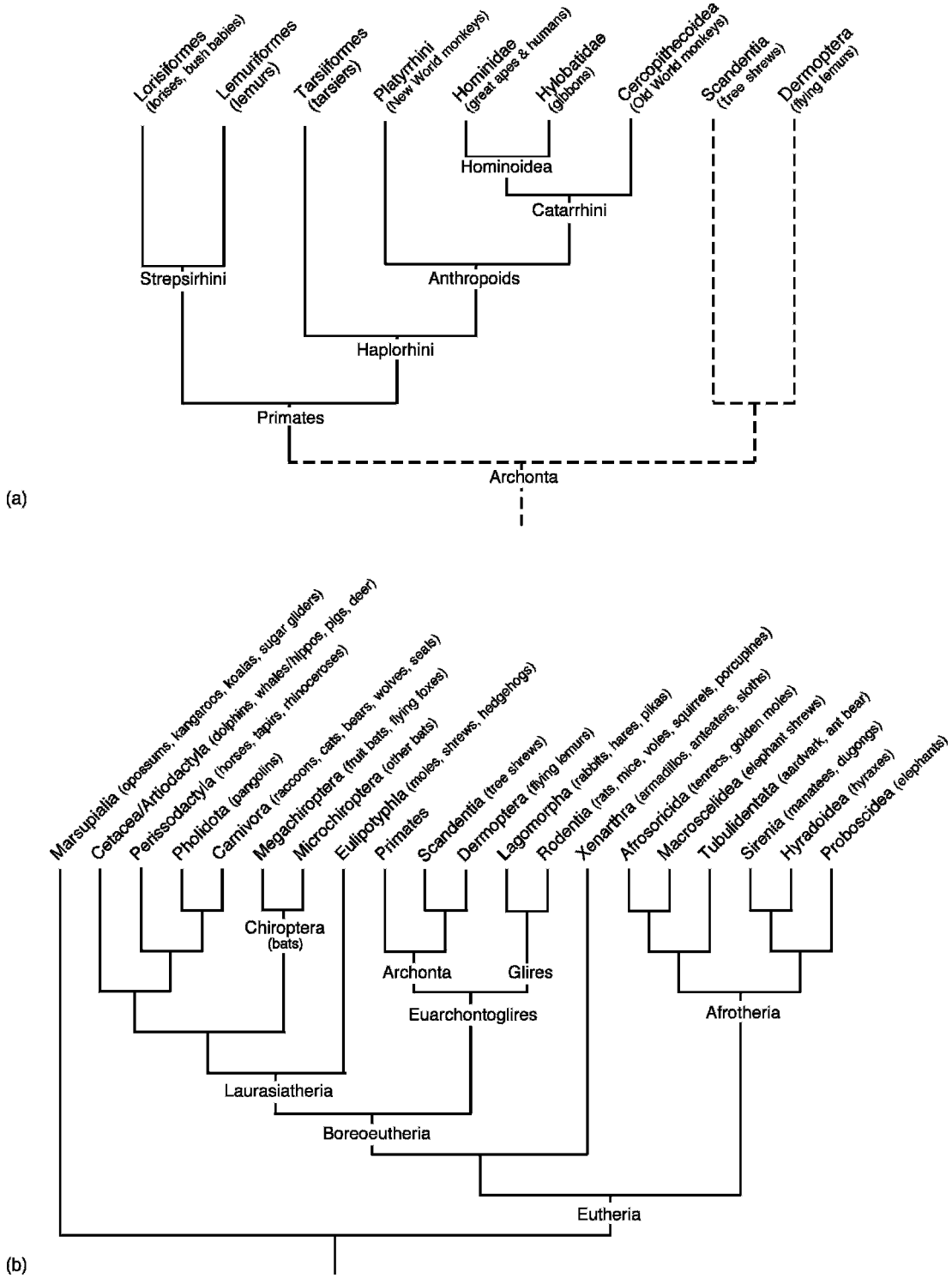

Figure 1 The evolutionary relationships of primates and mammals. a, The phyletic relationships of the major primate groups. Currently, tree shrews (order Scandentia) and flying lemurs (order Dermoptera) are the animals thought to be most closely related to primates. The Archonta is the higher-order group that includes Primates, Scandentia, and Dermoptera. b, A modern interpretation of the relationships of therian mammals, based on the comparative DNA studies of Murphy *et al.* (2001b). Murphy and colleagues recognized the close relationship between Primates, Scandentia, and Dermoptera (which they designated as 'Euarchonta' rather than Archonta). Note that the bats (order Chiroptera), in contrast to some other interpretations, are regarded here as monophyletic, but distantly related to primates.

are diurnal, live in social groups, and have a diet consisting primarily of plant material. By contrast to strepsirhines, the anthropoids are diurnally active (with the sole exception of the New World owl monkey, *Aotus*), and most live in sizable social groups, subsisting on fruit, flowers, gum, and leaves, and in some cases also insects and vertebrates. The tarsiers, although probably closely related to the anthropoids, resemble the smaller strepsirhines in being very small bodied, nocturnal, and solitary, and subsisting on a diet of invertebrates and small vertebrates. Tarsiers are readily identified by their enormous eyes.

35.2.2 Primate Evolution

35.2.2.1 Early views Not surprisingly, the history of ideas about primate and human evolution carries the very significant imprint of Charles Darwin (see especially Darwin, 1859, 1871). Darwin, of course, is a hero to modern evolutionary biologists, who tend to attribute to him a very modern view of evolution. In order to understand how Darwin influenced thinking about primate evolution, however, it is necessary to place him in proper historical context. Although Darwin is sometimes seen as a champion of the modern idea that phylogeny is like a branching tree rather than like an ascending scale – and there is indeed a prescient remark in one of his notebooks that suggests the primacy of the branching view of evolution – any fair reading of Darwin's large corpus of published writings makes it clear that he considered natural selection to be a process leading to progressive improvement, and that humans are, among animals, the most improved forms (Richards, 1992; Preuss, 1993, 1995b). Darwin, like most of the first generation of evolutionists, simply did not perceive a conflict between the branching-tree conception of evolution and the idea that evolution is a scale. As a result, early drawings of the tree of life generally have very stout vertical trunks, with humans at the top and little twigs branching off at lower levels of the trunk representing less-evolved (although nonetheless modern) forms. The archetype of this genre is the famous tree published by Haeckel (1874) and widely reproduced (see, e.g., figure 1 of Preuss, 2004b). Darwin, it should be said, was an unabashed fan of Haeckel (Richards, 1992).

For Darwin, then, an important part of the evolutionary story was about how to account for the ascendancy of humans among animals. In Darwin's view, the key to human success is not the human body, weak and feeble as it is, but rather the brain and the intellectual abilities it confers. It is important also to note, however, that Darwin – perhaps being concerned about drawing such a sharp line between humans and other animals as to create doubt about the theory of evolution – emphasized that the differences between the intellectual abilities of humans and other animals are matters of degree rather than kind. This is Darwin's 'Principle of Continuity', which has cast a very long shadow on the history of comparative psychology and neuroscience (Povinelli, 1993; Preuss, 1993, 1995b).

Theories of primate and human evolution developed during the early part of the twentieth century took their lead from Darwin, focusing on brain size and the conditions under which brain-size increases would be selected for (Cartmill, 1982). The British anatomists G. Elliot Smith and F. Wood Jones championed the idea that life in the trees selected for greater intelligence and enhanced vision (including the stereoscopic vision supposedly necessary for arboreal living). W. E. Le Gros Clark popularized these views in a series of books, including his widely read treatise, *The Antecedents of Man* (Le Gros Clark, 1959). Not only did Le Gros Clark promote the arboreal theory, he argued that the key to the success of primates was that they remained fundamentally unspecialized anatomically, the retention of a generalized, behaviorally adaptable skeleton serving as a better vehicle for the big brains of primates than the behaviorally limiting, specialized body forms evolved by terrestrial mammals. Thus, rather than being defined by a set of morphological specializations, primates were to be defined by a set of adaptive trends set in motion by an early commitment to arboreality: progressive enhancement of vision, reduction of olfaction, enlargement of the brain, and increasing intelligence. With respect to the brain, Le Gros Clark argued that as it became enlarged, the differences between regions became more sharply defined; however, the increased differentiation reflected the refinement of structural characteristics already present in more primitive forms rather the addition of new structural elements. This stance distinguishes Le Gros Clark from some other early neuroanatomists, notably Brodmann (1909), who believed that the greater histological differentiation of anthropoid and human cortex compared to other mammals represented the addition of new organs of mental function (Preuss, 1993).

Le Gros Clark also advanced a particular view of primate relationships and evolutionary history that proved extremely influential. He viewed primates as progressing through a series of ascending steps or grades reflecting the extent to which animals had

progressed along the adaptive trajectory initiated by arboreality, each grade being represented by some currently living forms. Arising from an ancestral insectivoran stock, the most primitive grade is represented by tree shrews, with lemurs, tarsiers, monkeys, apes, and humans representing successively higher evolutionary grades. Le Gros Clark did not think that humans are actually derived from modern lemurs, monkeys, or apes, of course, but rather that the modern forms have departed little from the ancient forms from which humans are descended. His inclusion of tree shrews among primates was controversial, although it enjoyed the support of the prominent paleontologist G. G. Simpson, and modern workers reject this view for reasons to be explained below.

The work of Le Gros Clark had a large impact on neuroscience. In part, this was because Le Gros Clark was himself an important neuroanatomist and had much to say about brain evolution. Perhaps more significant, however, was his treatment of the different groups of living primates as representatives of the stages of primate and human evolution. This led neuroscientists to bring insectivores (such as hedgehogs), tree shrews, and bush babies into the laboratory for study, particularly by Irving Diamond (Diamond and Hall, 1969; see also Hodos and Campbell, 1969). Diamond trained many students and had a strong hand in shaping modern comparative neuroscience. Le Gros Clark's impact is also apparent in the work of Heinz Stephan and his associates (Stephan and Andy, 1969) on evolutionary changes in the sizes of brain structures in primate evolution, who used hedgehogs and other supposedly 'basal' insectivores as stand-ins for the initial stage of primate evolution, tree shrews and other supposedly 'advanced' insectivores as stand-ins for the next higher stage, the living prosimians as representatives of the next stage, and so forth. Although the evolutionary concepts that inspired the work of Diamond, Stephan, and their colleagues are now considered to be flawed, the data generated remain invaluable.

35.2.2.2 Modern ideas about primate origins The 1960s and 1970s witnessed the emergence of a new movement in evolutionary biology, initiated by the writings of Willi Hennig (especially Hennig, 1966), and usually referred to as 'cladism'. Cladism comprises a set of methods for reconstructing the phyletic relationships of species based on comparative studies of their characteristics, and a set of rules for classifying organisms based on these relationships. (Not all who subscribe to cladistic ideas about reconstructing phylogeny adhere to these rules of classification.) The basic goal of a cladistic analysis is to identify groups of species that constitute the complete set of species descended from a common ancestral species; these groups are described as being 'monophyletic' and are called 'clades'. A clade is, simply, any complete branch of the evolutionary tree, complete with subsidiary branches. Cladistic analysis leads to the enumeration of sets of features of the last common ancestor (LCA) of a monophyletic group that distinguish that ancestor (and its descendants) from other taxa. These features are referred to as 'shared derived characters' or 'synapomorphies'. These are the features that define the group. Of course, the species making up the group will have many other features that arose much earlier in evolution and that are shared with a wide array of other species, but these features are not helpful for determining the relationships of the group with its close relatives. So, for example, the fact that primates and carnivores both have hair says nothing about whether or not carnivores are close relatives of primates, because hair is an ancestral feature of mammals and is present in most living mammalian forms. Cladism, then, inspires efforts to identify the set of shared, derived characters that define monophyletic groups (clades) and requires workers to grapple with the issue of whether characters shared by different groups of animals are shared by virtue of common descent, and thus should be considered synapomorphies, or as the result of independent, convergent evolution, and thus of no value in determining relationships (although convergence can be very helpful in understanding how organisms adapt to particular environmental circumstances).

With the rise of cladism, Le Gros Clark's approach to primate evolution became passé, his focus on grades giving way to an emphasis on identifying sets of characters that could serve as defining features of the primate clade and its subclades. This approach was explicitly adopted by Robert Martin and is now an accepted part of evolutionary primatology (see especially Martin, 1990, and the contributions in Ravosa and Dagosto, 2006). A survey of generally accepted primate synapomorphies would include: a divergent (grasping) big toe (hallux) and possibly also a grasping thumb; the presence of flat nails, rather than claws, on at least some digits; the presence of a complete ring of bone around the eye (i.e., a 'postorbital bar'); large, forward-facing eyes; brain enlargement; reduction of the olfactory apparatus; and enlargement of the visual apparatus.

One consequence of defining primates in this way was to remove tree shrews from the order

(see Martin, 1990, and the contributions in Luckett, 1980). Not only do tree shrews lack many of the defining anatomical characteristics of primates, but some of the ways in which tree shrews resemble primates, for example, the presence of an enlarged visual apparatus and of a complete orbital ring, came to be judged as convergences not relevant to common ancestry. Comparative studies of the nervous system have revealed many other ways in which tree shrews differ from undisputed primates (see especially the reviews of Campbell, 1980, and Kaas and Preuss, 1993).

Once one is focused on the character states that define a group, it is natural to try to reconstruct the anatomy and behavior of the LCA of the group and to ask why the defining features of the group evolved. The modern project of framing adaptive explanations of primate origins begins with the work of Cartmill (1972, 1974b) and Martin (1973) and continues today (for major reviews, see Martin, 1990; Cartmill, 1992; and the contributions in Ravosa and Dagosto, 2006). This led to a major re-evaluation of the arboreal hypothesis. By the 1970s, it was appreciated that the earliest primates were anatomically similar to some of the smaller-bodied living prosimians, such as the smaller bush babies (genus *Galago*), mouse and dwarf lemurs (*Microcebus*, *Cheirogaleus*), and tarsiers (*Tarsius*). Examination of their behavior suggests why primates have opposable first digits, and digits tipped with nails, rather than claws: animals such as *Galago* and *Microcebus* make their living in a particular kind of arboreal environment, specifically, in the fine, terminal branches of trees and shrubs (Charles-Dominique, 1977). Claws are of less utility for grasping fine branches than are opposable thumbs and big toes, and nails would provide support for the broad terminal segments for digital grasping. This, in brief, is the 'fine-branch niche' hypothesis (Martin, 1990).

Classically, the convergent, front-facing orbits of primates were explained as adaptations for binocular overlap and stereopsis, presumably required for safe arboreal locomotion. As Cartmill noted, however, there are many arboreal mammals (e.g., squirrels), that manage quite well in an arboreal environment despite having eyes on the sides of the head. Cartmill pointed out that living vertebrates such as raptors and cats that have convergent eyes tend to be predators. Recognizing that many of extant, small-bodied prosimians make their living at least in part by capturing insects and small vertebrates, often grabbing them with their hands, Cartmill proposed that the large, convergent eyes of primates are adaptations for predation, with stereoscopic vision enabling an accurate hand grab. Allman (1977) and Pettigrew (1978) helped flesh out this theory by suggesting that front-facing eyes might have less to do with stereoscopic vision than with establishing a direct light path between objects in the environment and the central retina, resulting in a sharper image. This advantage would be accentuated in a nocturnal environment.

Combining the arguments discussed above, we have a picture of the primate LCA as a small-bodied, nocturnal predator foraging in a fine-branched niche. This constitutes a sort of working hypothesis that frames modern research on primate origins. One matter of controversy is the relative importance of predation in shaping primate anatomy and behavior. Sussman, for example, has emphasized the importance of foraging for fruit and/or flowers – both available in terminal branches – in selecting for the grasping extremities of primates (Sussman and Raven, 1978; Sussman, 1991). Recent paleontological evidence suggests that the defining characteristics of primates did not emerge as a unitary adaptive package, but rather that grasping extremities evolved prior to orbital convergence (Bloch and Boyer, 2002).

35.2.2.3 Primates among mammals: Grandorder Archonta In order to understand primate specializations, it is necessary to reconstruct the ancestral condition from which the specializations evolved. In phylogenetics, one reconstructs ancestral organization by 'out group' analysis, that is, by studying the animals most closely related to the in-group. To understand primate specializations, then, we need to know who primates are related to. Recall that in the work of Le Gros Clark and G. G. Simpson, primates were regarded as emerging from an insectivoran stock. Indeed, for much of the twentieth century, insectivores such as hedgehogs and shrews were regarded as the wellspring from which all eutherian orders emerged, with the result that mammalian evolution was depicted as more like a bush than a tree. Not everyone took this view, however: early in the twentieth century, W. K. Gregory proposed that primates did not simply emerge from a generalized insectivore form, but instead were part of a small set of related mammalian taxa consisting of elephant shrews, tree shrews, bats, and the so-called 'flying lemurs', who are not lemurs at all but rather small animals adapted for gliding. He called this collection of primates and related mammals the grandorder Archonta (Gregory, 1910).

As cladism began to exert its influence, Gregory's ideas were resurrected by a number of workers, notably McKenna (1975). McKenna modified

Gregory's concept of a grandorder Archonta to consist of primates (order Primates), bats (order Chiroptera), flying lemurs (order Dermoptera), and tree shrews (order Scandentia). Subsequent research marshaled comparative data on many aspects of the biological organization of primates and their putative relatives, including skeletal anatomy, soft-tissue anatomy, and molecular biology, with the result that the Archonta concept is now widely endorsed (MacPhee, 1993; Ravosa and Dagosto, 2006). Despite this, there has been considerable wrangling over the pattern of interrelationships among the putative archontans as well as disagreements about which taxa should be included in Archonta. Some argued that bats were not a natural, monophyletic group and that only one group of bats, the megachiropterans, are closely related to primates, and are, indeed, the sister group of primates (Pettigrew, 1986; Pettigrew *et al.*, 1989). This is the 'flying primate hypothesis', which has spurred much debate (MacPhee, 1993).

Recently, comparative genomic studies were undertaken by O'Brien and colleagues with the goal of settling the question of how the different mammalian orders are interrelated (Murphy *et al.*, 2001a, 2001b). They examined 18 homologous DNA sequences from a wide array of mammals, drawn from genomic databases, and reconstructed the mammalian branching order using maximum-likelihood and Bayesian techniques (Figure 1b). It is perhaps too soon to conclude that these studies have settled all the really significant questions about mammalian relationships, but they have important implications for students of primate evolution. In particular, they support a modified version of the Archonta hypothesis. Interestingly, although their results support the monophyly of bats, they indicate that bats are distantly related to primates, and cannot be considered archontans, which in their system consist of primates, tree shrews, and flying lemurs. In addition, their results indicate that the rodents and lagomorphs form a monophyletic group (superorder Glires), which may be the sister group of the Archonta. Collectively, the clade consisting of Archonta (now also known as Euarchonta) plus Glires is referred to as Euarchontoglires.

35.2.2.4 Haplorhine/anthropoid origins and hominin origins In principle, any subsidiary branch of the evolutionary tree of primates can be subjected to the sort of analysis that has been applied to the tree as a whole, that is, to identify the shared, derived characters that define the group and to develop and test adaptive accounts to explain the evolution of those characters. Much effort has been devoted to exploring the origins of the haplorhine anthropoid primates (for reviews, see The Role of Vision in the Origin and Evolution of Primates and the contributions in Ross and Kay, 2004a). Among the derived features of haplorhines and anthropoids are several specializations of the eye: tarsiers and anthropoids possess a true avascular fovea and they lack the reflecting tapetum lucidum (presumably an adaptation for nocturnal vision) found in strepsirhines. In anthropoids, the eye is isolated in a bony cup that may help to stabilize the image on the retina and the concentration of cones in the central retina is much higher than in strepsirhines. These changes, plus the fact that all living anthropoids save *Aotus* are diurnal, suggest an early shift to diurnality in stem anthropoids and concomitant specializations for high-acuity vision under daylight conditions. The olfactory apparatus is reduced in haplorhines compared to strepsirhines. Anthropoids also tend to be larger bodied than strepsirhines and live in larger social groups, consisting of multiple adults. Early anthropoids known from the fossil record, however, were very small, suggesting that increased body size evolved after the emergence of stem anthropoids. The larger size of anthropoids implies dietary changes, as larger-bodied primates tend to meet their protein needs by consuming leaves more than insects. The masticatory apparatus of anthropoids is more robust than that of strepsirhines, which makes it possible for them to include harder food items (such as unripe fruit) in their diet.

An evolutionary scenario that has been proposed to account for these features of anthropoids holds that the earliest (stem) haplorhines were small, diurnal predators, which selected for high visual acuity, with later stem anthropoids evolving increased body size and increased amounts of plant material in the diet (Ross and Kay, 2004b). Increases in social group size may have evolved early: some early fossil species have sexually dimorphic canines, which suggests the existence of male–male intragroup competition. In this scenario, tarsiers are seen as an early offshoot of the lineage leading to anthropoids, in part because their visual systems share certain features of anthropoids, such as the presence of a fovea and the absence of a tapetum lucidum. The living tarsiers are also obligate small-animal predators. Although living tarsiers are nocturnal, they are thought to derive from diurnal ancestors. The scenario holds that although extant tarsier species are nocturnal, they evolved from a diurnal ancestor, resulting in loss of the tapetum lucidum, and have adapted to nocturnality by evolving enormous eyes, much as owl monkeys did.

Among anthropoids, the clade that has received the most attention with regard to its origins and adaptations is of course that consisting of humans and their close relatives. Humans (*Homo sapiens*) are a recent offshoot of the African apes, the branching date being 5–10 Mya (Fleagle, 1999). Our closest relatives are a clade consisting of the common chimpanzee (*Pan troglodytes*) and the bonobo (*Pan paniscus*), followed by gorillas (*Gorilla gorilla*). Traditionally, species connected to the branch leading to humans after its split from the African apes were classified as members of the family Hominidae, or colloquially, as 'hominids', and the domain of human origins was referred to under the rubric of 'hominid evolution'. The recent recognition of the propinquity of humans to the apes has led to the expansion of the category Hominidae to include the great apes and humans, and the expansion of the subfamily Homininae to African apes and humans. As a result, the branch leading uniquely to modern humans is usually classified today at the tribe level (a tribe being a sub-subfamily). Humans, therefore, belong to the tribe Hominini, and the study of human origins is referred to as 'hominin evolution'.

We are fortunate to have an extensive physical record of human origins; this permits us to document evolutionary increases in hominin brain size from the study of fossils and behavioral changes from the archeological record. Hominins underwent a fantastic increase in brain size – modern human brains are approximately three times larger in volume than those of chimpanzees – and the fossil record indicates that most of the increase in brain volume took place during the last 2 million years. By contrast, bipedalism, that peculiar way humans have of getting around in the world, appeared much earlier in hominin evolution.

After many decades of focusing on the similarities between humans and apes, students of cognition have recently begun to appreciate the many respects in which humans are specialized relative to our ape cousins. Humans, of course, have a unique and highly developed system of symbolic representation and communication – language (see The Evolution of Language Systems in the Human Brain). The evolution of language may well be related to the extreme degree of hemispheric asymmetry exhibited by humans, although language is by no means the only function that is strongly lateralized in humans (see The Evolution of Hemispheric Specializations of the Human Brain). Recent research points to the existence of a variety of additional higher-order cognitive systems that mediate our understanding of the physical interactions of objects and the our inferences about the causes and mechanisms of the behavior of other organisms, and of ourselves (see Neurological Specializations for Manual Gesture and Tool Use in Humans). The human propensity to concoct narrative accounts of events, and to maintain them even in face of contrary evidence, would seem to reflect the interaction of the language system with systems for representing causation. Human cognitive specializations can in many instances be viewed as components of a broader human adaptation for culture, permitting individual humans to acquire the know-how, ideas, and values of their community, and making it possible for communities to adapt to an extraordinary variety of environmental conditions (Richerson and Boyd, 2005).

35.3 Topics and Issues in Primate Brain Evolution

35.3.1 Encephalization and Gross Morphology

We think of primates as being highly encephalized creatures. This is true of modern anthropoid primates, which are about twice as encephalized as 'average' modern mammals (Jerison, 1973). Modern strepsirhines, however, are not notably encephalized compared to other extant mammalian groups. Nevertheless, there is reason to think that brain size expansion was an early feature of primate evolution. As with other groups of mammals, notably carnivores, ungulates, and cetaceans, the order Primates underwent expansion of the brain relative to body size (encephalization) throughout its history (Jerison, 1973). At any given time period in the Cenozoic, however, primates appear to have been more encephalized than most of their contemporaries (Jerison, 1973). This was the case even early in the Cenozoic, prior to the diversification of the anthropoids. Moreover, Sacher (1982) argued that primates (including strepsirhines) commit a disproportionately large fraction of metabolic resources to brain growth in early development, so that at birth, primate brains are about 12% of total body size, compared to about 6% for most other mammalian orders.

Just as relative brain size increased independently in different mammalian orders, it is likely that there was some degree of independent encephalization among primate groups. Early primates from the Eocene (~55–34 Mya) that resemble modern strepsirhines in many features of anatomy were substantially less encephalized than modern strepsirhines. Early anthropoids may also have been less encephalized than modern anthropoids. For example, it has been argued

that *Aegyptopithecus*, dated to about 33 Mya and generally accepted to be a catarrhine, had a brain about half the size of modern catarrhines of similar body size (Jerison, 1979). This suggests that although relative brain sizes are similar in New World and Old World anthropoids, some brain-size enlargement occurred independently in the two groups.

Primate brains are not simply uniformly enlarged versions of some common mammalian brain – particular regions, such as the neocortex, underwent disproportionate enlargement. The quantitative neuroanatomical studies of Stephan and colleagues, alluded to above, have documented differences in the sizes of brain components across a substantial range of primate and insectivoran species (Stephan and Andy, 1969; Stephan, 1972; Stephan *et al*., 1988), and their data set has been widely used to generate and test hypotheses about primate evolution. Unfortunately, however, most of the brain structures they measured are too large (e.g., neocortex, cerebellum) to correspond to meaningful functional units of the brains. Moreover, structures can undergo substantial modifications of internal organization without undergoing substantial size change. For example, the absolute size of the primary visual area is similar in humans and chimpanzees (Frahm *et al*., 1984), but the internal organization of the area is quite different in certain respects (Preuss and Coleman, 2002). Nevertheless, it is possible to say something about the relationship between the enlargement of particular brain regions in primate evolution and the evolution of the external form of primate brains.

The external morphology of primate brains reflects, to a considerable degree, the degree and pattern of enlargement of the visual cortex, which involved the expansion of the primary visual area, area V1 (an area common to most, if not all, other mammals), as well as the addition of new areas (see especially, Allman, 1977, 1982, 1999; Kass, 1977, 1982, 1987). In most strepsirhines and haplorhines, the cortex devoted largely or exclusively to the visual modality encompasses approximately half the cortical mantle (Allman, 1977, 1982). The expansion of area V1 was probably accompanied by the evolution of a distinctive sulcal configuration, the triradiate calcarine fissure, which consists of a retrocalcarine sulcus (the familiar 'calcarine fissure' of the neuroscientific literature) and ascending and descending branches expending from the anterior end of the retrocalcarine (Martin, 1990). This triradiate configuration is found in most living primates (Martin, 1990) and was probably present in early primates.

Expansion of the visual cortex is also reflected dramatically in the unusual morphologies of primate temporal lobes. In most extant strepsirhines and anthropoids, the temporal lobe forms a distinct ventral island of cortex, demarcated from the frontal and parietal lobes by a deep lateral (Sylvian) fissure. Much of the temporal lobe consists of visual cortex (the inferotemporal cortex) and multimodal cortex with major visual inputs (the superior temporal sulcal (STS) cortex). The inferotemporal and multimodal STS regions appear to be neomorphic in primates (Preuss and Kaas, 1999; Preuss, 2006). The ventral expansion of visual cortex influenced the morphology of adjacent regions. In most mammals, the hippocampus and associated tissues form an arc around the posterior end of the corpus callosum, whereas in strepsirhine and haplorhine primates, the hippocampus has a more inferior location, as though the temporal lobe in which it resides were rotated around the posterior pole of the callosum. The orientation of auditory cortex, located superiorly in the temporal lobe, appears to reflect the torque of the temporal lobe; in most mammals, the map of auditory frequencies in the primary auditory area (A1) is arranged with high frequencies anteriorly and low frequencies posteriorly, whereas in primates, high frequencies are represented in the superior and posterior part of A1 and low frequencies inferoanteriorly (Stiebler *et al*., 1997).

Although the visual cortex evidently underwent early expansion, it has been argued that the frontal lobe initially remained quite small, undergoing major expansion only later in primate evolution (Radinsky, 1970; Jerison, 1973; Gurche, 1982). Judgments of frontal lobe size depend, however, on how you determine the border between frontal lobe and its neighbors on the lateral surface, the parietal and temporal lobes, and this is not as simple a matter as one might suppose. In most anthropoid primates, there is a deep central sulcus that separates the frontal and parietal lobes. Tarsiers and most strepsirhines lack a central sulcus, however, and it is typically not apparent in the endocasts of early primates (Radinsky, 1970; Gurche, 1982), so the frontal–parietal boundary cannot be determined with any precision in the endocasts. We should therefore be cautious in drawing conclusions about the size of the frontal lobe relative to other cortical regions in early primates.

Identifying the frontal–temporal boundary would seem to a more straightforward affair: in most modern strepsirhine and anthropoid primates, a deep Sylvian fissure divides the temporal lobe from the frontal and temporal lobes. Some of the endocasts of early primates, such as the specimen of the

lemur-like *Adapis* illustrated by Radinsky (1970) and by Gurche (1982), also preserve an impression that is plausibly interpreted as a Sylvian fissure. Thus, it is tempting to conclude that the presence of a Sylvian fissure is an ancestral feature of primate organization.

Nevertheless, Sylvian fissure is evidently not, a feature of all primates, living and extinct. Tarsiers, for example, lack a Sylvian fissure (Le Gros Clark, 1959; Collins *et al*., 2005), so that the insular cortex, which is buried in the depths of the Sylvian fissure in most primates, is exposed on the surface of the tarsier brain (Woolard, 1925). The exposed insular cortex and adjacent portions of frontoparietal cortex appear to form a nearly vertical ridge where they join the remainder of frontal cortex. Le Gros Clark (1959) suggested that the peculiar morphology of tarsier brains results from the enormous enlargement of the orbits in these animals, effectively indenting the ventral portion of the frontal lobe and anterior portion of the temporal lobe. The morphology of tarsiers, however, may not be unique among primates: illustrations of endocasts of extinct primates from the Eocene show a diversity of sulcal patterns (Radinsky, 1970; Jerison, 1973; Gurche, 1982), some with typical Sylvian fissures and some that could have morphologies more like those of extant tarsiers. Moreover, the extant lemur *Daubentonia* (the aye-aye) also appears to lack a typical Sylvian fissure (Kaufman *et al*., 2005; Le Gros Clark, 1959), and the sulci that are present seem rather shallow for a brain of its size (Kaufman *et al*., 2005). Thus, we should probably be cautious in ascribing to the LCA of primates a Sylvian fissure morphology like that found in most extant primates.

A number of additional sulci have been described in primate brains. Primates, like other mammals, possess a rhinal fissure, which separates the hippocampal and entorhinal cortex from neocortex. Most primates possess a cingulate sulcus on the medial wall superior to the corpus callosum, although in smaller primates this may be just a shallow groove. Many extant strepsirhines possess on the lateral surface of the hemisphere an elongated, anteroposteriorly situated groove, termed the coronolateral sulcus (Radinsky, 1975), which in smaller-brained forms may be broken up into two or three segments, that have been termed from posterior to anterior, the intraparietal sulcus (IPS), sulcus e, and sulcus rectus (Connolly, 1950; Haines *et al*., 1974). The sulcus rectus, a term which has also been applied to the principal sulcus (PS) of Old World monkeys (Falk, 1978, 1982), has also been referred to as sulcus frontalis in lemurs (Brodmann, 1905, 1909). The use of the terms 'intraparietal sulcus' and 'sulcus rectus' (or 'principal sulcus') suggests that the cortical areas within which these grooves are found in strepsirhines are homologous to the areas within which the corresponding grooves are found in anthropoids, and specifically in Old World monkeys. Caution is in order, once again: Preuss and Goldman-Rakic (1991c), in their studies of frontal lobe organization in *Otolemur* (formally *Galago*) *crassicaduatus*, argued that the sulcus rectus marks the border between premotor and granular prefrontal cortex, and thus differs from the PS of macaques, which lies entirely within prefrontal cortex. Similarly, they noted that whereas the IPS of macaques separates area 7 and its subdivisions from area 5, the IPS of *Otolemur* lay entirely within the territory of area 7 (Preuss and Goldman-Rakic, 1991a). Thus, inferences about the areal organization of the cortex of different primates based on the configurations of sulci are problematic, especially when comparing relatively distantly related taxa such as *Macaca* and *Otolemur*.

35.3.2 Chemical Senses

Whereas primates are characterized by a well-developed visual system, evolution of the chemical senses mainly involved reductions or degradative changes (Figure 2). Evolutionary reductions are especially notable in the main and accessory olfactory systems, changes that were, however, probably concentrated in haplorhine or anthropoid phylogeny. By contrast to haplorhines, strepsirhines have comparatively well-developed olfactory systems, and it is likely that they retain many features shared with other mammalian groups. Unfortunately, we have very little modern information about the olfactory systems of the taxa most closely related to primates.

Evolutionary changes in the olfactory systems in primates were accompanied by changes in the anatomy of the primate face. Most mammals possess two distinct olfactory systems: a main system, with receptors in the epithelium of the nose, and an accessory system, with receptors located in a specialized zone of epithelium buried within the upper jaw known as the vomeronasal organ (VNO; also known as Jacobson's organ) (Martin, 1990). The accessory olfactory system is usually thought to be involved principally in the detection of pheromones. In most mammals, there is a physical connection between the two olfactory systems. Most mammals have a naked, glandular external nasal membrane – a wet nose, in other words. This membrane extends down to the mouth where it is anchored to the soft tissue of the upper jaw. Some mammals (including

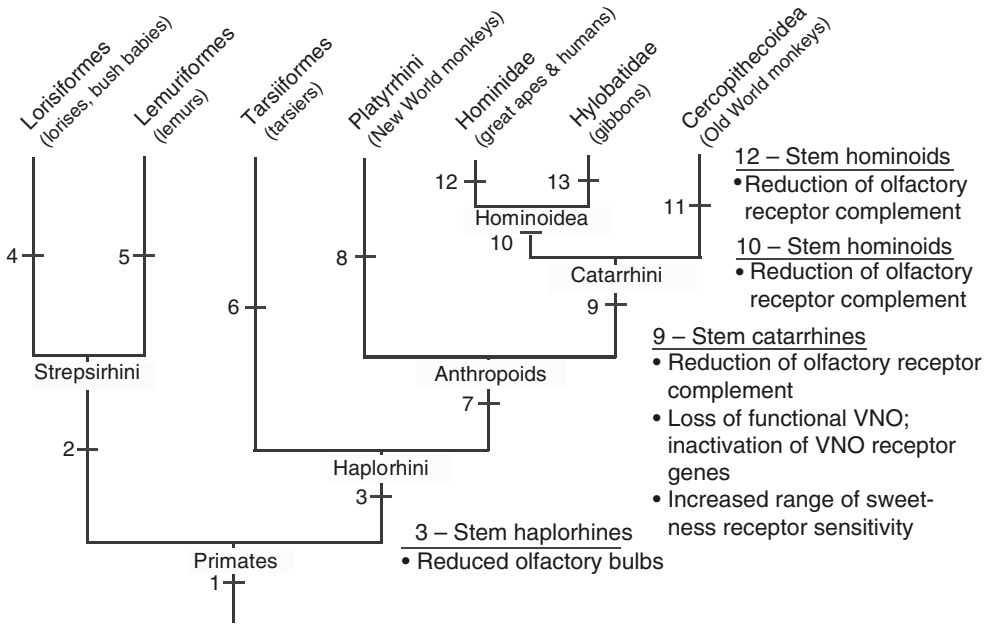

Figure 2 Evolutionary changes in the portions of the central nervous system devoted to the chemical senses. In this and following figures, characters interpreted as derived are mapped onto specific segments of the primate tree; the segments are numbered to facilitate comparisons across figures.

strepsirhine primates) have a well-developed median cleft extending from the external nose to the space between the upper incisors. Ducts in the anterior palate allow passage of liquid from external nasal membrane to Jacobson's organ. Ducts also interconnect Jacobson's organ with the nasal cavity, superiorly.

Although the familiar haplorhine/anthropoid primates lack an exposed rhinarium of the type just described, the living strepsirhine primates exhibit this ancestral mammalian condition. The retention of ancestral nasal morphology in strepsirhines is accompanied by a generally well-developed central olfactory systems: compared to haplorhines, the main olfactory bulbs are quite large (Stephan *et al*., 1988). The VNO and accessory olfactory bulbs are present, consistent with the apparent importance of pheromonal communication in extant strepsirhine primates (Charles-Dominique, 1977).

The VNO, however, underwent dramatic changes in anthropoid evolution. New World monkeys evidently possess functional VNOs, but they appear to be greatly reduced in Old World anthropoids, to the point of being vestigial (hominoids) or absent (in Old World monkeys). This doesn't necessarily imply that pheromonal communication is absent in Old World anthropoids, as it is possible that pheromones can be detected by receptors of the main olfactory system (Wysocki and Preti, 2004).

Information from olfactory receptors in the nasal epithelium reaches the brain through projections to the olfactory bulbs, which project in turn to the thalamus, amygdala, and ultimately to portions of the orbital and insular cortex. Taste inputs are relayed through the brainstem and thalamus, from which they also reach orbito-insular cortex. We know relatively little about evolutionary changes in the central pathways or structures representing the chemical senses in primates. One change that is well documented, however, is the evolutionary reduction in the size of the olfactory bulb in haplorhines: by comparison to haplorhines, the olfactory bulbs of strepsirhines are enormous (Stephan and Andy, 1969; Stephan, 1972; Stephan *et al*., 1988). The olfactory systems of strepsirhines are clearly more conservative than those of haplorhines.

The sequencing of the genomes of a variety of mammalian species has spurred comparative studies of olfactory receptor (OR) and VNO receptor genes. These make ideal subjects for comparative genomics, because mammals possess more than 1000 different OR genes, comprising multiple gene families, and on the order of about 250 VNO receptor genes. These investigations indicate that changes in olfactory and VNO receptor genes parallel the morphological changes described above. The morphological reduction of the olfactory system was accompanied by the accumulation of mutations that transformed functional OR genes into

pseudogenes. The fraction of OR genes that is nonfunctional is higher in Old World monkeys than New World monkeys, higher still in hominoids, and highest in humans, among the hominoids that have been examined (Rouquier *et al*., 2000; Gilad *et al*., 2004). In humans, more than 50% of the OR genes have mutations that should render them nonfunctional (Gilad *et al*., 2005). This suggests a relaxation of selection pressures on OR genes in catarrhine primates, at least. Despite this, there is also evidence for positive selection in certain subsets of OR genes, even in humans (Gilad *et al*., 2005). Similar to the situation in the olfactory system, the morphological diminution or loss of the VNO in catarrhines was accompanied by the fixation of mutations that render inactive the *TRPC2* and *V1R* genes, which code for proteins essential for transducing signals from VNO receptors (see also Liman and Innan, 2003; Zhang and Webb, 2003).

We know very little about the evolution of taste in primates, although comparative molecular and genetic studies are providing some new insights. In humans, five basic tastes are recognized: sweet, sour, bitter, salty, and umami (a savory flavor). The number of taste-receptor genes is evidently much smaller than the number of OR genes. Three genes in the *TAS1R* family are believed to be involved in the perception of sweet and umami, with the protein products of the different gene family members combining to form heterodimers with different sensitivities. Bitter perception is mediated by a set of approximately 25 genes in the *TAS2R* family. The basis of salty and sour perception is poorly understood (Drayna, 2005).

Glaser has investigated the evolution of sweet perception in primates and found that humans and other catarrhines perceive more chemical compounds as being sweet than do other primates. The basis for this increased range of sensitivity reflects modifications of the structure of *TAS1R* receptors rather than the addition of new receptors.

35.3.3 The Visual System

35.3.3.1 Eye and retina Ancestral primates are thought to have been nocturnal animals, and the eyes of modern strepsirhine primates, most of which are nocturnal, retain the hallmarks of this heritage. For example, strepsirhines, in contrast to haplorhines, lack a retinal fovea and most strepsirhines have a reflective membrane, the tapetum lucidum, that is found in many nocturnal mammals and presumably enhances vision under low-light conditions (Figure 3). The tapetum is absent in some modern diurnal lemurs (Martin, 1990). The complement of photoreceptor cells in strepsirhine primates is also typical of nocturnal mammals: in addition to rods, which respond to light over a broad spectrum of wavelengths, strepsirhines have two varieties of cones, which respond to short-wavelength light (S cones) and medium- to long-wavelength light (M/L cones). Most nonprimate mammals that have been examined also have two-cone systems (Jacobs, 1993). Interestingly, mutations of the gene coding for the S-cone visual pigment have rendered this gene nonfunctional in the lorisiform (loris–bush baby) group of strepsirhines, which are nocturnal, and also in the nocturnal New World owl monkey, *Aotus* (Jacobs *et al*., 1996a; Kawamura and Kubotera, 2004). Although lacking a fovea, strepsirhines do possess a central specialization with a high density of photoreceptors, so that visual acuity is greatest in the central part of the visual field. The central specialization is populated by small rods and cones. In contrast to anthropoids, in which rods and cones are distinguishable by the morphology of their outer segments, strepsirhine rods and cones are similar in appearance and the existence of cones in strepsirhines was not definitively demonstrated until antibodies for cone-specific opsins were developed (Wikler and Rakic, 1990).

The eyes of tarsiers and of anthropoids differ markedly from those of strepsirhine primates. As both taxa lack a tapetum lucidum, this was probably lost prior to the tarsier–anthropoid divergence. A fovea is present in *Tarsius* and in anthropoids, and presumably evolved in stem haplorhines (Ross, 2004) although the density of retinal ganglion cells (the cells that transmit visual information to the brain) in the tarsier fovea is more typical of a nocturnal strepsirhine than a diurnal anthropoid (Collins *et al*., 2005). Tarsier foveae contain both rods and cones (Hendrickson *et al*., 2000), whereas the central retina (containing the fovea) of diurnal anthropoids is populated by small cones to the virtual exclusion of rods.

The ancestral cone complement of haplorhines was most likely a two-cone system (Heesy and Ross, 2001), although it may have been a polymorphic system with some individuals having two cones and other individuals having three (Tan and Li, 1999). Most New World monkeys have only a single M/L gene, located on the X chromosome, but there are different alleles for this gene, each allele producing a protein with a different spectral sensitivity. Thus, most New World monkey populations are polymorphic for cone opsins, and because the M/L alleles are located on the X chromosome, many

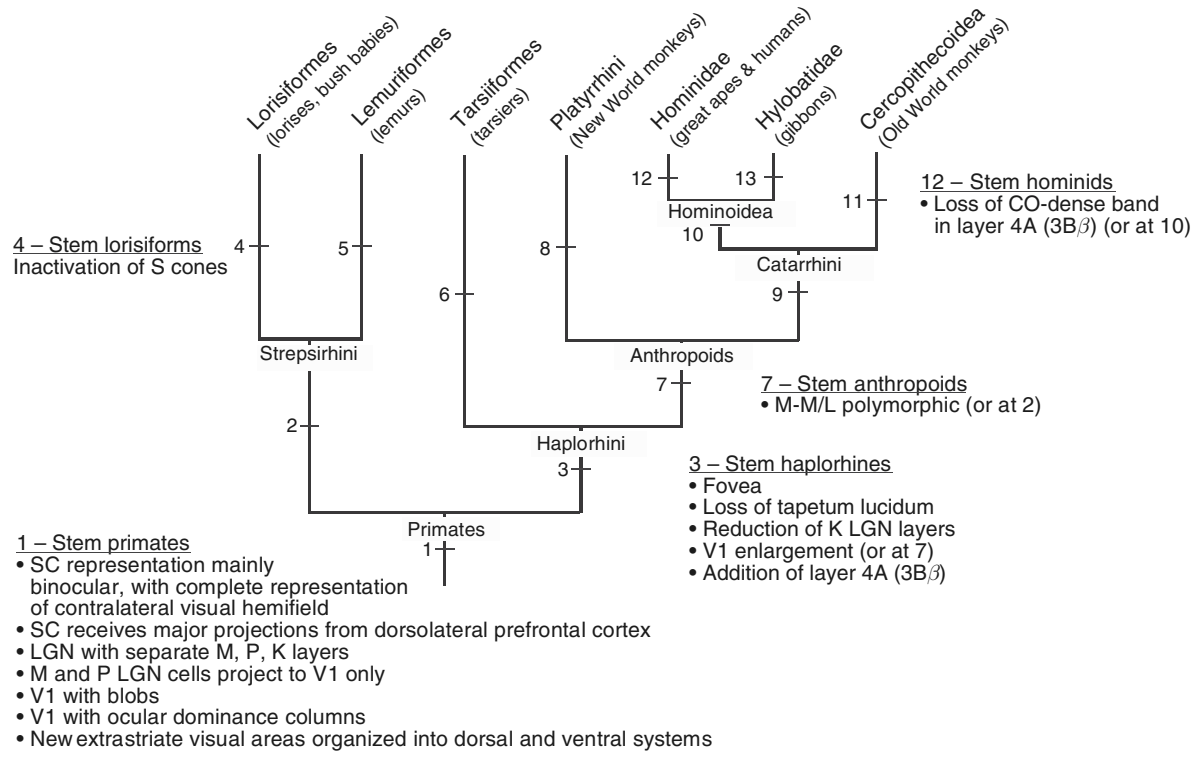

Figure 3 Interpretation of the shared derived features of the visual system in primates and major primate subgroups. For more details of the visual system specializations of hominoids, see Preuss and Coleman (2002) and Preuss (2004a).

females have two different M/L alleles. These individuals are psychophysically trichromatic. Catarrhine primates have a three-cone system, the ancestral M/L opsin gene having duplicated to yield different M and L genes, both located on the X chromosome. (A similar event occurred independently in the New World howler monkeys, *Alouatta* spp.; Jacobs *et al.*, 1996b). Extant tarsier species have two-cone systems, but there is sufficient variation in the M/L genes of different tarsier species to prompt the suggestion that the ancestral condition for tarsiers (and thus for haplorhines, given the situation in New World monkeys), was polymorphic (Tan and Li, 1999).

The recent appreciation that many nocturnal primates have functional cones has had a substantial impact on thinking about color vision and its role in primate evolution. For one, it is now quite common to see primates referred to as 'trichromats', 'dichromats', or 'monochromats', based on their complement of cone pigments. For another, the likely existence of a two-cone system in ancestral primates is regarded by some as being incompatible with the claim that the primate LCA was nocturnal (Tan *et al.*, 2005). The latter conclusion would seem to assume that the function of cones is to permit color discrimination of the sort anthropoid primates are capable of. Anthropoid color discrimination certainly requires conditions of high illumination, so that color discrimination is essentially lost under nocturnal conditions. The relationship between cone types and color perception is by no means direct, however. So, for example, the ability to discriminate green and red in catarrhine primates is not simply a consequence of the fact that catarrhines have dedicated green and red photoreceptors. Although it is tempting to think of the M and L receptors as green and red receptors, in fact, the sensitivity peaks of the M and L pigments are about 530 and 560 nm, respectively, which correspond to the green and yellow-green parts of the visible spectrum. The discrimination of red and green depends on the opponent (subtractive) interactions in the retina between M and L cones. These kinds of interactions evidently require high cone densities, such as those which occur in the anthropoid fovea (Dacey, 2000; Callaway, 2005). Yet anthropoids have cones in the retinal periphery, where they occur at much lower density than in the fovea, and many other mammals, both nocturnal and diurnal, have cones present at low density across the retina. The comparative psychophysical data suggest that nocturnal species have very poor color discriminative abilities, even those that have functioning S and M/L cones (see Jacobs and

Deegan, 2003). Indeed, even diurnal lemurs have very poor color discrimination (Blakeslee and Jacobs, 1985). It seems likely that the cones of most mammals are mainly doing something other than generating color-opponent signals such as those that form the basis for the fine color discrimination of anthropoids, for example, enhancing detection sensitivity in certain parts of the spectrum (e.g., Martin, 1990, p. 303; Winter *et al.*, 2003). It would be useful to have more experimental behavioral data about the vision of strepsirhine primates and nocturnal mammals; genetic, physiological, and anatomical studies of the visual systems of nonhuman species have advanced in recent years, but psychophysical and behavioral studies have not kept pace. We should be very cautious about making inferences about the color-vision capabilities of animals based on the presence or absence of cones expressing particular photopigments. To do so is, in effect, to adopt the anthropoid fovea – as specialized a bit of neural machinery as any we know of – as a general model of the mammalian retina.

35.3.3.2 Lateral geniculate nucleus and superior colliculus The retina sends visual information to numerous brainstem structures, of which we will consider only two here, the lateral geniculate nucleus (LGN) and the superior colliculus (SC), both of which underwent important changes in primate origins and evolution. The output from the retina is conveyed by the axons of retinal ganglion cells (RGCs). Currently, three main classes of RGCs are recognized, which in primates are usually termed M, P, and K cells, because they project to the magnocellular, parvocellular, and koniocellular layers of the LGN, respectively. These are probably homologous to the Y-, X-, and W-type cells described in other mammals (see The Evolution of Parallel Pathways in the Brains of Primates). The different types of RGCs convey different types of visual information. At least some of the P-type cells carry color-opponent information, but it is likely that some P-type cells are not color selective and there is evidence that some K-type cells have a special relationship to S cones, so these may be involved in color processing as well (Callaway, 2005).

The LGN is important as a relay for visual information to the cerebral cortex. The structure typically appears to be composed of separate cell laminae, and in primates the lamination is very conspicuous. Moreover, primate LGNs are laminated in distinctive ways (see especially Kaas *et al.*, 1978; Kaas and Preuss, 1993; see The Evolution of Parallel Pathways in the Brains of Primates and citations therein). In most mammals that have been studied in detail, M- and P-like cells are mixed in at least one layer of the LGN. In primates, however, M and P cells are strictly segregated, and there is a pair of M and P layers, one for each eye, yielding a fundamental four-layered pattern. In catarrhines, including humans, the P layers subdivide and interleave, yielding the 'six-layered' pattern described in neuroanatomy textbooks. Strepsirhine primates also have a pair of distinct K layers, sandwiched between the two P layers. In anthropoids, the K cells do not form distinct layers, but rather mainly occupy territories between the M and P layers. The nocturnal owl monkeys are unusual among anthropoids in having a well-developed single K zone located between the outermost M layer and the innermost P layer. Interestingly, tarsiers are reported to resemble anthropoids more than strepsirhines in their pattern of LGN lamination, and like owl monkeys, have a relatively well-developed K zone between the M and P layers (Collins *et al.*, 2005).

The SC occupies the most superior part of the midbrain. It is homologous to the structure referred to as the 'optic tectum' in most vertebrates, a name that reflects the fact that this structure plays an important role in visually guided behavior. The SC is a laminated structure; the upper layers receive visual inputs while the deeper layers receive auditory and somatosensory inputs (Huerta and Harting, 1984). Outputs from the SC descend to motor nuclei in the brainstem, especially those involved in eye movements, while ascending projections target the K layers of the LGN and the inferior pulvinar nucleus of the thalamus. Large regions of the neocortex project to the SC, although primates are distinctive among mammals in having a massive projection to the SC arising from the dorsolateral prefrontal cortex (Preuss, 2006).

Primates are also distinctive in the pattern of retinal projections to the SC. In most mammals, the SC receives its visual inputs mainly from the retina of the contralateral eye, with a small and variable contribution from the ipsilateral retina. In primates, by contrast, the SC receives strong inputs from both retinas, but only from the portion of each retina that represents the contralateral visual field. Thus, each SC in primates contains a 'map' of the contralateral half of visual space, whereas nonprimate SCs represent both contralateral and ipsilateral visual space, since the contralateral retina represents both parts of visual space (Lane *et al.*, 1973). This primate–nonprimate difference is well established (reviewed by Kaas and Preuss, 1993; Preuss, 2006). Pettigrew and colleagues have argued that

megachiropteran bats have a primate-like SC, and cite this and other evidence that megachiropteran bats should be considered the sister group of primates (Pettigrew, 1986; Pettigrew *et al*., 1989). The primate-like character of megachiropteran bats has been disputed (Thiele *et al*., 1991), and defended (Rosa *et al*., 1996), but in any event, there is now considerable evidence from comparative genomic studies indicating that megachiropteran bats are not closely related to primates (Murphy *et al*., 2001b). To the extent that megachiropterans resemble primates, therefore, those similarities are probably the result of evolutionary convergence. Preuss (2006) suggests that the adaptive significance of the primate specialization of SC organization lies in the fact that individual SC neurons receive inputs from both eyes, and speculates that the primate SC uses binocular disparity to make precise adjustments of eye movements and visually guided reaching and grasping movements.

35.3.3.3 Primary visual area The principal target of projections from the visual system to the cortex is area V1, also known as area 17 (after Brodmann, 1909) and as the striate area, so-called because of the horizontal stripe of myelin characteristic of this area in primates. Area V1 is present in all mammalian groups that have been studied and is undoubtedly one of the 'heritage' areas that was present in the early mammals (Kaas, 1995). In primates, as in other mammals, visual information reaches area V1 via the LGN and from the pulvinar nucleus of the thalamus (in primates, from the inferior part of the pulvinar, specifically). Notwithstanding these commonalities, primates possess specializations of V1 that distinguish them from other mammals, and there are also prominent differences in V1 organization within the primate order (see The Evolution of Parallel Pathways in the Brains of Primates; Kaas, 1993; Preuss, 2004a, 2006).

One distinctive feature of primate V1 is its pattern of inputs from the LGN (reviewed by Preuss, 2006). Virtually the entire cortical projection of the LGN reaches area V1 exclusively; only the K layers of the LGN project to other, 'extrastriate' cortical visual areas, and those projections are rather weak. In the nonprimates that have been examined, there are major LGN projections to extrastriate areas. The responsiveness of extrastriate cortex to visual stimulation depends more strongly on V1 in primates than in other mammals.

Another distinctive feature of primate V1 is its appearance when stained for cytochrome oxidase (CO), a metabolic enzyme. CO staining reveals a regular, repeating series of dark patches in the upper layers of area V1; these patches are technically known as 'blobs'. Blobs have been described in all strepsirhine and haplorhine primates that have been examined with appropriate histological material (Preuss and Kaas, 1996), including, most recently, tarsiers (Collins *et al*., 2005). Tree shrews lack blobs, as do representatives of many other mammalian orders that have been examined for blobs (reviewed by Preuss, 2006). Some carnivores exhibit blob-like staining, but because carnivores are not closely related to primates, this is almost certainly a case of convergence (Preuss and Kaas, 1996; Preuss, 2000a). Although blobs are a common feature of primate organization, their connections and functions are matters of debate. Published descriptions of connectivity indicate that blobs receive direct projections from the LGN, including the K layers, but details of these connections differ between studies and there could be genuine species differences (see The Evolution of Parallel Pathways in the Brains of Primates).

The strongest projections to V1 from the LGN in primates, as in most other mammals examined, terminate in the middle levels of cortex, specifically within a stratum of very densely packed small cells (granule cells) designated as cortical layer 4. Projections from the P layers target the deep part of layer 4, whereas projections from the M layers target its more superficial part. Interlaminar connections relay connections from layer 4 to more superficial and to deeper layers of cortex. This pattern of organization is common to the strepsirhine and anthropoid primates that have been examined. Anthropoid primates, however, have elaborated the organization of the upper layers of cortex. In particular, anthropoids possess an additional band of densely packed small cells above layer 4 that is separated from it by a narrow band of larger, more sparsely arranged cells. There are different ways of naming these layers: most workers follow (Brodmann, 1909) in referring to the upper band of small cells as layer 4A, the sparse band of larger cells as layer 4B, and the deeper, thick band of small cells as layer 4C. Others believe that the bands called 4B and 4A by Brodmann should be considered as subdivisions of layer 3; in this terminology, Brodmann's layer 4A corresponds to layer 3Bβ (see The Evolution of Parallel Pathways in the Brains of Primates). In most New World and Old World monkeys that have been examined, layer 4A/3Bβ receives a direct input from the parvocellular layers of the LGN, and there is a band of dense CO staining coincident with this input. Among New World and Old World monkeys, only *Aotus* is known to lack a direct LGN projection to layer 4A/3Bβ, and it lacks the corresponding CO-dense band, also

(Horton, 1984). Although we do not have information about the connectivity of tarsier V1, histologically, the lamination of this area resembles that of anthropoids more than strepsirhines (Collins *et al.*, 2005). We also have virtually no information about the connectivity of area V1 in apes or humans, but its histology differs from that of monkeys: a band of small cells corresponding to layer 4A/3Bβ is present, but it lacks a CO-dense band (Preuss *et al.*, 1999), which suggests that its connectivity differs from that of monkeys. Additionally, layer 4A/3Bβ of humans exhibits histological features that differ markedly from those of apes (Preuss *et al.*, 1999; Preuss and Coleman, 2002). Since area V1 is a major source of visual information for extrastriate visual areas, these species differences in the processing of visual information in V1 could ramify through the cortical visual system.

In primates, the projections of the LGN to layer 4 of area V1 are segregated by eye, forming a set of alternating, elongated ocular dominance columns (Hubel and Wiesel, 1969). Although the degree of ocular segregation in adult individuals varies across species, some degree of ocular segregation has been found in every primate species examined, while ocular dominance is absent in the nonprimate species that have been examined with the exception, again, of carnivores (Horton and Hocking, 1996).

35.3.3.4 Extrastriate visual cortex Primates possess a large region of extrastriate visual cortex comprised of multiple, retinotopically organized visual areas (see the reviews of Kaas, 1995; Tootell *et al.*, 1996; Rosa, 1999; Orban *et al.*, 2004; Sereno and Tootell, 2005). These have been extensively studied in the New World and Old World monkeys, and to a lesser extent in strepsirhine primates, using microelectrode mapping techniques, tract-tracing experiments, and histochemical (architectonic) methods. Histochemical and functional imaging techniques have recently been applied to the study of extrastriate cortex in humans. From these studies, it is clear that there is a large number of extrastriate visual areas in anthropoids – at least 15 and perhaps many more. The diversity of opinion regarding the numbers of areas reflects in part the application of different criteria for assigning areas to the visual realm – does an area need to be exclusively visual to qualify, or need it only have a prominent visual input? – as well as differing interpretations of experimental data. Within the primate extrastriate cortex, investigators have determined that the extrastriate areas and their interconnections form two broad information processing pathways: a dorsal stream, which begins in V1, traverses the middle temporal (MT) visual area, and ends in the posterior parietal cortex, and a ventral stream, which begins in V1, traverses area V4 (also called the dorsolateral visual area, DL, in New World monkeys), and ends in the inferior temporal (IT) region. These pathways are functionally specialized: the ventral and dorsal pathways have been characterized as the 'what is it' and 'where is it' systems, respectively (Ungerleider and Mishkin, 1982). While recent work supports the idea that the ventral pathway is specialized for the visual identification of objects, it has been argued that the functional specialization of the dorsal pathway pertains to 'vision for action', that is, for organizing eye and hand movements to objects in nearby space (Goodale and Milner, 1992).

Present evidence does not provide a definite indication of differences in the complement of extrastriate areas between Old World and New World monkeys. Moreover, the evidence from humans suggests a pattern of extrastriate organization similar in many important respects to that of Old World and New World monkeys (Orban *et al.*, 2004; Sereno and Tootell, 2005). One might reasonably suppose, however, that anthropoid primates have more visual areas than strepsirhines. For one thing, the evolution of haplorhines and anthropoids was accompanied by the appearance of a fovea and modifications of retinal receptor distribution and circuitry supporting color vision. For another, the visual region makes up a large fraction of the cortical mantle in strepsirhines and haplorhines, but strepsirhines are about half as encephalized as anthropoids. Nevertheless, at present there is no clear evidence that strepsirhines have fewer visual areas than do New World or Old World monkeys. This may reflect the fact that much less effort has been devoted to the study of visual cortex in strepsirhines than in anthropoids. This said, however, the studies that have been carried out in strepsirhines indicate that they possess many areas in common with anthropoids (e.g., Rosa *et al.*, 1997; Lyon and Kaas, 2002), and there is evidence from histological and connectional studies that the strepsirhine extrastriate region is divisible into dorsal and ventral streams, as in anthropoids (Preuss, 2006). (It would be very valuable to compare the organization of inferotemporal cortex in strepsirhines and in anthropoids, since this region would likely have been affected by the evolution of specializations of foveal vision that characterize anthropoids.) Moreover, cortical enlargement in anthropoids probably reflects in part the increased size of individual areas: area V1, in particular, is much larger in anthropoids than in strepsirhines of similar body size (Frahm *et al.*, 1984).

The organization of primate extrastriate cortex differs markedly from that of nonprimate mammals. Most of the nonprimates that have been studied – including even tree shrews of the genus *Tupaia*, which, as Le Gros Clark emphasized, are diurnal animals with well-developed visual systems – appear to have a small complement of visual areas, with homologues of the first and second visual areas (V1, V2), and perhaps four or five additional areas (Lyon *et al.*, 1998; Rosa, 1999). Certain groups of mammals, notably carnivores, independently evolved large complements of cortical visual areas. It follows, then, that many of the extrastriate areas of primates evolved after the separation of the primate lineage and therefore have no homologues in other mammals. Moreover, the visual areas of nonprimate mammals (even carnivores) do not appear to be comprised of functionally distinct dorsal and ventral streams, as they are in primates (Preuss, 2006).

Although the living primates share many common features of visual cortical organization, there are also differences. The best documented differences involve the intrinsic organization of particular visual areas. As noted above, the layering of area V1 varies markedly across primate taxa. V2 exhibits differences as well: when stained for CO, most anthropoids exhibit well-demarcated thick and thin stripes that extend from the caudal border to the rostral border of V2, while strepsirhines show much less distinct banding (Preuss *et al.*, 1993). The functional consequences of these variations in area V2 organization have not been investigated. Humans also are reported to have indistinct V2 bands (Tootell and Taylor, 1995). There are, in addition, differences in the functional properties of homologous areas between anthropoid primates. For example, area V3 of macaques is more motion sensitive than its homologue in humans, whereas area V3A of humans is more sensitive to motion and contrast than area V3A of macaques (see the reviews in Preuss, 2004a).

35.3.4 Somatosensory and Motor Systems

It is useful to consider the central somatosensory and somatic motor systems together (Figure 4), as they are intimately related structurally and functionally (see the reviews of Kaas and Pons, 1988; Kaas, 2004; see The Evolution of Sensory and Motor Systems in Primates). For example, the fine control of grasping forces is mediated by transcortical processing loops: sensory signals originating in the hands and feet are relayed through the spinal cord to the brainstem, thalamus, and somatosensory cortex, from there to motor cortex, and from motor cortex through the corticospinal tract (CST) to the motor neurons controlling digit flexion and extension (e.g., Evarts and Fromm, 1981). Similarly, the 'haptic' sense involves the assessment of somatosensory feedback about the shape, texture, compressibility, and other physical characteristics of objects resulting from grasping and manipulating them (e.g., Lederman and Klatzky, 2004).

35.3.4.1 Peripheral mechanisms Primates are characterized by specializations of the extremities related to grasping, including opposable first digits. Grasping abilities vary widely among primates, however (Bishop, 1964; Napier, 1993). Strepsirhines and most New World monkeys have little, if any, independent digit control. Catarrhine primates, as well as the New World *Cebus* monkeys, have a much greater degree of independent digit control. In apes and humans, the grasping ability of the thumb is greatly increased: the pad of the thumb can be opposed to the pads of other digits, yielding a precision grip. In humans, this capacity is extremely well developed and is accompanied by modifications of the hand bones and muscles (Susman, 1994). Primate grasping is facilitated by the presence on the ventral surfaces of the hands and feet of large regions of hairless (glabrous) skin with complex patterns of epidermal ridges. This 'dermatoglyph' skin, which is thought to reduce slippage, is also present in tree shrews and in arboreal marsupials (Cartmill, 1974a; Martin, 1990). Some New World monkeys have increased their grasping abilities by evolving prehensile tails, the pads of which also possess dermatoglyph skin. Primates also appear to differ from most other mammals in their fundamental pattern of gait: most primates have a 'diagonal-sequence' walk, in which a hind footfall is followed by the fall of the opposite forefoot, whereas most other mammals have a 'lateral-sequence' walk, in which a hind footfall is followed by the fall of the forefoot on the same side (Cartmill *et al.*, 2006; Lemelin and Schmitt, 2006).

We know rather little about the neurological bases of distinctively primate grasping and gait patterns at the level of spinal mechanisms. There is, however, evidence for a primate specialization on the sensory side. The sense of touch is mediated by several types of receptors, of which the Meissner corpuscles deserve special note. Meissner corpuscles consist of a nerve ending ensheathed in a capsule of non-neuronal cells, and linked to surrounding tissue in such a way as to make them exquisitely sensitive to deformation of the skin (Martin, 1990; Hoffmann *et al.*, 2004). They are concentrated in the epidermal ridges, especially of digits 1–3, which

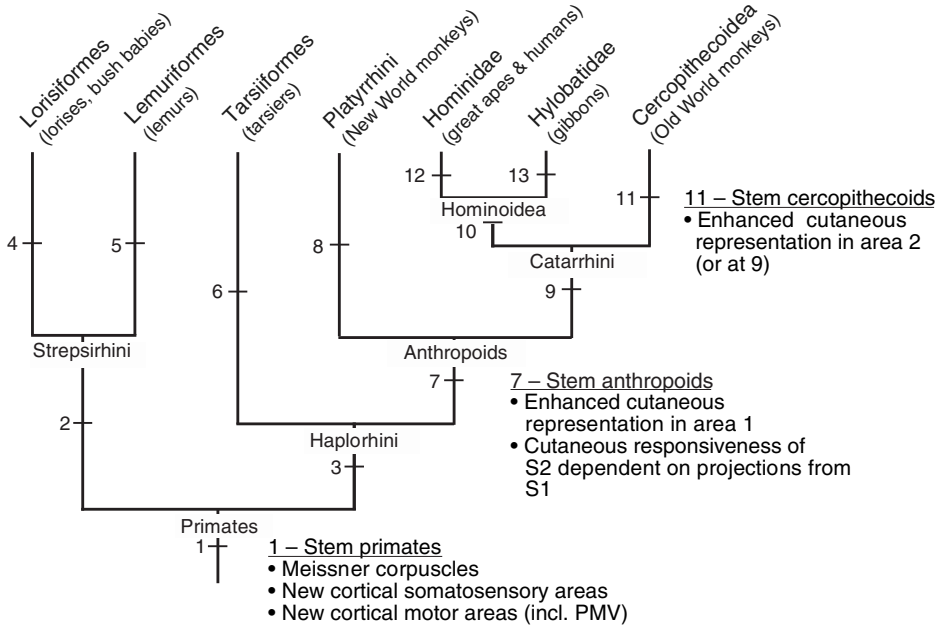

Figure 4 Interpretation of the shared derived features of the somatic sensory and motor systems in primates and major primate subgroups.

have been proposed to constitute a 'tactile fovea' (Hoffmann *et al.*, 2004). The placement of Meissner corpuscles suggests that they play an important role in the sensory control of grasping (e.g., by detecting slippage and initiating compensatory changes in grip force). Additionally, Meissner corpuscles could play an important role in the use of the hand as a haptic organ: some primates, especially catarrhines, appear to use active touch to investigate the mechanical properties of food items and other objects. Hoffmann *et al.* (2004) suggest specifically that Meissner corpuscles provide information for assessing the texture (and hence ripeness) of fruit. Meissner corpuscles are not, strictly speaking, unique to primates: morphologically similar organs are also present in the dermatoglyph skin of marsupials and on the finger-like tips of elephant trunks (Hoffmann *et al.*, 2004). They are, however, absent in tree shrews and most other mammals that have been examined (Martin, 1990; Hoffmann *et al.*, 2004). It seems likely that Meissner corpuscles evolved independently in several mammalian groups as the elaboration of a simpler type of mechanoreceptor.

35.3.4.2 Cortical somatosensory systems Mapping of the cortical somatosensory and motor fields has a long history, going back to the beginnings of experimental electrophysiology in the last half of the nineteenth century. It was clear from an early date that these fields are organized in an orderly, somatotopic fashion, with the tail and hindlimb represented medially in the cortex, near or within the interhemispheric fissure, and the forelimb and face represented laterally. In anthropoid primates, the principal somatic fields are located along the central sulcus, the somatosensory region posteriorly and the motor region anteriorly. In nonprimates, which lack the large territory of dorsolateral prefrontal cortex present in primates, the somatic regions are located on the lateral surface near the frontal pole. Early comparative mapping research culminated in the work of Woolsey (1958). Woolsey concluded that there were two somatosensory areas, the large primary area, SI, located along the central sulcus, and a smaller area, SII, located posterior and inferior to SI along the dorsal bank of the lateral sulcus. He also acknowledged two motor areas, the primary area, MI, located immediately anterior to SI, and a secondary area, MII (also called the supplementary motor area, SMA), located anterior and medial to MI along the mesial surface of the frontal lobe. Woolsey believed these four areas were present in most if not all eutherian mammals. The well-known neurosurgeon Wilder Penfield promulgated a similar understanding of human motor and somatosensory cortex (Penfield and Roberts, 1959). The elegance of Woolsey's schematic figures, with their artful drawings of somatosensory and motor homunculi, ensured that they would remain staples of neurobiology textbooks for many decades.

In the 1970s, the large surface electrodes that had been used for cortical mapping studies were replaced with fine-diameter electrodes that were inserted into the cortex to record from or stimulate neurons. The use of intracortical microelectrodes made it possible to map cortical areas in much greater detail, and with their application came the appreciation that Woolsey's interpretations of the somatosensory and motor regions were simplistic (Merzenich *et al*., 1978; Kaas *et al*., 1979). For example, the territory encompassed by Woolsey's 'SI' in anthropoid primates turned out to contain four separate representations of the body, two areas that receive inputs mainly from cutaneous receptors (including the Meissner corpuscles) and that correspond to cytoarchitectonic areas 3b and 1, and two areas that receive inputs mainly from muscle and joint receptors, corresponding to areas 3a and 2. Of these areas, the somatotopy of area 3b most closely resembled Woolsey's SI, so it was dubbed 'SI proper' (see the review of Kaas, 1983).

In contrast to SI, modern research has largely substantiated Woolsey's concept of an SII, an area of principally cutaneous representation located posteriorly and laterally to SI along the dorsal bank of the lateral sulcus (see the reviews of Kaas, 2004; see The Evolution of Sensory and Motor Systems in Primates). This research, however, has identified additional somatosensory representations in the lateral sulcus anterior and posterior to SII, as well as in the posterior part of the insular cortex, ventral to SII. These areas include the so-called parietal ventral (PV) and parietal rostral (PR) areas. There are also additional territories of somatosensory representation posterior to area 2, in the region termed area 5.

Comparative studies of strepsirhines (mainly *Galago* and *Otolemur*) and a variety of New World and Old World monkeys suggest that the areas discussed above are common to all major primate groups and were likely present in stem primates. It is also likely that at least some of these areas are neomorphic in primates, as most nonprimate mammals that have been studied, including tree shrews, have only a single main cutaneous representation in the region of SI, presumably homologous to area 3b/SI of primates (Kaas, 1983), and only a small region of somatosensory representation in the parietal cortex posterior to SI, which in primates contains areas 1, 2, and 5 (see The Evolution of Sensory and Motor Systems in Primates; Kaas, 1983; Beck *et al*., 1996).

There are some important variations in cortical somatosensory organization between primate groups. For one, New World monkeys that have prehensile tails, such as capuchins (*Cebus*) and spider monkeys (*Ateles*), possess prominent representations of the tail pads in SI (Pubols and Pubols, 1971; Felleman *et al*., 1983). In addition, cutaneous representation appears to be more extensive in anthropoids, and particularly in catarrhines, than in strepsirhines. Most strepsirhines that have been studied lack the second strip of cutaneous representation in area 1 caudal to area 3b/1 that is present in most anthropoids, although the slow loris, *Nycticebus coucang*, is reported to have a second representation of the glabrous skin of the hand (Carlson and Fitzpatrick, 1982). Although present in most New World and Old World monkeys, cutaneous representation in area 1 is reduced or lacking in tamarins (Carlson *et al*., 1986) and marmosets (Krubitzer and Kaas, 1990), both New World monkeys of the family Callithricidae that re-evolved claws in place of nails. Also, whereas areas 2 and 5 in strepsirhines and platyrrhines show little responsiveness to cutaneous stimulation, Old World macaque monkeys have a zone of cutaneous responsiveness extending across these areas (Pons *et al*., 1985). Finally, there is evidence for changes in the connectivity of the forebrain somatosensory network in primates. In galagos, there are parallel thalamic projections of cutaneous information to areas SI and SII, so that lesions of SI do not greatly impair the responsiveness of SII to cutaneous stimulation intact. This appears to be the primitive condition for eutherian mammals (Garraghty *et al*., 1991). In anthropoids, the cutaneous responsiveness of SII is the consequence of projections from area SI, lesions of which leave SII unresponsive (e.g., Pons *et al*., 1992).

There are interesting functional parallels in the organization of the cortical somatosensory and visual networks. The pathway from SI to SII is considered to be an important stage in a stream of somatosensory information processing that terminates in the hippocampus and provides the substrate for tactile recognition (Friedman *et al*., 1986). This constitutes an analogue of the ventral stream of visual processing – a somatosensory 'what' pathway. Information also flows from SI and neighboring areas in the central region posteriorly to area 5 and adjacent posterior parietal zones that control the movements of the limbs in space. This may be more than merely an analogue of the dorsal visual (vision-for-action) pathway, as the posterior parietal cortex is a locus of interaction of the somatic sensory and motor systems with the visual system.

35.3.4.3 Cortical motor systems Among the principal users of information processed through the somatosensory cortex are the areas of the motor

cortex. Woolsey, as already noted, believed there were two cortical motor areas, MI and MII, and that these were present in many eutherian groups. Even in Woolsey's day, however, his conception of the MI as a single, large area, spanning the entire territory between the somatosensory and prefrontal region to include both areas 4 and 6 of Brodmann, was controversial. Other workers identified area 4 with the primary motor area, while area 6, which is less responsive to electrical stimulation and was thought to be involved in higher-order aspects of motor control, was termed 'premotor' cortex (reviewed by Wise, 1985; Preuss *et al*., 1996). Recent research has shown both these interpretations of the motor cortex to be overly simplistic, with modern researchers recognizing MI, which appears to consist of at least two major subdivisions, a dorsal and a ventral premotor subdivision (PMD and PMV, which, like MI, appear to include major internal divisions), an SMA (MII) located on the mesial wall of the hemisphere, a presupplementary motor area, located anteriorly to SMA, and several cingulate motor areas, buried within the cingulate sulcus (reviewed by Dum and Strick, 2002; Kaas, 2004; see The Evolution of Sensory and Motor Systems in Primates). Most of the evidence for these areas comes from New World and Old World monkeys, but recent work in the strepsirhine *Otolemur* suggests a similar set of areas, so it seems very likely that a similarly large complement of areas was present in stem primates. Among primates, there appears to be at least one notable difference in areal organization: in macaque monkeys, area PMV consists of two subdivisions, distinguishable on functional and anatomical grounds (Gentilucci *et al*., 1988; Rizzolatti *et al*., 1988), whereas in the strepsirhines and New World monkeys that have been examined, PMV appears to be a unitary field (Preuss *et al*., 1996; Wu and Kaas, 2003; Fang *et al*., 2005).

By contrast to primates, the motor region of the nonprimate mammals that have been investigated evidently consists of only a very few areas (see The Evolution of Sensory and Motor Systems in Primates). Of the areas present in primates, MI, at least, is probably a common feature of eutherian mammals. There is likely an additional, very small region of premotor cortex in nonprimate mammals, but there is very little indication of anything approaching the seven or more nonprimary motor areas present in primates, so presumably many of those areas are neomorphic in primates. The clearest case for a neomorphic motor area in primates can probably be made for area PMV. It includes an anatomically discrete field of corticospinal projections that is not seen in tree shrews or in other mammals (Nudo and Masterton, 1990). It also has a distinctive somatotopy, representing the face and forelimb virtually exclusively, and seems to play an important role in organizing grasping movements of the hands and mouth, which led Preuss (1993) to suggest that this area evolved to organize the visually guided reaching and grasping behaviors of stem primates in the fine-branch niche.

35.3.4.4 Corticospinal tract The cortex can affect motor activity via a number of routes, the most direct of which are the direct projections to cranial nerve nuclei in the brainstem (corticobulbar projections) and to the spinal cord (corticospinal projections). These projections arise from a broad territory of frontal and parietal cortex in primates and other mammals, spanning the motor and somatosensory regions.

The CST has attracted a great deal of attention from comparative neurobiologists. Early research noted that lesions of the cortex had profound and lasting effects on movement and motor control in primates, whereas in many other mammals the effects were minimal. Damage to the motor cortex in humans and other anthropoid primates results in paresis; with time, most aspects of motor control recover, but individuated movements of the digits are refractory to recovery (Kuypers, 1981).

Comparative anatomical studies of the CST reinforced the idea that the CST plays a special role in movement control in primates. The CST is reported to vary markedly across mammals in its location within the brainstem and spinal cord, the number and diameter of fibers it contains, the levels of spinal cord to which it reaches, and the location of terminals with respect to the interneurons and motor neurons of the spinal cord.

In some mammals, the CST is reported to descend only to the level of the cervical or thoracic spinal cord (reviewed by Kuypers, 1981). In these animals, the CST can reach motor neuron populations controlling the forelimbs, but can have no direct effect on control of the hindlimb or tail, the motor neurons for which are located at lumbar and sacral levels of the cord. Tree shrews are said to have CST projections that reach no further than the mid-thoracic cord (Jane *et al*., 1965; Verhaart, 1966; Shriver and Noback, 1967). Other mammals, including primates, carnivores, bats, and rodents, are reported to have projections that extend into the lumbosacral cord (Kuypers, 1981). Primates, it has long been argued, are distinguished from most other mammals by having corticospinal fibers that terminate directly on the

motor neurons that innervate the muscles of the hands and feet; among nonprimates, only raccoons were said to have comparable, monosynaptic innervation of hand muscle motor neurons (Kuypers, 1981). Moreover, it was argued that the extent to which the CST extends beyond cervical levels in the spinal cord, and the extent to which motor neurons receive direct, monosynaptic input from the cortex, determines the digital dexterity of animals, with primates and raccoons having these conditions and being the most dexterous animals (Heffner and Masterton, 1975, 1983; see also the review in Striedter, 2005).

One problem with the studies of corticospinal connectivity cited above is that for the most part they employed techniques for studying connections (lesion-degeneration methods) that are not considered very reliable today. It seems likely that the conclusions reached about the level of the spinal cord reached by the CST in a given species are trustworthy in most cases, but conclusions about whether or not CST projections reach the territory of spinal motor neurons in the ventral horn should be considered cautiously. A study by Bortoff and Strick (1993), using a modern tracer substance (WGA-HRP), reported a result quite in line with classical considerations. They compared the distribution of corticospinal terminations after injections of WGA-HRP into area M1 of two closely related New World monkeys: capuchins (*Cebus apella*), which are noted for their manipulative abilities, and squirrel monkeys (*Saimiri sciureus*), which are not. They reported that CST terminations are much more extensive in the ventral horn motor neuron territory of *Cebus* than *Saimiri*. Reliable conclusions about the evolution of the CST will require more investigation using modern tracing techniques. It is also necessary to apply better analytical techniques to multispecies data sets than have been used in the past. For example, the regression techniques used to relate CST anatomy to digital dexterity by Heffner and Masterton (1975, 1983) are no longer considered adequate; improved methods ('independent contrasts') make it possible to remove the confounding effects of phylogeny in correlations, such as the over-representation of primates in the sample. Using these techniques to re-analyze the Heffner–Masterton data set, Iwaniuk *et al*. (1999) concluded that the length of the CST was related to digital dexterity, but not the extent to which it invaded the territory of motor neurons controlling the distal extremities. This is certainly not the last word on the subject (as Iwaniuk *et al*., 1999, would likely acknowledge), but it does highlight the need for neuroscientists to apply the best methods of phylogenetic inference, as well as the best techniques for studying neural organization, to problems of nervous system evolution.

35.3.5 Auditory System

The auditory system has not been studied by comparative biologists with anything like the effort devoted to the visual system. There is presently little indication that the origins of primates were accompanied by marked modifications of the auditory sensory apparatus, although changes in central auditory systems are known and will be discussed below. Small, nocturnal strepsirhine primates, as well as tarsiers, use hearing along with vision to localize insect prey, and if stem primates were nocturnal predators this would likely have been true of them as well. It is noteworthy that some nocturnal primates (bush babies and tarsiers in particular) have large external ears (pinnae), that are ridged and can be moved and shaped to assist sound localization (Charles-Dominique, 1977). The orientation of the pinnae modulates sound frequency, and this may play an important role in sound localization, which in small mammals relies more on frequency differences than interaural timing differences (Heffner, 2004).

Glendenning and Masterton (1998) compared the volumes of auditory brainstem nuclei in a variety of mammals and reported that primates are fairly typical among mammals in terms of the sizes of the different nuclei relative to each other. The dorsal cochlear nucleus, however, appears to have undergone a reduction of lamination in the origin of anthropoid primates, with further reduction in hominoids (Moore, 1980; Johnson *et al*., 1994).

As with other neocortical sensory systems, the cortical auditory system of primates is characterized by a large number of areas (see The Evolution of the Primate and Human Auditory System). The areas are arrayed in a distinctive fashion: along the upper part of the temporal lobe (partly or completely buried within the lateral sulcus), there is a 'core' of areas that share similar histology, with a well-developed layer 4 densely packed with granular cells. The posterior-most core area is the primary auditory area (A1). A1 is bordered rostrally by the rostral (R) area, which can be difficult to distinguish from A1 cytoarchitectonically, and area R is replaced rostrally by the rostrotemporal (RT) area. The core is surrounded on all sides by a 'belt' of auditory areas; currently, there are thought to be eight belt areas. The lateral part of the belt is bordered by

several 'parabelt' areas, which occupy the superior temporal gyrus. Posterior to the core and belt is an additional field involved in auditory processing known as area Tpt. Many of the core and belt areas are known to represent auditory frequencies in systematic fashion, that is, they are 'tonotopically' organized. As with other cortical sensory systems, the cortical auditory system exhibits a hierarchical organization, with areas in the core relaying auditory information to surrounding areas. The auditory system, however, exhibits a greater degree of parallel processing than the visual and somatosensory systems: the core and belt areas all receive inputs from the main auditory nucleus of the thalamus, the medial geniculate nucleus (MG), the three core areas receiving inputs from the ventral MG, and the belt areas from the dorsal MG (see The Evolution of the Primate and Human Auditory System; see also Rauschecker *et al.*, 1997). As the organization of primate auditory cortex has come to be better understood, it has been suggested that there are at least partly separate processing streams specialized for analysis of information about the identity of sound sources and about their spatial localization, that is, 'what' and 'where' systems (Romanski *et al.*, 1999; see also Kaas and Hackett, 1999; Rauschecker and Tian, 2000).

The organization detailed above has now been documented in both Old World and New World monkeys (see The evolution of the primate and human auditory system). Comparative architectonic studies indicate that an identifiable core is present in strepsirhine primates (Preuss and Goldman-Rakic, 1991a), and in chimpanzees and humans (Hackett *et al.*, 2001). Also, Tpt has been identified architectonically in strepsirhines, Old World monkeys, and humans (Galaburda and Pandya, 1982; Preuss and Goldman-Rakic, 1991a). Area A1 is probably common to all mammals, and most nonprimate mammals have at least five or six additional auditory areas (see, e.g., Stiebler *et al.*, 1997). It is not clear which of the primate auditory areas these are homologous to, nor is it clear that nonprimates exhibit the core-belt-parabelt system present in primates.

One of the major mysteries of human evolution is how auditory cortex was modified in relation to the evolution of language. One possibility is that humans evolved new areas to support language, but there is at present no evidence of this (Preuss, 2004b). Indeed, the cortical territory most strongly identified with Wernicke's area, the cortex of the planum temporale, posterior to area A1, contains architectonic area Tpt, which as discussed has also been identified in nonhuman primates (Galaburda and Pandya, 1982). Presumably, the cortex of Tpt must have undergone changes in its internal organization and/or its relationship to other cortical areas to support human language. Since humans tend to be left-hemisphere dominant for language, the fact that the planum temporale tends to be larger in the left hemisphere than the right might be seen as a language-related modification (Galaburda *et al.*, 1978). This cannot be the entire story, however, as great apes exhibit a pattern of posterior temporal asymmetries at least qualitatively similar to those of humans (Gannon *et al.*, 1998; Hopkins *et al.*, 1998), although the possibility of quantitative species differences remain. To date, the only clear difference to have been documented between the posterior temporal region of humans and apes is an asymmetry of cortical minicolumns in area Tpt: humans have more widely spaced columns on the left than on the right, whereas no asymmetry is present in chimpanzees or macaques (Buxhoeveden *et al.*, 2001).

35.3.6 Limbic System

The limbic system comprises a ring of cortex that makes up the lateral and medial margins of the cortex, including the hippocampus and parahippocampal cortex, retrosplenial and posterior cingulate cortex, the anterior cingulate and prelimbic areas (which are sometimes now considered portions of the medial prefrontal cortex), and orbital and insular cortex (including regions of olfactory and taste representation), along with noncortical structures that are connected with these regions, such as the amygdala and portions of the hypothalamus. What unites these regions are their roles in motivation and emotion, mediated by connections with the autonomic system. One might think that the limbic system would be a hotbed of comparative neuroscientific investigation, if only because modern evidence indicates that the limbic system is critically involved in social cognition. There is also the very interesting question of how the specialized cortical systems of primates came to interact with the limbic region, which is composed of structures that for the most part have homologues not only in other mammals but in nonmammalian vertebrates (see, e.g., Rolls, 2004). Presumably, it is because these structures have been viewed as ancient (we apply terms like 'paleocortex' and 'archicortex' to the limbic cortex) that they have received so little attention from an evolutionary viewpoint, although of course old structures can undergo evolutionary changes just as new ones can.

In fact, there is good evidence that cortical components of the limbic system were modified in

primate evolution (Figure 5). Primates possess divisions of posterior parahippocampal cortex (areas TH and TF) that have no obvious counterparts in other mammals (Preuss, 2006). These areas are important way stations between the neocortex and the memory systems of the hippocampus. In addition, the posterior cingulate region of primates contains a territory, area 23, that is not recognized in most other mammals (Preuss, 2006). Area 23 underwent further modification in anthropoid evolution, with the addition of a well-developed internal granular layer (Zilles *et al*., 1986; Preuss and Goldman-Rakic, 1991a). Also, subdivisions of area 23 and retrosplenial area 29 identifiable in anthropoids are not distinguishable in strepsirhines, and strepsirhines possess divisions of retrosplenial area 30 that are not distinguishable in anthropoids (Zilles *et al*., 1986). Tarsiers appear to share some features of anthropoid posterior cingulate cortex not present in strepsirhines (Zilles *et al*., 1986). New World monkeys differ from other primates in the thickness and cell density of the outer layers of retrosplenial area 29 (Armstrong *et al*., 1986). Evolutionary changes of the posterior cingulate and retrosplenial cortex are of special interest, as in humans these territories are believed to be part of the system for representing conscious self-reflection (Fink *et al*., 1996; Vogt and Laureys, 2005).

There is also evidence of changes at finer levels of organization in the limbic system. Humans and great apes possess an unusual class of large, spindle-shaped neurons in layer 5 of anterior cingulate and orbitoinsular cortex (Nimchinsky *et al*., 1999). These 'spindle cells' or 'Von Economo neurons' are especially large and prominent in humans, and they have been suggested to have a role in human social judgment. In addition to spindle cells, great apes and humans have a specialized class of calretinin-containing pyramidal cells in anterior cingulate cortex (Hof *et al*., 2001).

35.3.7 Higher-Order Forebrain Systems

I include among the 'higher-order' regions of the forebrain the cortex of the STS, the posterior parietal cortex, and portions of the 'prefrontal' cortex (Figure 6). These are regions that receive information from the sensory cortical systems, integrating multiple inputs to perform specific, supramodal cognitive and behavioral functions. I also include in this discussion the dorsal pulvinar, a division of the thalamus that has extensive connections with higher-order cortical regions. These four anatomical regions are noteworthy, first, because they either have no homologues in nonprimate mammals or underwent major modifications in primate evolution (Preuss, 2006), and second, because they enlarged out of proportion to other brain regions in human evolution (Preuss, 2000b, 2004b).

35.3.7.1 Superior temporal sulcal cortex The STS of Old World macaque monkeys contains a series of anteroposteriorly elongated architectonically distinct zones that extend much of the length of the sulcus anterior to visual area MT. This region was dubbed the superior temporal polysensory (STP)

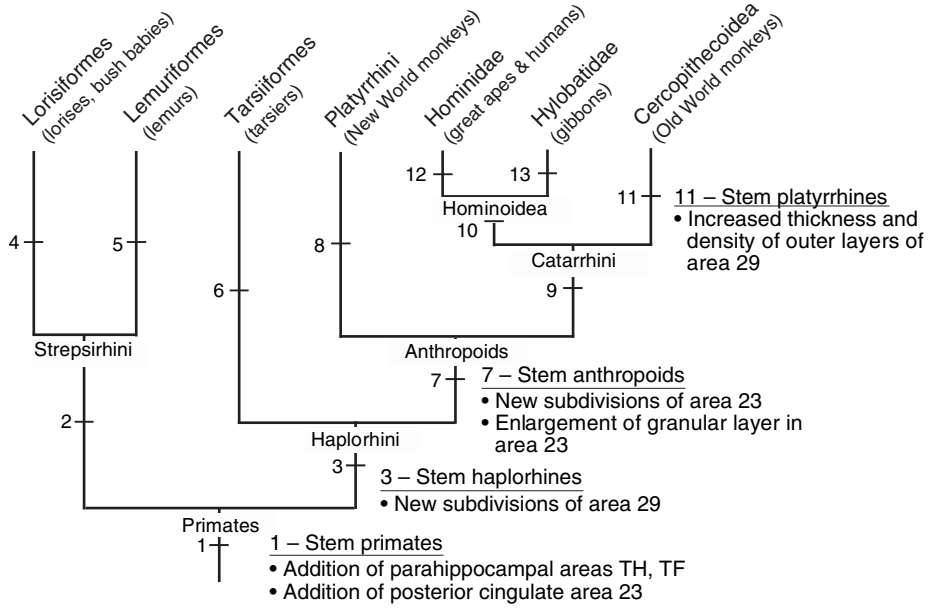

Figure 5 Interpretation of the shared derived features of the limbic system in primates and major primate subgroups.

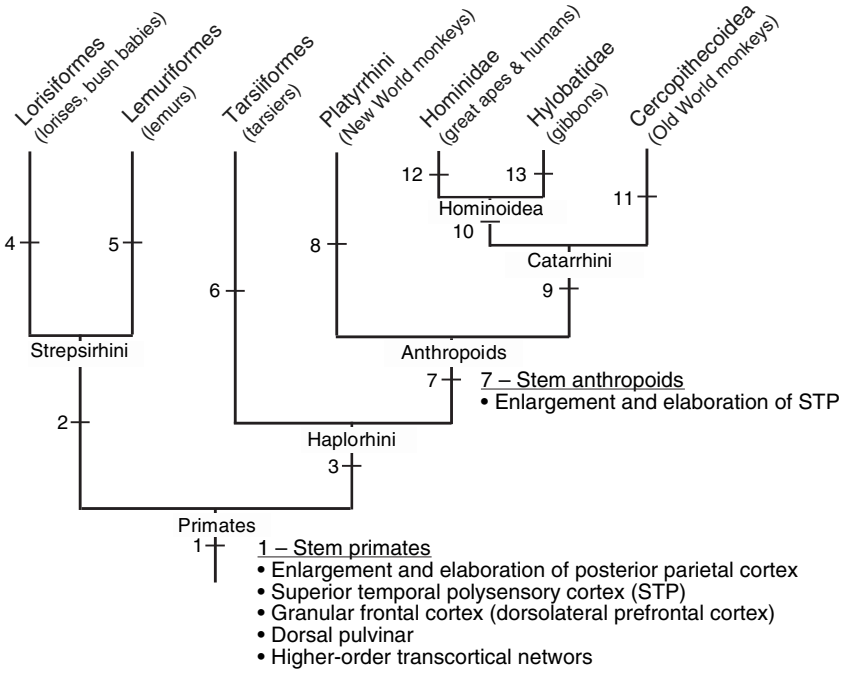

Figure 6 Interpretation of the shared derived features of higher-order forebrain systems in primates and major primate subgroups.

area by Bruce *et al*. (1981), and it receives inputs from both the dorsal and ventral visual streams (Boussaoud *et al*. 1990; Cusick 1997). STP has attracted special attention since the discovery in macaques that its neurons are active in response to viewing biological motion (moving faces, eyes, limbs, and bodies) and that individual STP neurons can be very selective for particular actions (Perrett *et al*., 1985; see also the review of Puce and Perrett, 2003). Currently, STP is regarded as a key node in a network of cortical areas, also including posterior parietal and prefrontal cortex, involved in the analysis of conspecifics' social behavior (Frith and Frith, 1999; Puce and Perrett, 2003). Functional imaging studies strongly suggest that a homologue of STP exists in the posterior part of the STS of humans. Evidence from strepsirhine primates, however, suggests that the anatomy of the STS region differs markedly from monkeys: Preuss and Goldman-Rakic (1991a, 1991b) confirmed that the macaque STS was comprised of multiple architectonic zones, but could distinguish only a single zone in *Otolemur*. This implies that major changes in STS organization took place during anthropoid or catarrhine evolution. There is no evidence that a homologue of STP or its constituent areas exists in nonprimate mammals.

35.3.7.2 Posterior parietal cortex The posterior parietal cortex corresponds to areas 5 and 7 of Brodmann (1909) in monkeys and lemurs. In macaques, areas 5 and 7 occupy the superior and inferior parietal lobules (SPL, IPL), respectively. In humans, Brodmann maintained that the IPL was occupied by areas 40 and 39, for which he recognized no homologues in monkeys. Other authorities, however, have regarded areas 40 and 39 as homologous to the anterior and posterior parts of area 7, designated as areas 7b and 7a or as areas PF and PG, in nonhuman primates (see, e.g., Economo and Parker, 1929; Bonin and Bailey, 1947; Bailey and Bonin, 1951; Eidelberg and Galaburda, 1984). Neurologists have long appreciated that posterior parietal cortex plays an important role in spatial attention and in action control. In humans, these functions are strongly lateralized: lesions involving right parietal cortex result in hemispatial neglect (inattention to objects and events to the patient's left), while lesions involving left parietal cortex yield a bilateral manual apraxia, a deficit in the ability to produce skilled hand movements, such as those required to use tools or to gesture (see Neurological Specializations for Manual Gesture and Tool Use in Humans; Haaland *et al*., 2000; Muhlau *et al*., 2005). Modern studies of nonhuman primates indicate that posterior parietal cortex receives information from the dorsal stream of the visual system as well as information about eye movements and limb position, and furthermore, that the region is a patchwork of small, functionally specialized territories involved in controlling the

direction of attention, eye movements, and reaching and grasping movements of the hands (Andersen *et al*., 1997; Colby and Goldberg, 1999; Grefkes and Fink, 2005). Results of functional imaging studies in humans are consistent with these studies, and reveal that posterior parietal subdivisions are involved in transcortical networks that mediate spatial attention (e.g., Corbetta *et al*., 1998; Kastner and Ungerleider, 2000) and attention to and control of action (e.g., Rushworth *et al*., 2001; Schluter *et al*., 2001; Johnson-Frey *et al*., 2005; see Neurological Specializations for Manual Gesture and Tool Use in Humans).

Although comparative studies indicate many commonalities among primates in the organization of posterior parietal cortex, it was by no means static in primate evolution. For one thing, there is no indication that any nonhuman primate exhibits the extreme degree of functional hemispheric specialization present in humans (see The Evolution of Hemispheric Specializations of the Human Brain; Corballis, 1991). Furthermore, functional imaging studies of human and macaque subjects viewing identical stimuli indicate that moving stimuli activate more discrete zones of parietal cortex in humans (Vanduffel *et al*., 2002; Orban *et al*., 2004; see also Orban *et al*., 2006). This might indicate that humans possess more parietal areas than macaques or, alternatively, that certain areas became more sensitive to certain classes of visual stimuli in humans than in macaques (Preuss, 2004a). At present, it is not clear whether these differences are true human specializations, or whether they evolved early in the history of the hominoid (ape-human) group, or (as could well be the case) specializations of macaques. It is noteworthy that the posterior parietal cortex of primates is very different than that of other mammals. In rats, for example, there is a small region of cortex between the visual and somatosensory regions that receives input from both and that been likened to posterior parietal cortex, but if this is the homologue of primate posterior parietal cortex, the region must have undergone extensive modification in primate evolution, with the addition of many new subdivisions specialized for primate-characteristic behaviors (Preuss, 2006).

35.3.7.3 Prefrontal cortex Prefrontal cortex in primates, as currently understood, includes several different territories: a large region with a well-developed granular layer 4 that occupies mainly the dorsolateral surface of the frontal lobe (i.e., granular frontal cortex, or more commonly, dorsolateral prefrontal cortex); an orbital region, the anterior parts of which are granular, but which grades off posteriorly into agranular cortex; and a medial region, which also is granular anteriorly but grades off posteriorly into agranular cortex. Classically, the medial agranular regions were classified as anterior cingulate cortex (Brodmann, 1909), rather than as prefrontal cortex, but for reasons to be discussed below, it has come to be thought of as 'prefrontal'.

Among mammals, only primates have a region of cortex with a well-developed granular layer on the dorsolateral surface of the frontal lobe (Brodmann, 1909). The region is present in all primates that have been examined, and is much larger in anthropoids than in strepsirhines (Brodmann, 1909; Preuss and Goldman-Rakic, 1991c). Owing in part to the influence of Brodmann, the granular dorsolateral prefrontal cortex initially came to be regarded as a hallmark of the primate brain. The fact that some neurologists in the early part of the twentieth century regarded this region as the seat of higher-order cognitive functions reinforced this view. Modern experimental studies in nonhuman primates (reviewed by Goldman-Rakic, 1988; Preuss and Goldman-Rakic, 1991b; Pandya and Yeterian, 1996; Barbas, 2000; Petrides, 2000) reveal it to have strong connections with the higher-order parietal and temporal areas discussed above, and functional studies in humans and nonhuman primates indicate that different parts of the granular frontal cortex are involved in attention, working memory, and planning (Passingham, 1993; Goldman-Rakic, 1996; Fuster, 2000; Miller and Cohen, 2001; Tanji and Hoshi, 2001; Miller *et al*., 2002; Passingham and Sakai, 2004; Petrides, 2005).

The idea that dorsolateral prefrontal cortex is special to primates has, nevertheless, been challenged (see the reviews of Preuss, 1995a, 2006). With the introduction of the first generation of techniques for studying cortical connectivity (lesion-degeneration techniques), it became clear that the cortical regions differed in their patterns of connectivity as well as their histology. Early research on the forebrain connections of the cortex focused on connections with the thalamus because cortical lesions produce degeneration in thalamic nuclei that project to them; most other connections could not be reliably resolved until improved methods became available in the 1970s. Rose and Woolsey (1949) championed the idea that regions of cortex could be defined by the thalamic nuclei that projected to them. As the dorsolateral prefrontal cortex, the largest prefrontal region in primates, receives its major thalamic inputs from the mediodorsal thalamic (MD) nucleus, prefrontal cortex came to be defined as MD-projection cortex (Rose

and Woolsey, 1948). As it happens, all mammals that have been examined have a MD nucleus and a cortical territory to which it projects, so by this reasoning, all mammals possess a homologue of dorsolateral prefrontal cortex, even though the MD-projection cortex of nonprimates lacks the well-developed granular layer that marks this region in primates (Rose and Woolsey, 1948; Akert, 1964). It was also reported that dopamine-containing nuclei of the brainstem project very strongly to MD-projection cortex in both primates and nonprimates, and this has also been used to identify homologues in different mammals (Divac *et al.*, 1978). Attempts have also been made to refine this analysis by identifying homologues of specific subdivisions of primate dorsolateral prefrontal cortex in nonprimates (Akert, 1964). A region of special interest has been the cortex that lines the principal sulcus of macaques (principalis cortex), because lesions of this region impair performance on spatial working memory tasks, a set of cognitive tasks that have been adapted for use in a wide range of mammals. Using the criteria of MD projections, dopamine projections, and involvement in spatial working memory tasks, homologues of macaque principalis cortex have been proposed in nonprimate species, and most importantly in rats, which are the most widely used model animals in mammalian neuroscience. In rats, the principalis homologue has usually been localized to the medial surface of the frontal lobe, and some workers have identified it specifically with area 32 (the prelimbic area) (Brito *et al.*, 1982; Passingham *et al.*, 1988; Dalley *et al.*, 2004; Vertes, 2004).

This might seem a satisfactory account of prefrontal homologies, but there are difficulties with both the evidence and the reasoning (Preuss, 1995a). For one thing, in primates, MD projects not only to the granular, dorsolateral prefrontal frontal cortex, but also to agranular regions, including orbital cortex, the classical anterior cingulate areas (areas 24 and 32 of Brodmann), and even to insular and premotor cortex. For another, while dorsolateral prefrontal cortex receives dopaminergic inputs, the strongest dopamine projections in primates are actually to the motor region and the orbital and medial cortex. Finally, in primates, lesions of the medial frontal cortex, involving the cingulate region and sparing the dorsolateral region, produce impairments on spatial working memory tasks. Thus, none of the features that have been used to identify homologues of granular prefrontal cortex in nonprimates are actually diagnostic of granular prefrontal cortex in primates. In fact, the medial frontal cortex of rodents very closely resembles the agranular parts of the medial frontal cortex of primates on a variety of structural and functional grounds – both are limbic regions, after all. It is true that the medial frontal cortex of rodents resembles primate granular frontal cortex in certain respects, but these are also the ways that the medial frontal cortex of primates resembles the dorsolateral prefrontal cortex of primates; the similarities are not diagnostic. Moreover, primate granular frontal cortex has additional features of areal organization and connectivity that do not match any known region of frontal cortex in any nonprimate mammal (Preuss, 1995a).

On present evidence, then, there are good grounds for concluding that dorsolateral prefrontal cortex is in fact one of the distinctive features of the primate brain. In addition, there is evidence that this region underwent extensive modification during primate history. A comparative study of the connectivity and histology of this region in galagos and macaques indicated that the latter have many more subdivisions of granular frontal cortex (Preuss and Goldman-Rakic, 1991b, 1991c). It was concluded that galagos share with macaques homologues of frontal eyefield and related areas, but that galagos lack homologues of the areas located within and surrounding the principal sulcus of macaques. This result suggests that the principalis areas are derived features of anthropoid or catarrhine cortex, and reinforces the view that a principalis homologue is absent in nonprimates.

35.3.7.4 Tying it all together – the dorsal pulvinar As noted above, the higher-order temporal, parietal, and frontal regions of primates are strongly interconnected, making up a collection of distributed networks that subserve specific cognitive functions (Goldman-Rakic, 1988). In primates, these regions are all connected with a particular region of the thalamus known as the medial pulvinar, or more appropriately, the dorsal pulvinar (see Gutierrez *et al.*, 2000, and the review of Preuss, 2006). Neuroanatomists have long noted the great size of the pulvinar in primates compared to other mammals (e.g., Le Gros Clark, 1959). The pulvinar consists of several different territories, however, and most attention has been paid to those regions of the pulvinar that are specifically related to the visual system, receiving input from the retina and SC and projecting to striate and extrastriate visual cortex. In primates, the visual pulvinar proper consists of the inferior pulvinar and part of the lateral pulvinar; the homologous territories in nonprimate mammals are referred to as the pulvinar or as the pulvinar-lateral posterior complex. The dorsal pulvinar is

most likely neomorphic in primates, as there are no structures in a similar location or with similar connections in other mammals (Preuss, 2006). By virtue of its connections, the dorsal pulvinar is in a position to coordinate the activities of the distributed, higher-order cortical systems of primates, although the nucleus has received very little experimental investigation and its functions are presently unknown.

35.4 Conclusions and New Directions

This review documents numerous evolutionary changes in the nervous systems of primates, localizing them wherever possible to their period of origin in primate evolutionary history. It is clear that primate brain evolution cannot be understood simply as a matter of enlargement and general differentiation; rather, the record indicates that primate brains changed in very specific ways at particular time points in evolutionary history. Ultimately, one would like to see the evidence for evolutionary modifications of the nervous system woven into a broader account of primate evolution, an account that relates changes in the nervous system to changes in other aspects of anatomy and, of course, to behavior. We are a long way from realizing this goal. Without question, many of the nervous system specializations described here are, in a way, rather unremarkable in light of what we know about primate evolution generally. For example, given the emphasis placed in primate origins research on the importance of high-acuity nocturnal vision and visually guided grasping, it is not surprising that we find that stem primates underwent changes in central systems involved in vision, eye-movement control, and grasping. It is not possible at present, however, to make very strong claims about why primates evolved the particular nervous system features they possess, such as, why primate visual systems have such a strong hierarchical organization compared to other mammals, or why primates have CO-rich blobs in area V1. Current theories of structure–function relationships in the nervous system are not sufficiently well developed to provide us with much insight into issues at this level of detail.

How do we get better theories of structure–function relationships? In my view, we need more comparative research. Comparative studies have not been a high priority for the institutions that fund neuroscience, but there is some reason to think this will change. Driven by the need to make sense of data from dozens of different species coming from the various gene-sequencing projects, molecular biology has begun to incorporate the concepts and methods of evolutionary biology. Bioinformatics is, in a very real way, computerized evolutionary biology. We can expect the transformation of molecular genetics to affect the neurosciences. The demonstration of differences in the genomes and proteomes of different mammalian and primate species will naturally lead to the question: what are their phenotypic consequences? Already, we have seen how comparative genetic studies can inform our understanding of the evolution of primate sensory receptors (see Gilad *et al.*, 2004). What's more, we can use the information provided by comparative molecular studies about changes in the sequences and tissue-specific expression patterns of specific macromolecules to drive 'phenotype discovery' (Preuss *et al.*, 2004). For example, by knowing which molecules have undergone change, we can employ such routine methods as *in situ* hybridization and immunohistochemistry to localize those molecular changes in their anatomical context.

While we can expect comparative molecular biology to provide new insights into the primate brain evolution, the foregoing review draws attention to one really fundamental need: correlative comparative studies of the brain, behavior, and cognition. Consider this: many of the features that distinguish human brains from, say, those of rats, evolved very early in primate evolution. We possess them because our ancestors possessed them. To understand why our brains have these design features, we need to understand what they contributed to the behavior of early primates. To understand that, we need comparative studies of brain, behavior, and cognition in a variety of mammals, including especially anthropoid and strepsirhine primates, along with the animals to which primates are thought to be closely related, such as tree shrews, rodents, and rabbits. No such comparative science exists today.

In addition to sharing many design features with other primates and other mammals, humans presumably possess features that are uniquely human and that provide the basis for our distinctive cognitive and behavioral characteristics. We currently have very little reliable information about brain specializations of humans. One bright spot in this area is that imaging technologies have now developed to the point where we can examine humans and nonhuman primates on something like a level playing field (see, e.g., Orban *et al.*, 2004). The problem is that most of these studies involve

comparisons of humans and macaque monkeys. It is important to recognize that the demonstration of a human–macaque difference is not a demonstration of a human specialization. For example, a macaque–human difference could have arisen early in hominoid evolution, long before humans diverged from the African apes; this would be a hominoid specialization. Alternatively, an observed difference could be a macaque or a catarrhine specialization, with humans conserving the ancestral condition. Demonstrations of human specializations, therefore, require comparing humans to a wider range of primate species. Of central importance is the comparison of humans to chimpanzees: since chimpanzees are our closest relatives, any claim of human specialization requires the demonstration that humans differ from chimpanzees (Preuss, 2004b). Although there has recently been a renaissance of human–chimpanzee comparative psychological research we know almost nothing of the differences in brain organization between humans and chimpanzees that would provide the basis for human cognitive and behavioral specializations. There is, therefore, a pressing need for comparative studies of the brain and cognition in humans and chimpanzees.

Acknowledgment

The author is grateful for the generous support of the James S. McDonnell Foundation (JSMF 21002093). This article is dedicated to the memory of Professor Patricia S. Goldman-Rakic.

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36 The Role of Vision in the Origin and Evolution of Primates

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Glossary

Anthropoidea Monophyletic group (clade) consisting of living monkeys, apes, and humans, their last common ancestor, and all fossil taxa more closely related to living anthropoids than other primates.

APA Arcuate premotor area.

cathemeral Active during the day and at night.

CMAd and CMAv Dorsal and ventral cingulate motor areas, located in the cingulated sulcus on the medial aspect of the cerebral hemisphere.

corticospinal tracts Fiber bundles consisting of axons from cell bodies in the cerebral cortex that connect to motor neurons and interneurons in the spinal cord.

crepuscular Active in the evening and morning.

diurnal Active principally or solely during the day.

euprimates or primates of modern aspect Monophyletic group consisting of Haplorhini, Strepsirrhini, Omomyiformes and Adapiformes. The taxon was erected to distinguish primates of modern aspect from archaic primates, or plesiadapiforms.

frontation Caudal angle between the nasion inion chord and the intersection of the midsagittal plane with the orbital plane.

hallux Big toe, first toe.

Haplorhini Monophyletic group (clade) consisting of Anthropoidea and tarsiers, their last common ancestor, and all fossil taxa more closely related to living haplorhines than to other primates.

MI Primary motor cortex.

nocturnal Active principally or solely at night.

orbital convergence Degree to which orbits face in the same direction, or converge on each other. Convergence is the caudal dihedral angle between the plane of the orbit and the midsagittal plane.

PMv Ventral premotor area.

pollex Thumb; first, or radial digit.

SMA Supplementary motor area, motor cortex located on the medial aspect of the cerebral hemisphere, dorsal to the cingulate sulcus.

stereopsis Seeing objects as solid, or three dimensional.

Strepsirrhini Monophyletic group (clade) consisting of living lemurs, lorises, and galagos, their last common ancestor, and all fossil taxa more closely related to liv ing strepsirrhines than to other primates.

36.1 Introduction

The visual system features prominently in adaptive explanations for the divergence of primates from other mammals and the origin of anthropoid or simian primates from their prosimian ancestors. However, the origin and radiation of primates was associated with modification of a number of other sensory and motor complexes, including the auditory, feeding, and locomotion systems. The integrated nature of these modifications demands that considerations of the role of the visual system in primate evolution include changes in these other systems. Many current explanations for primate origins do not take this integration into account. Here, we consider the role of vision in association with other functional systems. After briefly reviewing the taxonomy of extant primates, we begin by enumerating the features distinguishing primates from other mammals, especially their close relatives. We then review the hypotheses advanced to explain the evolution of these features, evaluating those hypotheses with special reference to the neuroscience literature dealing with the visual system, motor control of hand movements, and eye–hand coordination.

36.2 What Is a Primate?

Living primates are classified into three universally accepted groups (Figure 1): Anthropoidea (monkeys, apes, and humans), Tarsiiformes (tarsiers), and Strepsirrhini (Malagasy lemurs together with lorises and galagos) (Martin, 1990; Fleagle, 1999; Hartwig, 2002). (Many publications spell this with one 'r', i.e., Strepsirhini. However, two 'r's are preferable because, although the Zoological Code of Nomenclature does not codify spelling of taxonomic names above the family level, the original spelling of the term was 'Strepsirrhini' (Geoffroy Saint-Hilaire, 1812a) and this is also the correct derivation from the Greek (Jenkins, 1987).) The phylogenetic position of *Tarsius* is controversial, with some researchers placing it as the sister taxon of anthropoids, making a clade Haplorhini (e.g., Cartmill, 1980; Martin, 1990; Kay *et al.*, 1997, 2004; Ross *et al.*, 1998) and others placing it as the sister taxon of strepsirrhines, making a clade Prosimii (e.g., Eizirik *et al.*, 2001). Many researchers accept Haplorhini as a valid clade, but use the term 'Prosimii' to refer to the paraphyletic group consisting of strepsirrhines and tarsiers. Here we follow the classification of Fleagle (1999).

Living strepsirrhines include the Malagasy primate families united in the Lemuriformes (or Lemuroidea) and the African and Asian strepsirrhines, grouped together in the Lorisiformes (or Lorisoidea). The Lemuriformes includes Cheirogaleidae, Daubentoniidae, Indriidae, Lemuridae, and Lepilemuridae (or Megaladapidae). Lorisiformes includes the African Galagidae, and the African and Asian Lorisidae.

Anthropoids (also known as simians) are divided into two major clades: the Platyrrhini, or New World monkeys; and the Catarrhini, including Old World monkeys, apes, and humans. There is general agreement on the family or subfamily groupings of most of the platyrrhines – Callithrichidae (marmosets and tamarins), Atelinae (spider, woolly, and woolly spider monkeys), Alouattinae (howler monkeys), Pitheciinae (sakis, bearded sakis, and uacaris), Cebinae (including *Cebus* and *Saimiri*), Aotinae (owl or night monkeys), and Callicebinae (titi monkeys) – but the relationships among these groups are debated. The Catarrhini are divided into two major clades, the Cercopithecoidea, including cercopithecines and colobines, and the Hominoidea, including the apes and humans.

It is generally believed that the closest living relatives of primates are scandentians (tree shrews) and dermopterans (flying lemurs), although the phylogenetic relationships of these animals to primates continue to stimulate debate. The hypothesis of a grouping of dermopterans, scandentians, primates, bats, and elephant shrews in a superorder Archonta (Gregory, 1920) is not supported by recent analyses. Bats instead are included with carnivores, ungulates, and whales in a clade Laurasiatheria, while primates group with tree shrews, dermopterans, and the rabbit–rodent clade, Glires, in a larger clade, Euarchontoglires (Springer *et al.*, 1997; O'Brien *et al.*, 1999). Recent molecular trees for mammals either place Scandentia and Dermoptera in a clade that is the sister taxon to primates (Springer *et al.*, 1997; Murphy *et al.*, 1999; Eizirik *et al.*, 2001, 2004) or group Scandentia and Primates in a clade with Dermoptera as the sister taxon (Liu *et al.*, 2001). The long-term robustness of these phylogenetic groupings remains to be seen.

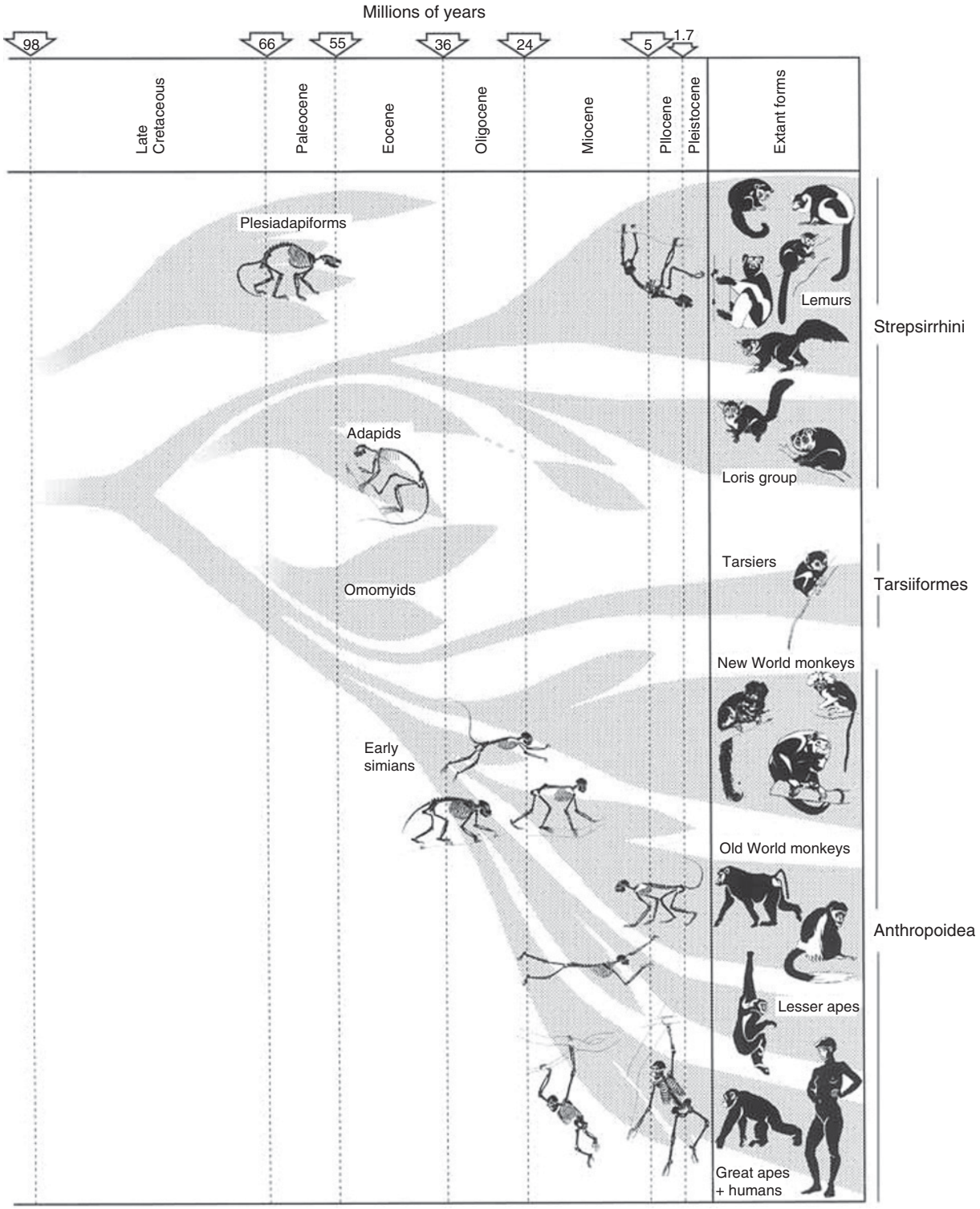

Figure 1 Outline phylogenetic tree of primates (modified from Martin, 1993). The generally accepted groups of living primates are shown on the right. Two groups of fossil primates that appear and radiate in the Eocene adapids and omomyids are of uncertain affinities to living primates (Martin, 1993). Plesiadapiforms and their relatives, including carpolestids (Bloch and Boyer, 2002), are also of uncertain affinities to primates. When plesiadapiforms are included in Primates, living primates, omomyids, and adapids are grouped together as Euprimates. Reproduced from Martin, R. D. 1993. Primate origins: Plugging the gaps. *Nature* 363, 223 234, with permission from Nature Publishing Group.

36.3 Early Explanations for Primate Origins

Primates have long been distinguished from other mammals by their grasping hands and feet, various enhancements of the visual system, and their relatively enlarged brains (Geoffroy Saint-Hilaire, 1812a, 1812b; Elliot Smith, 1924). Like many of the explanations to follow, Grafton Elliot Smith's early explanations for primate origins invoked functional benefits of these features in an arboreal habitat, but Elliot Smith also emphasized that changes in primate locomotion and grasping were integrated with changes in the somatic, auditory, and visual sensory systems. In an address to the Anthropological Section of the British Association for the Advancement of Science delivered in 1912 (Elliot Smith, 1924, chapter 1), Elliot Smith identified the neopallium (later termed neocortex) as the most salient feature distinguishing mammalian brains from those of nonmammals. The neopallium of mammals not only receives input from the visual, auditory, tactile, and kinesthetic senses, providing a substrate for merging and associating of the information streaming in from the periphery, but also contains the motor areas that put into effect the decisions made on the basis of these associations. Thus, Elliot Smith saw the neopallium as the organ that made it possible for mammals to learn and adapt to their surroundings.

The adaptability conferred on basal mammals by the neopallium was lost by many descendant lineages when they became specialized for cursorial, flying, aquatic, or burrowing environments. Primates, in contrast, retained their primitive adaptability, plasticity, and flexibility, primarily because they were arboreal. Arboreal mammals, Elliot Smith argued, require a balanced emphasis of the senses, with enhancement of vision, hearing, and touch. The agility of movement required in the trees "necessitates an efficient motor cortex to control and coordinate such actions as an arboreal mode of life demands . . . and also a well-developed muscular sensitivity to enable such acts to be carried out with precision and quickness" (Elliot Smith, 1924, p. 30). This general enhancement of the special senses, as well as the somatic sensory and motor systems used in locomotion, accounted for the general enlargement of the brain characteristic of primates.

Elliot Smith also emphasized the integrated nature of changes in the visual and tactile senses. The integrated nature of the neopallium meant that enhancement of the visual system in primates affected the whole neopallium, not just the visual areas.

> The sense of touch also shared in the effects, for tactile impressions and the related kinaesthetic sensibility, the importance of which to an agile tree living animal is obvious, assist vision in the conscious appreciation of the nature and the various properties of the things seen, and in learning to perform agile actions which are guided by vision (Elliot Smith, 1924, p. 32).

This correlated development of visual and tactile senses led to integrated development of improved eye–hand coordination, linking up the tactile, kinesthetic, and visual cortical areas. Thus, for Elliot Smith, primate arboreality was not the only factor responsible for their adaptability, plasticity, and ability to learn, but it also resulted in enhanced development of their visual and grasping abilities, and in the integration and co-evolution of the two systems.

Wood Jones's theory of primate evolution included many of Elliot Smith's conclusions, but he also discussed specific features that are the focus of current explanations for primate origins. Wood Jones (1916) explained forward-facing eyes and postorbital bars as secondary consequences of a shift to arboreality, not specializations for it. He argued that, with progressive adoption of arboreal habits, the hindlimb became specialized for supporting the body weight during climbing, liberating "the fore-limb from any such servile function as supporting the weight of the body: it becomes a free organ full of possibilities," a process Wood Jones referred to as "emancipation of the fore-limb" (Wood Jones, 1916, p. 17). The emancipated forelimb could then take over from the jaws the role of food acquisition, allowing the snout to be reduced in size. As the snout recedes, the orbits are dragged around toward the front of the face, and postorbital ossifications (bar and septum) develop between the orbit and the temporal fossa (Wood Jones, 1916, p. 99). Echoing Elliot Smith, Wood Jones noted that one incidental benefit of the combination of a dextrous forelimb with forward-facing eyes is the ability to simultaneously manipulate and view an object in front of the face, making it advantageous to merge tactile and visual information in the newly expanding cortical association areas created by the expanding brain.

Wood Jones and Elliot Smith's arboreal theory of primate evolution (Howells, 1947) was adopted by Le Gros Clark (1934, 1959) as the explanation for general trends in primate evolution. The sensorimotor integration integral to Wood Jones' and Elliot Smith's theory was embodied in Le Gros Clark's total morphological pattern, "the integrated combination of unitary characters which together make up the complete functional design of a given anatomical structure" (Le Gros Clark, 1959, p. 13). The lack

of specialization and the retention of adaptability were attributed by Le Gros Clark to:

> . . . an arboreal habitat, a mode of life which among other things demands or encourages prehensile functions of the limbs, a high degree of visual acuity, and the accurate control and coordination of muscular activity by a well developed brain (Le Gros Clark, 1959, p. 43).

Overlapping visual fields and high visual acuity were argued to confer the ability to judge distances necessary for leaping in an arboreal environment. Le Gros Clark also re-emphasized the importance of eye–hand coordination for primate evolution identified by Elliot Smith, arguing that the enhancement of the tactile senses that accompanied the changes to the visual system were related to improved ability for manual manipulation and appreciation of the environment.

36.4 Primates in the Fine-Branch Niche

Le Gros Clark's theory of primate evolution was promulgated to the next generation of primatologists and became the received view (Cartmill, 1982). In the 1960s and 1970s, field research on behavior and ecology of nocturnal strepsirrhine primates in Madagascar and West Africa by R. D. Martin and P. Charles-Dominique suggested to them some refinements of the arboreal theory. Their fieldwork revealed similarities between cheirogaleids and galagids in a number of features, including nocturnality, small body size, hindlimb-dominated locomotion utilizing grasping extremities in the fine-branch and creeper niche, and an omnivorous diet including fruit, insects caught with the hands, and gum obtained with the help of the toothcomb (Charles-Dominique and Martin, 1970; Martin, 1972, 1973). They interpreted these commonalities as retentions from the common ancestor of strepsirrhines at least, and possibly primates as a whole, suggesting that occupation of the fine-branch niche might be the adaptive shift that characterized primate origins.

The advantages of the distinctive features of the primate visual system to an occupant of the fine-branch niche were not precisely articulated, although Martin addressed them briefly in 1979:

> Occupation of the "fine branch niche" by a relatively small bodied ancestral primate would hence explain the emphasis on the grasping foot characteristic throughout the order Primates and at the same time provide a reason for the emphasis on vision and replacement of the primitive prehensile function of the snout by mobile, grasping hands. (Leaping between adjacent fine branches and grasping of small animal prey on nearby supports with the hands would explain the relatively large eyes, the uni versal possession of a postorbital bar, and the reduction of the snout and anterior teeth among primates.) (Martin, 1979, p. 64).

Martin subsequently argued that forward rotation of the orbits enhances stereoscopic vision that would be advantageous for "[a]ctive locomotion in a network of fine arboreal supports" (Martin, 1990, p. 657). This fine-branch niche hypothesis for primate origins included only very general explanations for the origins of orbital convergence and a postorbital bar in stem primates, made no mention of eye–hand coordination, and emphasized the importance of locomotion in terminal branches over predation or food acquisition (Martin, 1979).

36.5 Orbital Convergence, Postorbital Bar, Manual Grasping, and Visual Predation

Cartmill (1970, 1972) took issue with the arboreal theory of primate evolution on the grounds that arboreality alone cannot explain the origins of grasping extremities, convergent orbits, and nails on the digits, because a variety of active, leaping arboreal animals, such as squirrels, lack these features altogether. He argued:

> If the primate evolutionary trends have not been characteristic of other lineages of arboreal mammals, we may conclude that there is something wrong with the arboreal theory in its received form and any explanation of the primate trends must involve a more detailed description of the habitus of the ancestral primate (Cartmill, 1972, pp. 102 103).

Cartmill (1974) noted that many nonprimate animals with forward-facing eyes, such as cats, owls, and hawks, are "visually directed predators," and many nonprimate animals with grasping extremities, such as chameleons and small marsupials, engage in "prolonged and stealthy locomotion on slender terminal branches in pursuit of insects." Cartmill's hypothesis was significant in that it demonstrated that arboreality alone could not explain the evolution of optic convergence and grasping extremities in primates; something more specific was needed. Cartmill invoked adaptation to visual predation in the fine branches of the shrub layer of tropical rainforests to explain both grasping hands and convergent orbits. An integral component of the hypothesis was the importance of eye–hand coordination originally identified by Elliot Smith:

> The prehensile forelimbs necessary for stalking insects along thin branches serve also, among living insectivorous prosi mians, as prey seizing organs analogous to the tongue of a chameleon. The importance to primates of hand eye coordi nation, which [Elliot] Smith was the first to stress, can be plausibly traced to an ancestral habitus in which the hand was used for striking prey (Cartmill, 1972, p. 116).

Cartmill (1970) formally defined orbit orientation in terms of two variables (convergence and frontation) (Figure 2), which he measured in a wide sample of arboreal mammals. Subsequent morphometric work by Ross (1995), Noble *et al.* (2000), Heesy (2003, 2005), and Ravosa and Savokova (2004) has expanded the available data and the most extensive data set (Heesy, 2003, 2005) is shown in Figure 3. Primates certainly have more convergent orbits than do dermopterans and scandentians, but many other mammals overlap with primates in their degree of orbital convergence, including a number of carnivorans, bats, and marsupials. However, few mammals share the combination of high degrees of frontation and convergence seen in primates, and when allometric factors are taken into account, primates have more convergent orbits for their relative orbit size than other mammals (Noble *et al.*, 2000; Heesy, 2003).

Refinements to Cartmill's visual predation hypothesis were necessary. Pettigrew (cited by Allman, 1977, p. 29; Pettigrew, 1978) and Allman (1977) pointed out that orbital convergence has advantages for nocturnal animals that are not applicable to diurnal animals. The

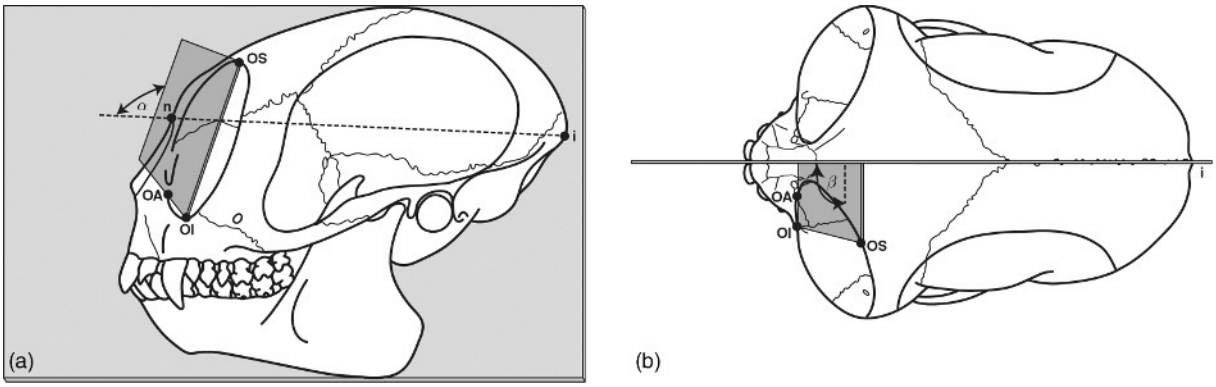

Figure 2 Diagram illustrating definitions of orbital convergence and frontation from Cartmill (1970, 1972) and subsequently used by Ross (1995), Noble *et al.* (2000), Heesy (2003, 2005), and Ravosa and Savakova (2004). The midsagittal plane is lightly shaded, the orbital plane heavily shaded. a, Frontation is the caudal angle between the nasion inion chord and the intersection of the midsagittal plane with the orbital plane (i.e., 180° α). b, Convergence is the caudal dihedral angle between the plane of the orbit and the midsagittal plane (i.e., 180° β).

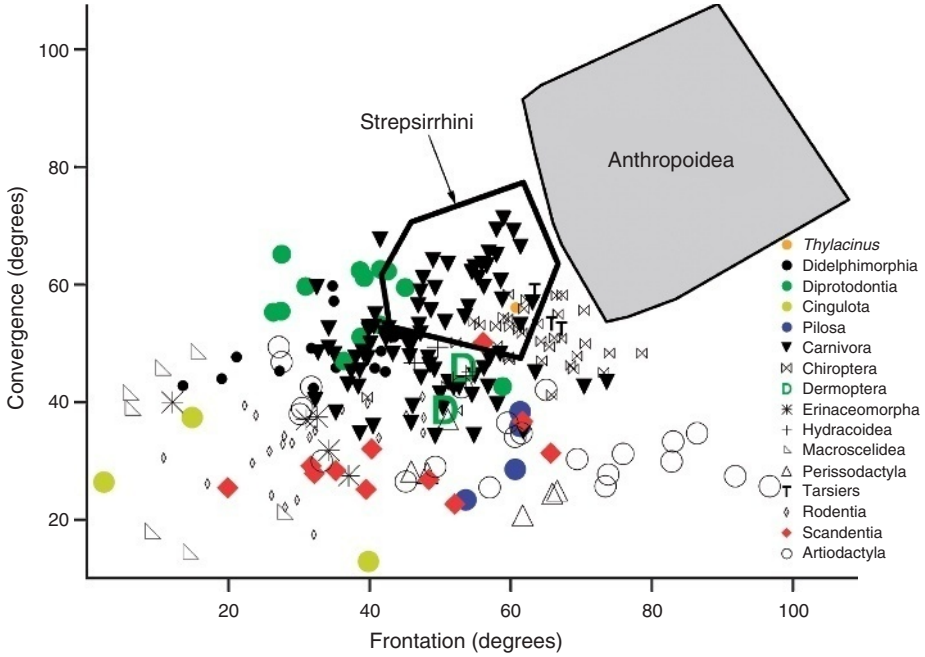

Figure 3 Bivariate plot of orbital convergence angle (degrees) against orbital frontation angle (degrees) in mammals. Individual data points for primates are excluded and replaced by minimum convex polygons (*sensu* Jerison, 1973). Data are from Heesy, C. P. 2005. Function of the mammalian postorbital bar. *J. Morphol.* 264, 363 380.

Allman–Pettigrew model notes that orbital convergence is associated with convergence of the optic axes on the visual axes, a means of improving retinal image quality that is necessary for nocturnal animals but not for diurnal ones. Whereas diurnal animals can ensure high retinal image quality by decreasing pupil diameter, thereby restricting incoming images to the paraxial region of the dioptric apparatus, nocturnal animals must maintain large pupil apertures in order to preserve image brightness. Consequently, nocturnal animals must improve image quality in the area of visual field overlap by optic and orbital convergence. This suggested to Allman (1977) that, if the first primates had high degrees of orbital convergence, they were probably nocturnal (Figure 4).

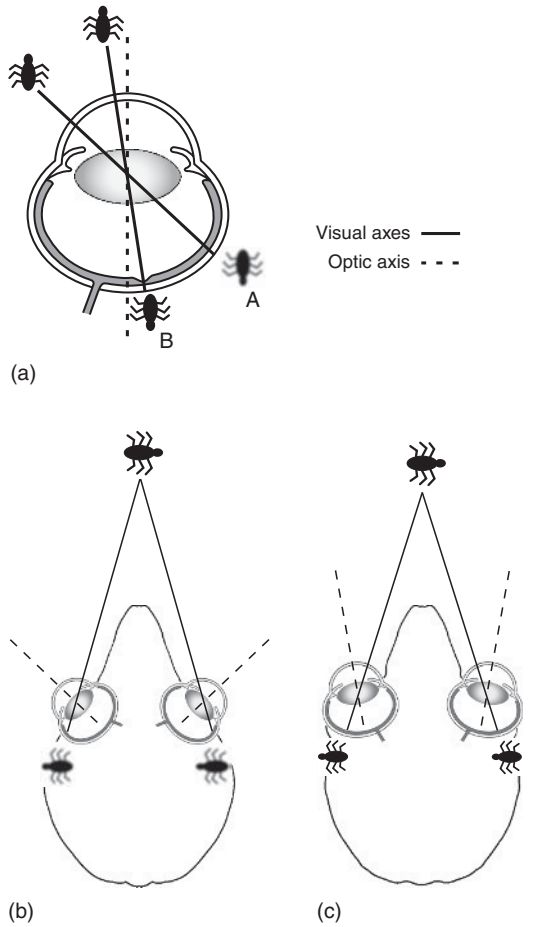

Figure 4 Diagrams illustrating functional significance of orbital convergence in nocturnal primates. a, Diagram of eye illustrating the effect of relative orientation of optic and visual axes on image quality. Image quality is best when the visual axis is more closely aligned with the optical axis. b, Diagram of visual and optic axis orientation in an animal with laterally facing orbits. c, Diagram of visual and optic axis orientation in an animal with convergent orbits. The quality of the image of the area in front of the animal is lower in (b) than in (c) because the optic and visual axes are less closely aligned.

36.6 The Primate Postorbital Bar

Primates all have postorbital bars which, while not unique to primates, do serve to separate them from their nearest putative fossil relatives, the plesiadapiforms. Cartmill (1970) and Heesy (2003) list a variety of other mammals with postorbital bars and processes. Dermopterans have postorbital processes (i.e., incomplete bars), while tree shrews have complete postorbital bars. Cartmill (1970, 1972) hypothesized that the primate postorbital bar functions to protect the orbital contents against movements originating from the chewing muscles in the temporal fossa. These movements might occur in all chewing animals, but Cartmill hypothesized that they were particularly problematic in animals with convergent orbits. Orbital convergence brings the plane of the orbit out of the plane of the temporal fossa, such that distortions of the postorbital ligament caused by contraction and bulging of the temporal muscles impinge upon the orbital contents (Figure 5).

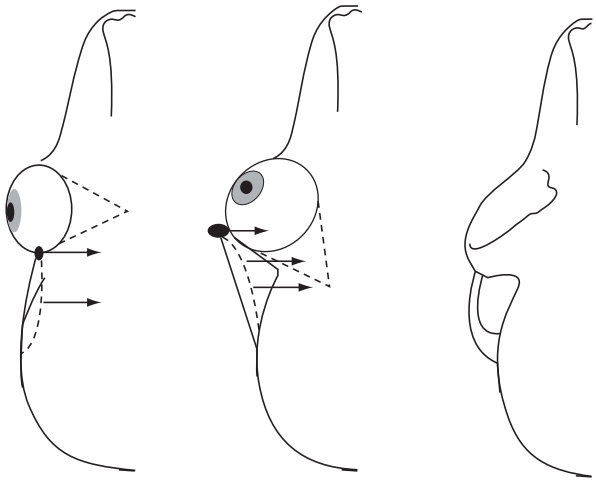

Figure 5 Diagram illustrating the function of the postorbital bar according to Cartmill (1972). In the left figure, the orbit is laterally directed and contractions of the temporalis muscle that pull the temporalis fascia and postorbital ligament medially do not impinge upon the eye (medially directed arrows). In the middle figure, the effects of moderate orbital convergence are illustrated. Convergence of the orbits is achieved by anterolateral displacement of the postorbital ligament. This brings the ligament lateral to the eye so that medial displacement of the ligament moves the eye around. As shown in the right figure, to prevent unwanted eye movements, the ligament is ossified into a postorbital bar to stiffen the lateral orbital wall. Adapted from Heesy, C. P. 2003. The Evolution of Orbit Orientation in Mammals and the Function of the Primate Postorbital Bar. PhD thesis, Stony Brook University, with permission of the author.

This hypothesis receives support from recent comparative morphometric analyses of orbit orientation in nonprimate mammals. Increased orbital frontation (roughly equivalent to verticality) in animals with moderate degrees of orbital convergence also causes the orbital and temporal planes to diverge, necessitating evolution of a postorbital bar (Noble *et al.*, 2000; Ravosa and Savokova, 2004). Heesy (2003, 2005) showed that the degree of postorbital ossification across a wide range of mammals is correlated with the degree to which the planes of the orbital aperture and of the temporal fossa diverge, regardless of whether that divergence is caused by increased orbital convergence, frontation, or displacement (Figure 6). This suggests that the evolution of the postorbital bar in primates represents an instantiation of a general principle identified by Cartmill that applies across all mammals: when the orbit and temporal fossa are not coplanar, movements in the temporal fossa are more likely to disturb the orbital contents and some kind of postorbital ossification is necessary to insulate the orbit.

The precise source, magnitude, and nature of the eye movements originating in the temporal fossa are unknown. Lemme *et al.* (2005) measured deformation in the postorbital ligament of pigs during stimulation of the temporalis and masseter muscles, and during mastication. They found that deformation of the ligament was primarily caused by contraction of the ipsilateral superficial masseter. In nonanthropoid primates, the chewing muscles, including the superficial masseter, are recruited much more vigorously on the working side than the balancing side, producing higher bone strain magnitudes on the postorbital bar of the working side than that of the balancing side (Ravosa *et al.*, 2000). Together, these results suggest that any disturbances suffered by the eyes during chewing would be asymmetrical. It might be difficult to offset or tolerate this asymmetry (Ravosa *et al.*, 2000), although this would depend on the nature of the movements. If the eyes were primarily protruded, the resulting diplopia would be less than if the eyes were abducted or adducted. Heesy *et al.* (2006) measured eye movements in anesthetized cats and galagos during stimulation of the masticatory muscles and found varying amounts of protrusion and abduction. Whether these movements occur in awake, chewing primates has not been established.

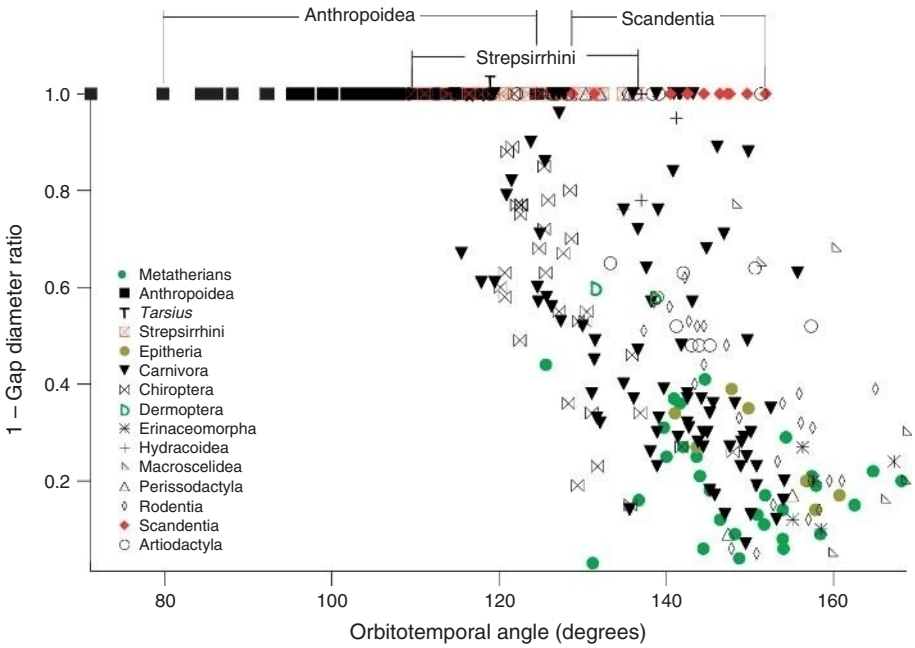

Figure 6 Bivariate plot of 1 postorbital gap:orbit diameter ratio against orbitotemporal angle. The gap:diameter ratio is the distance between the tips of the postorbital processes divided by orbit diameter. This value is subtracted from 1.0 so that animals with longer processes or bars are higher on the *y*-axis. Animals with values of 1.0 at least have complete postorbital bars. Orbitotemporal angle is the dihedral angle between the plane of the orbit and the plane of the temporal fossa. This angle quantifies the internal angle between the plane of the orbit and the plane of the temporal fossa. This plot shows that as the orbit becomes less coplanar with the temporal fossa (i.e., as the orbitotemporal angle decreases), the length of the gap between the postorbital processes decreases. Only animals with postorbital bars can have orbital planes that are strongly divergent from the plane of the temporal fossa. Note that only animals with postorbital septa (i.e., tarsiers and anthropoids) have extreme values of orbitotemporal angle. The data are from Heesy, C. P. 2005. Function of the mammalian postorbital bar. *J. Morphol.* 264, 363 380.

36.7 Criticisms of the Nocturnal Visual Predation Hypothesis

The visual predation hypothesis was the most widely accepted explanation of primate origins until counter-arguments began to appear in the 1990s. Critiques of the nocturnal visual predation (NVP) hypothesis can be grouped into three categories of argument: that the ancestral primates were not nocturnal; that the predatory adaptations of the ancestral primates were not visual; and that the visual adaptations of the ancestral primates were not predatory.

36.7.1 Ancestral Primates Were Not Nocturnal

Several researchers have argued against the NVP hypothesis on the grounds that basal primates were not nocturnal. Tan and Li's (1999; Li, 2000) hypothesis that the ancestral primates were trichromatic and diurnal is unparsimonious in the context of a more comprehensive analysis of the data (Heesy and Ross, 2001). More recently, Ni *et al.* (2004) reported the discovery of a skull of the basal omomyiform primate *Teilhardina asiatica* from the earliest Eocene deposits of the Lingcha Formation, China (Figure 7). On the basis of the relative orbit size of this specimen, Ni *et al.* suggested that *T. asiatica* was diurnal. The use of relative orbit size as an indicator of activity pattern in fossil primates was pioneered by Walker (1967), but fully developed by Kay and Cartmill (1977; Kay and Kirk, 2000). This work showed that, in living primates with skull lengths below approximately 75 mm, nocturnal species generally have larger orbits than diurnal species. This separation of nocturnal and diurnal species in relative orbit size makes it possible to discriminate activity pattern in fossil species by plotting orbit size against body size to see whether the fossil resembles living nocturnal or diurnal primates. Applying this technique to interpret the activity pattern of the tiny *T. asiatica* necessitates extrapolation below the range of skull lengths exhibited by living primates. Ni and colleagues used a least-squares regression model to estimate the orbit dimensions of nocturnal and diurnal taxa at the skull length of *T. asiatica*, and argued that the relative orbit size of *T. asiatica* suggests that it was diurnal. Optimizing activity pattern onto a phylogenetic tree of primates and their relatives, Ni *et al.* reconstructed diurnality at the stem primate node, hence calling the NVP hypothesis into question.

Ni *et al.*'s analysis suffers from the difficulty of extrapolating the relationship between relative orbit size and activity pattern below the body size range of living primates (Martin and Ross, 2005). The

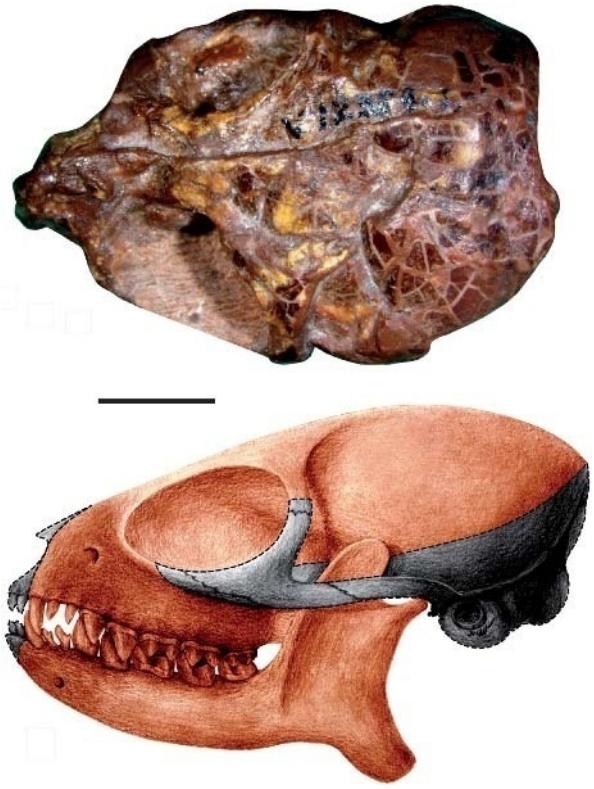

Figure 7 Skull of *T. asiatica* in dorsal and reconstructed lateral view; (IVVP V12357), earliest Eocene Lingcha Formation, Hengyang Basin, China (Ni *et al.*, 2004). Scale bar: 5 mm. Reproduced from Ni, X., Wang, Y., Hu, Y., and Li, C. (2004). A euprimate skull from the early Eocene of China. *Nature* 427, 65 68, with permission from Nature Publishing Group.

relationship between eye size and body size in mammals has been claimed to be nonlinear, such that eye size declines rapidly at body sizes below the range of extant primates (Ross, 2000; Kiltie, 2000; Martin and Ross, 2005). In Figure 8, corneal diameter of the eye is plotted against head-and-body length in mammals. The line that best fits the data is a fourth-degree polynomial, and a quadratic explains the data better than a linear least-squares line, but none of these lines is significantly different from any others, making it difficult to determine what kind of regression line should be used at small body sizes. This calls into question the hypothesis that *T. asiatica* was diurnal and raises the thorny issue of how to reconstruct activity pattern in fossil primates at body sizes below those of extant forms.

Various lines of evidence point to a nocturnal origin for basal euprimates (reviewed by Ross *et al.*, 2006). Charles-Dominique and Martin (1970) argued that the ancestral primate was probably nocturnal because nocturnality characterizes galagids, cheirogaleids, and lorisids, and these animals are probably the most primitive members of their respective lineages. Later, Martin (1973)

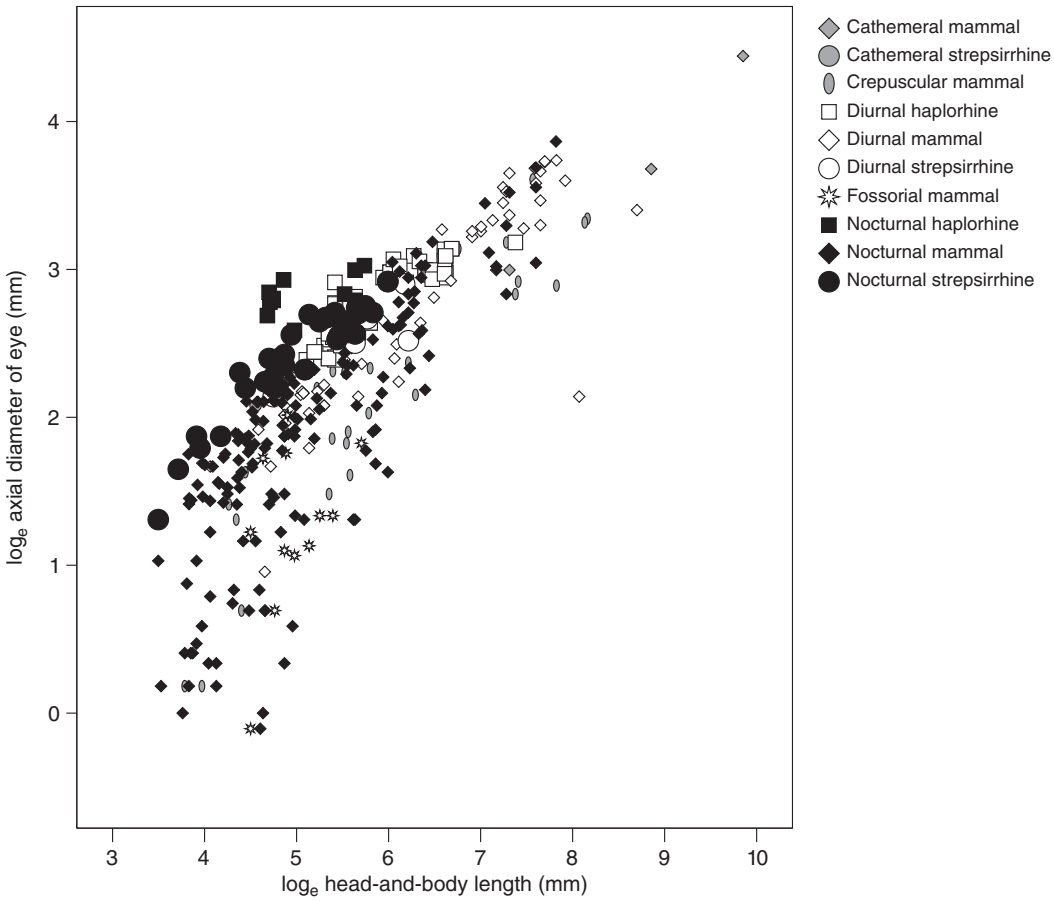

Figure 8 Plot of axial diameter of the eye against head-and-body length in mammals. Data from Ritland, S. 1982. The Allometry of the Vertebrate Eye. Unpublished PhD dissertation, Department of Biology, University of Chicago and Ross (unpublished data).

bolstered this argument by pointing out that the presence of the tapetum found in diurnal lemurids is best explained as a primitive retention from a last common ancestor of strepsirrhines that was nocturnal. Ross (2000) hypothesized that the earliest primates also probably possessed a tapetum. Explicitly cladistic reconstructions of the evolution of activity pattern in primates and their relatives corroborate the hypothesis that nocturnality characterized the first euprimates (Heesy and Ross, 2001). The possibility that certain early primates were extremely small, even around 10 g in size (Gebo *et al.*, 2000; Gebo, 2004), also suggests that these animals were nocturnal, as most living mammals in this size range are nocturnal. The possibility that basal primates were smaller than any living primates is not universally accepted, but some of them certainly were. It is therefore important to ask what the visual systems of such animals would have been like. Most extant mammals in this size range are olfactory-dominated animals. What kind of eye could a 10 g primate have carried and how would its brain organization have been affected (Kaas, 2000)? Theoretical investigations of such issues, combined with future fossil discoveries, promise to provide important clues as to the visual adaptations of early primates.

36.7.2 Predatory Adaptations of the Ancestral Primates Were Not Visual

The most common criticism of the NVP hypothesis is that primates use nonvisual senses to locate prey (Rasmussen, 1990; Sussman, 1991; Crompton, 1995). Sussman (1991) reviewed relevant data, pointing out that *Galagoides demidoff* and *Tarsius* can localize prey using hearing (Charles-Dominique, 1977; Niemitz, 1979), whereas lorises localize prey with olfaction. However, as has been noted previously, the fact that primates use nonvisual senses to localize prey does not necessarily mean that their visual sense is not important for prey localization (Dominy *et al.*, 2004; Ross *et al.*, 2005). Both galagos and lorises have been reported to use visual cues to localize moving prey (Charles-Dominique, 1977, p. 39; Schulze and Meier, 1995).

Moreover, it is also clear that, among extant mammals, increases in sound-localization acuity are associated both with increases in width of the binocular visual field and with narrowing of the field of highest visual acuity (Heffner and Heffner, 1985, 1992): animals with the highest auditory acuity also have large binocular visual fields and narrow fields of high-acuity vision. These data led Heffner and Heffner to suggest that the function of sound localizing is "directing the attention of other senses toward the sound-producing object" (Heffner and Heffner, 1992, p. 711). Primates notably have increased sound-localizing ability, increased binocular field width, and narrow fields of high visual acuity, and the work of Heffner and Heffner suggests that these features are interrelated. It cannot therefore be argued that use of auditory information for prey localization falsifies the hypothesis that visual cues are used as well (Sussman, 1991; Crompton, 1995). On the contrary, it suggests that if early primates were indeed nocturnal visual predators, they were probably auditory predators as well, and vice versa.

36.7.3 Visual Adaptations of Ancestral Primates Were Not Predatory

The most important criticism of the NVP hypothesis proposes alternate explanations for the origins of the high degrees of orbital convergence characteristic of primates. Two alternate reasons for orbital convergence have been suggested: localizing small fruits in terminal branches and locomotion in the fine-branch niche.

Sussman (1991) agreed with Cartmill that the divergent hallux and pollex and flattened nails are grasping organs, noting:

> It is generally agreed that these adaptations would have allowed Eocene prosimians far greater access to fruits and flowers, as well as plant visiting insects, making them much more efficient at locomoting and foraging in the small terminal branches of bushes and trees than were the plesiadapoids (Sussman, 1991, p. 219).

But Sussman went on to suggest that the evolution of orbital and optic convergence is not explained either by locomotion or by predation on small insects, which he saw as being captured using hearing and olfaction. Instead, Sussman notes that fruit bats also appear to have convergent orbits, like primates, and implicitly suggests that in primates this might be related in some way to eye–hand coordination:

> these nocturnal animals [i.e., fruit bats and primates] were feeding on and manipulating items of very small size (e.g., fruits, flowers and insects), at very close range, and under low light conditions. This might require acute powers of discrimi nation and precise coordination (Sussman, 1991, p. 219).

Rasmussen's (1990) study of the feeding and locomotor behavior of *Caluromys* led him to suggest that there might be elements of truth to both Cartmill's and Sussman's models. He suggested that the stem primates were lured out onto the terminal branches by:

> . . . fruit and flowers with associated coevolving insect faunas . . . Once up into the swaying terminal branches, those indivi duals that could best meet their arthropod requirements by visual predation probably had a selective advantage over those whose visual, locomotor and manual coordination abilities were less suited for such a complex task (Rasmussen, 1990, p. 274).

Thus, Rasmussen argues that early primates were lured out into the terminal branches for the reasons advocated by Sussman, but the visual specializations were adaptations for the NVP suggested by Cartmill.

Crompton (1995) argued that stereopsis in the fine-branch niche "cannot readily be ascribed to the need to detect cryptic, immobile insects, since they are not the typical prey" (Crompton, 1995, p. 25). Instead, in a modified version of the fine-branch niche hypothesis, Crompton argued that foraging, leaping, and climbing among the dense supports of the fine-branch niche would benefit from stereopsis and grasping hands because this environment:

> . . . provides a visually complex, confusing background against which to distinguish a variety of mobile and immobile targets, both dietary items (fruit, as well as insects) and locomotor substrates (Crompton, 1995, p. 25).

In the end, Crompton invoked a multifactorial explanation for the origins of the orbital convergence.

> Orbital frontality is more likely to have first appeared as a con sequence of the more general benefit that accrues, for a small bodied primate similar to *Microcebus*, in the fine branch niche. This is provision of scotopic acuity and depth perception for the location of diverse targets, fruit and branches as well as insects in a complexly shaded environment (Crompton, 1995, p. 26).

The importance of the grasping hand for Crompton lies not only in climbing and manipulation of food, but also in securing a safe landing after short leaps. Once again, eye–hand coordination is implicit in Crompton's argument, although the relevance of this coordination for landing after a leap is not clear.

Thus, the adaptive significance of the distinctive features of the primate visual system is debated. Cartmill (1972, 1974) and Rasmussen (1990) agree that orbital convergence facilitates NVP on insects, captured with the hands in the fine-branch milieu; Sussman (1991) argues that orbital convergence is linked to manipulating small fruits, flowers, and insects under low light levels; Martin (1990) links orbital convergence to locomotion in a fine-branch niche, and Crompton (1995) invokes both

feeding on small food objects and locomotion to explain the evolution of orbital convergence.

These debates over the ecological significance of increased orbital convergence stimulated additional comparative morphometric research on orbit orientation in mammals. Heesy (2003) measured orbit orientation in a large sample of metatherian and eutherian mammals, and found strong effects of locomotor substrate, activity pattern, and diet on orbital orientation. Orbital convergence and frontation are higher in arboreal taxa than terrestrial or aerial taxa, and frontation and verticality are higher in faunivorous and omnivorous taxa than in opportunistic and nonpredatory animals. When these analyses were performed on eutherians exclusive of primates, nocturnal and cathemeral/crepuscular animals were found to have more convergent orbits than diurnal animals, and faunivorous taxa to have more convergent orbits than nonpredators. When all possible categories of locomotor substrate, activity pattern, and diet were considered, arboreal, nocturnal faunivores were ranked as having the highest degrees of orbital convergence. Heesy's analyses suggest that, across a wide range of mammals, nocturnal, arboreal faunivores tend to have more convergent orbits than other ecological categories. In a similar study, Ravosa and Savakova (2004) showed that, when allometric factors are taken into account, pteropodid bats do not have orbits that are as convergent as those of primates, negating one of Sussman's criticisms of the NVP hypothesis. Moreover, felid carnivorans (which are predominantly nocturnal) have primate-like degrees of orbital convergence, while nocturnal visual predatory tree shrews (*Ptilocercus*) and nocturnal procyonid carnivorans have more convergent orbits than diurnal predatory close relatives.

Both of these studies (Heesy, 2003; Ravosa and Savakova, 2004) corroborate the NVP hypothesis, but neither study explicitly evaluates the hypothesis relative to the fine-branch niche locomotion hypothesis. Ravosa and Savokova show that felids – NVPs not living in the fine-branch niche – have primate-like levels of orbital convergence, suggesting that NVP is sufficient to produce orbital convergence, but they do not exclude the possibility that fine-branch living also would produce this effect, even in the absence of NVP. Similar issues emerged from Lemelin's (1999) comparison of hand morphology in didelphid marsupials and primates. Although he confirmed that locomotion on fine terminal branches is associated with convergent similarities in hand and foot anatomy and proportions in marsupials and primates, the animals concerned also fed on small fruits and insects in the terminal branches. This makes it difficult to factor out the relative importance of feeding versus locomotion and of insectivory versus frugivory for hand and foot morphology.

To demonstrate that NVP is necessary and sufficient to explain orbital convergence and the unique hand morphology of primates, but fine branch locomotion or fruit feeding are not, NVPs living in the fine-branch niche need to be compared with non-NVPs living in the fine-branch niche. Variation in degrees of predation, hand morphology, and orbital convergence within primates provides one source of appropriate comparisons. Lemelin (1996, p. 173) reports preliminary results of analyses that demonstrated "significant and positive covariation between amount of insectivory, selection to catch styles, and relative lengths of the digits among closely related prosimians."

36.8 Comparative Neuroscience

In parallel with these developments in primatology, comparative neuroscience has revealed a series of distinctive features of the primate nervous system, which, judging by their common occurrence in most primates, can be hypothesized to have evolved along the primate stem lineage, after the divergence of any sister group, such as tree shrews and dermopterans.

36.8.1 Visual System

The high degree of orbital convergence characteristic of primates increases the size of the binocular field (Ross 2000; Heesy, 2004) and improves the potential and actual quality of the image falling on the central retina. These changes make it worthwhile increasing relative eye size to increase image size (Ross *et al.*, 2006), increase the density of photoreceptors and ganglion cells in the central retina to increase sampling frequency, and increase representation of the central retina in the visual structures of the brain (Allman, 1977). Barton (2004) has shown that, while controlling for body size, increases in relative orbital convergence are associated with increases in the relative volume of the lateral geniculate nucleus, relative area of the primary visual cortex, and relative neocortex size in general. Barton also shows that these increases are primarily attributable to increases in parvocellular rather than magnocellular pathways, suggesting that they reflect adaptations for improved fine-grained stereopsis, rather than increased sensitivity to movement. He suggests that:

> . . . the increase in visual brain size in primates with more convergent orbits might reflect enhancements of stereo acuity and vergence control mechanisms specifically related to the visually guided grasping and close range manipulation of food items (Barton, 2004, p. 10115).

Barton's analysis treats variation in the visual system within primates, not across mammals, as a whole, so caution must be exercised when extending the results back into the primate stem lineage. To the extent that such extrapolation is valid, many of the changes to the visual system that occurred in the primate stem lineage can be hypothesized to have been not only integrated with each other, but also associated with improvements in fine-grained stereopsis and visual acuity in the center of the visual field. However, it is important to remember that the visual systems of stem primates were also characterized by an array of changes related to other functions, including improved sensitivity to movement, and improved ability to locate movements and sounds in space. Primates exhibit extensive projections from each retina to its ipsilateral lateral geniculate nucleus and superior colliculus, and both the visual cortex and superior colliculus contain representations of only the contralateral visual field. The superior colliculus provides the substrate for the visuomotor response, in which the eyes are directed to novel objects entering the visual field (Schiller and Stryker, 1972) and the unique arrangement of the projections to the superior colliculus in primates removes ambiguity regarding the position of those objects (Allman, 1999). As noted above, increased overlap of the visual fields across mammals is also associated with increased ability of the auditory system to localize sounds in space, suggesting that such abilities also characterized basal primates (Heffner and Heffner, 1982, 1992; Heffner, 2004). Primates are characterized by expansion and multiplication of their extrastriate visual areas, including not only areas that process information on fine-grained stereopsis and acuity (the ventral information stream in temporal cortex), but also areas such as the middle temporal (MT) area devoted to analysis of movement in the contralateral visual field (see reviews in Allman, 1977, 1999; Allman and McGuinness, 1988; Kaas, 2002). Thus, there is evidence that the basal primate visual system was modified not only to enhance fine-grained stereopsis (Barton, 2004), but also to improve the ability to detect and localize sources of movement and sound in the visual field. These latter attributes would be of particular benefit to NVPs, but of little obvious use for finding fruits and berries.

36.8.2 Hand Motor Control

In vertebrates, control of voluntary limb movements is mediated by descending pathways from the brain to the motor neurons in the spinal cord. All vertebrates possess reticulospinal, rubrospinal, tectospinal, and various other pathways from the brain to the spinal cord (Nudo and Masterson, 1988), but corticospinal tracts (CSTs) are found only in mammals. Simian primates and carnivores have larger CSTs than other mammals (Phillips and Porter, 1977; Figure 9), and the lateral CST of primates is unusual in both the degree to which it penetrates to caudal spinal cord segments and in the directness of its connections with motor neurons of the muscles of the distal extremities (Phillips and Porter, 1977; Heffner and Masterson, 1983). Across mammals and within primates, increased CST penetration down the spinal cord and increasingly ventral termination of CST connections within the cord are correlated with progressive increases in the degree of digital dexterity (Heffner and Masterson, 1975). This suggests that the emergence of these features in basal primates was associated with increased manual digital dexterity. Extant primates use their hands for many things, including grasping branches during locomotion, acquiring food, and social grooming (Bishop, 1964). Precisely which of these functions originally demanded enhanced dexterity is not immediately obvious from the anatomical data.

One question arising from these data is why there is a relationship between manual digital dexterity and CST penetration beyond those cervical spinal cord segments that supply the muscles of the forelimb (Heffner and Masterson, 1975). The answer to this conundrum may lie in a Wood Jonesian emancipation of the forelimb accompanying increased coordination of the hindlimbs and forelimbs. One benefit of this is illustrated in Figure 10, a photograph of a *Mirza coquereli* cantilevering from a

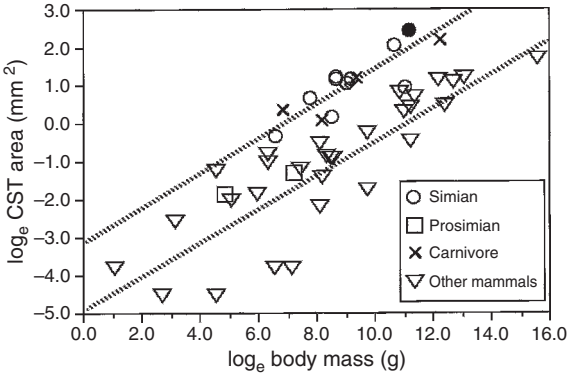

Figure 9 Bivariate plot of the natural log of CST area against the natural log of body mass in mammals. The least-squares regression lines for simian primates and for rodents are shown. Humans are shown by the solid circle. Simian primates have larger CSTs than other mammals, a feature in which they resemble carnivores. Data from Heffner, R. and Masterton, B. 1975. Variation in form of the pyramidal tract and its relationship to digital dexterity. *Brain Behav. Evol.* 12, 161 200.

Figure 10 *M. coquereli* adopting a cantilever posture. Image of Coquerel's dwarf Lemur, *M. coquereli*, Kirindy Forest, Madagascar, © Manfred Eberle, www.phocus.org.

vertical branch to grasp something out of the air. Various prosimians (cheirogaleids, galagos, and tarsiers) have been reported to manually acquire flying prey while holding onto branches with their feet (Crompton and Andau, 1986; Gebo, 1987; Martin, 1990). We hypothesize that extension of the CST down to lumbar and sacral spinal cord segments provides the anatomical connections necessary for arboreal mammals to coordinate a secure hold on the substrate with their hindlimbs or tail while they use their hands for catching insects, harvesting fruits, or other tasks requiring manual dexterity.

Another distinctive aspect of the primate corticomotor (CM), system is the degree of multiplication of premotor areas in frontal cortex. Macaques, for example, exhibit at least six separate premotor areas that project not only into primary motor cortex (MI), but also give origin to corticospinal neurons. In the three areas in which this has been studied, these corticospinal neurons include CM fibers that run directly from the cortex to the motor neurons in the ventral horn of the cervical and lumbar regions of the spinal cord (Dum and Strick, 2002). Five of the six premotor areas have distinct projections to both upper and lower cervical spinal cord segments. Three of these areas (supplementary motor area (SMA), dorsal cingulate motor areas (CMAd), ventral cingulate motor areas (CMAv)) project to lower cervical spinal cord segments, specifically to the intermediate zone and ventral horn, the latter of which contains the motor neuron cell bodies for the hand muscles. Each premotor area receives inputs from a different combination of posterior parietal and prefrontal cortical areas, "each participates in distinct loops with the basal ganglia and cerebellum" (Dum and Strick, 2002, p. 681), and each projects in parallel to the spinal cord. Just as the multiplicity of prestriate visual areas serves as the substrate for a multiplicity of diverse visual functions, so each of these multiple premotor areas is argued to be "a functionally distinct efferent system that differentially generates and/or controls specific aspects of motor behavior" (Dum and Strick, 2002, p. 677). The anatomical and physiological relationships between these areas and the control of hand movements suggest that the increased dexterity characteristic of primates is related to the multiplication and increased functional diversity of these cortical premotor areas.

Nudo and Masterson (1990b) showed that the size of CST cortex is highly correlated with body mass, brain mass, and the area of the neocortex, with the strongest relationship between CST cortex area and overall neocortex area. After they factored out the effect of increased cortex size, they found primates to show a constant proportion of CST cortex to overall cortex area, while raccoons show relative increases in CST cortex compared to other carnivorans. They attributed the enlargement of the CST cortex in primates to overall neocortical enlargement. Whatever the mechanism of enlargement, the size of the cortical areas giving rise to CSTs increases along the lineages leading to humans and raccoons from basal mammals in parallel with their dexterity (Nudo and Masterson, 1990b).

36.8.3 Eye–Hand Coordination

Elliot Smith noted that eye–hand coordination is an important component of the basal primate adaptations, but most explanations for primate origins in the literature have neglected to emphasize this basal attribute. Recent studies in comparative neuroscience have revealed distinctive anatomical features of the primate brain that are involved in mediating this coordination.

Although nonprimate mammals have premotor areas that give origin to CSTs, primates are unique in having CSTs arise from a distinct subregion of ventral premotor cortex not found in other mammals: region C of Nudo and Masterson (1990a) or the arcuate premotor area (APA) of Dum and Strick (2002). Allman (1999) synonymized region C with the ventral premotor region, PMv, but region C in macaques at least is only the rostral part of PMv lying within the posterior bank of the inferior limb of the arcuate sulcus. Regardless of terminology, primates are unique in possessing areas PMv and APA/region C, both of which appear to be important in the control of visually guided reaching and grasping movements (i.e., eye–hand coordination).

APA is unusual among the six premotor areas discussed above in that it exhibits very dense and numerous connections to the hand representation in MI, and to upper cervical segments supplying the muscles crossing the shoulder and elbow joints, but does not project to lower cervical spinal cord segments where hand motor neurons are located. Nevertheless, stimulation of this area commonly elicits movements of the fingers and thumb, but less commonly movements of more proximal joints, such as the wrist, elbow, and shoulder (Martino and Strick, 1987; Dum and Strick, 1991; He *et al.*, 1993). Dum and Strick (2002, p. 681) suggested that APA/region C "is primarily involved with control of distal forelimb movements" and the anatomical data presented above suggest that this control involves coordination of the movements in joints of the upper arm as well.

Preuss (1993) reviewed the evidence available at that time that PMv plays "a role in visually guided reaching and prehension." The work of Rizzolati *et al.* had revealed that neurons in PMv respond not only to tactile stimuli applied to the hands and face, but also to visual stimuli, especially to stimuli within reaching distance. Neurons in this region are active, "specifically during purposive, prehensive movements of the face and forelimbs" (Preuss, 1993). Preuss argued that integrated use of the mouth and the hand may have been important components of early primate feeding adaptations, whether for visually guided manual predation on insects as suggested by Cartmill (1970) or "visually guided grasping and manipulating fruits and flowers" as advocated by Rasmussen and Sussman (Preuss, 1993, p. 355). Preuss' hypothesis receives support from more recent observations that when PMv caudal to the inferior limb of the arcuate sulcus – close to the origin of the CST – is stimulated, coordinated movement of the hand to the mouth is elicited, accompanied by opening of the mouth (Graziano *et al.*, 2002a). This suggests an important role for PMv in visually guided movements of the arm and hand during feeding. However, PMv also functions in the integration of tactile, auditory, and visual information in the control of arm movements (Graziano and Gandhi, 2000; Graziano *et al.*, 1999, 2002a, 2002b). Graziano's work has revealed a polysensory zone that integrates visual, auditory, and tactile information into the planning of hand movements in space (Graziano *et al.*, 1999; Graziano and Gandhi, 2000). Integration of visual, auditory, and tactile information is plausibly related to capturing flying or moving prey, whereas auditory information is not obviously necessary for coordination of movements associated with locomotion or grasping fruits.

Improved sensorimotor coordination in control of primate hand movements is also indicated by expansion and elaboration of somatosensory areas (ventral somatosensory area (VS), the parietal rostral area (PR), and the retroinsular area (Ri)) and areas in the posterior parietal cortex that are important for visual and visuomotor processing (Wu *et al.*, 2000; Kaas, 2004). The latter areas connect forward into the array of new premotor areas in the frontal lobe, including the multiple premotor areas controlling hand and digit movements (e.g., PMv, SMA). Stimulation of the rostral half of posterior parietal cortex in *Otolemur* (Stepniewska *et al.*, 2005) and macaques (Their and Andersen, 1998; Cooke and Graziano, 2003) elicits complex movements that "seem to be components of ethologically meaningful behavioral patterns such as feeding and defense" (Stepniewska *et al.*, 2005, p. 4882). To the extent that these attributes and connections of PMv characterized stem primates, PMv was probably an important component of a neural system adapted not only for foraging for small fruits and berries, but also for NVP. Unfortunately, the available data do not allow definitive statements as to the original function of PMv. Graziano's research was carried out on macaques, and it is not clear to what extent nocturnal primates such as galagos and lorises possess a polysensory zone in PMv. Moreover, although the origin of PMv may have been more important for mediating eye–hand coordination used in feeding than in locomotion, the other premotor areas distinctive of primates (Kaas, 2004) may well have had locomotor-related functions originally, and the precise order in which they arose cannot currently be discerned. Indeed, it may be that the neurological adaptations associated with eye–hand coordination are either interchangeable with or so extremely similar for both NVP and fine-branch locomotion as to make it impossible to discriminate between these competing hypotheses regarding primate origins. However, both scenarios imply that improved eye–hand coordination was a fundamental adaptation in basal primates.

36.9 Locomotor System

As reviewed above, it was F. Wood Jones who suggested that specialization of the hindlimb for supporting body weight during climbing emancipated the forelimb from supportive functions, freeing it for specialization to perform other tasks. Several attributes of the primate locomotor system suggest that Wood Jones' hypothesis contains some nuggets of truth. The feet of primates are adapted for grasping as part of a distinctive grasp-leaping

pattern of locomotion (Szalay and Dagosto, 1988). Changes to the joint between the first metatarsal and the entocunieform allow the hallux (big toe) to be held in an abducted position, divergent from the rest of the digits, and to be stable under high forces during grasping. The proximal end of the first metatarsal manifests a robust process that not only buttresses the enotcunieform joint but also provides a hypertrophied area of attachment for the powerful peroneus longus muscle that plantarflexes and everts the foot at the ankle. The primate upper ankle joint is adapted for stability across an enhanced range of flexion and extension, as would be encountered during leaping, and the tarsal elements are elongated to enhance the length of the hindlimb, facilitating a more powerful leap (Martin, 1979). The lower ankle joint evinces adaptations for increased inversion and eversion of the foot necessary during climbing (Dagosto, 1988).

All digits of primates typically bear nails, rather than claws, although some species have re-evolved claw-like nails. Callitrichids and *Daubentonia* have claws on all digits except the hallux, and prosimians have a toilet claw on one pedal digit. The skin of the distal digits is expanded into pads sporting cutaneous ridges for increasing friction on arboreal supports. These ridges are also associated with Meissner's corpuscles for enhanced tactile sensitivity (Martin, 1986). The phalanges of the hands and feet are lengthened relative to the metapodials to improve grasping abilities on fine branches, an adaptation evolved convergently with didelphid marsupials (Lemellin, 1999).

Primate locomotor gaits are also distinctive (Martin, 1990; Schmitt, 2003). Primates typically employ diagonal sequence gaits in which the footfall of the forefoot always follows the contralateral hindfoot, ensuring a secure grasp of the substrate with the hindfeet before moving the forefoot (Cartmill *et al.*, 2002). Primates also walk with a compliant gait, characterized by more elbow flexion, less vertical displacement of the center of mass, and longer stride lengths than other mammals. These traits are hypothesized to have arisen as adaptations to locomotion on small compliant branches (Cartmill *et al.*, 2002; Schmitt and Lemelin, 2002). Convergent evolution of diagonal sequence gaits in the arboreal woolly possum, *Caluromys*, corroborates the hypothesized link between this trait and locomotion on fine supports (Lemelin *et al.*, 2003). Primates also have greater peak reaction forces at their hindlimbs than their forelimbs and they display a more protracted forelimb at touchdown than other mammals (Larson *et al.*, 2001). Convergent evolution of this complex of features in the arboreal kinkajou (*Potos flavus*), a carnivore that also possesses a prehensile tail, provides support for Wood Jones' suggestion that the forelimb function becomes more diverse when the hindlimbs bear the majority of the body weight. Kinkajous not only support more of their body weight with their hindlimbs than their forelimbs, and exhibit highly protracted forelimbs at touchdown, resembling primates (Larson *et al.*, 2001), but they also possess CM connections to the ventral horn of the spinal cord, and relatively dextrous forelimbs (Petras, 1969).

36.10 The Fossil Record of Primate Origins

Although it may not be possible to determine from studies of extant primates alone whether the visual and grasping adaptations of early primates originally functioned as adaptations for locomotion or for feeding on insects and small fruits in light-limited environments (Allman, 1977; Pettigrew, 1978; Martin, 1990; Cartmill, 1982; Crompton, 1995), more direct evidence from the fossil record can provide insight.

The lineage leading to extant primates is traditionally thought to have diverged from other mammals close to the Cretaceous/Tertiary boundary. The first members of this primate lineage were long thought to be the plesiadapiforms, a radiation of fossil mammals that thrived in the Paleocene and Early Eocene of the northern continents. The traditional interpretation is that plesiadapiforms, or archaic primates, gave rise to a single stem lineage for euprimates, which quickly divided into two lineages, the omomyiforms and adapiforms, which appear in northern continents at the beginning of the Eocene (*c.* 55 Mya). Compared with plesiadapiforms, these latter two clades manifest closer anatomical similarities and phyletic affinities with extant primates and are grouped with them as Euprimates. In the 1980s and 1990s, many researchers excluded plesidapiforms from Primates because they are not adaptively similar to euprimates, and because cladistic analyses identified at least some plesiadapiforms as basal dermopterans (Beard, 1990; Kay *et al.*, 1992). Recent fossil discoveries and reinterpretation of old fossils have called into question the possibility of dermopteran relationships for plesiadapiforms and have once again identified them as the fossil group most closely allied with euprimates, placing them even closer to extant primates than tree shrews (Silcox, 2001; Bloch and Boyer, 2002).

The best-preserved plesiadapiform skull, that of the paromomyid, *Ignacius graybullianus*, is illustrative of plesiadapiform skulls in general (Figure 11). The braincase is relatively small and the orbits are small, superiorly facing, and completely confluent with the temporal fossa. The infraorbital foramen, like that of *Palaechthon* (Kay and Cartmill, 1977), is relatively large, suggestive of the importance of the vibrissae in detecting prey (Kay *et al.*, 1992). Skulls of *Plesiadapis* are similar, suggesting that the pronounced visual adaptations of euprimates were not shared by plesadapiforms. In contrast, postcranial fossils of many plesiadapiforms display a range of adaptations for arboreality (Szalay and Dagosto, 1988). Recently, a grasping foot with a nail on the hallux was reported from the carpolestid plesiadapiform *Carpolestes,* a putative close relative of primates (Bloch and Boyer, 2002). If *Carpolestes* is indeed representative of the stem lineage of euprimates, it suggests that the manual and pedal grasping abilities of primates evolved prior to their visual specializations, potentially supporting Rasmussen's hypothesis that early primates originally ventured into the small terminal branches in search of small fruits and only subsequently developed the visual adaptations characteristic of living primates (Bloch and Boyer, 2002).

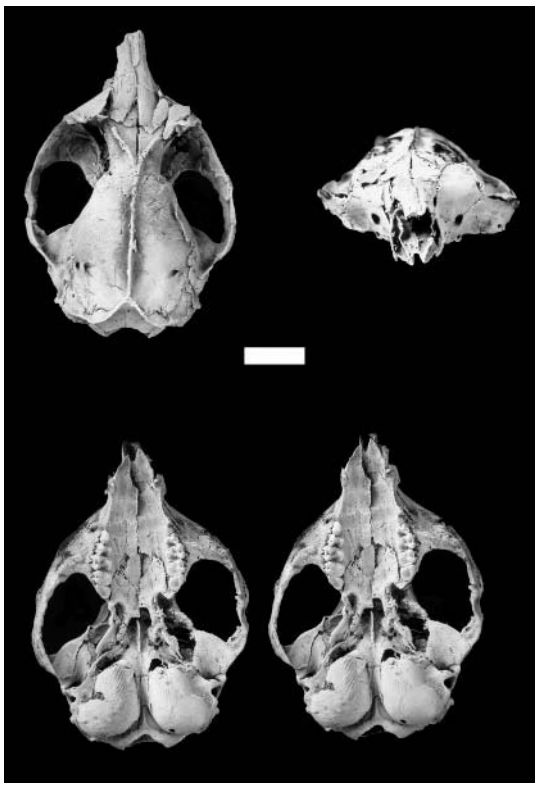

Figure 11 Skull of *I. graybullianus* in dorsal, rostral, and ventral (stereopair) view. Scale bar: 1 cm. Images courtesy of R. F. Kay.

Although *Carpolestes* may have resembled the antecedents of the ancestral primates in some respects, several problems dictate caution in basing interpretations on a direct reading of the fossil record. First, the fossil record is notoriously incomplete. Tavaré *et al.* (2002) have estimated that less than 7% of the species in the primate crown clade have been recovered such that major gaps are present. Hence, even when fossil evidence of extinct species of primates and their relatives is available, these species can be separated from events of interest by significant lengths of time, diminishing their relevance as direct indicators (Ross *et al.*, 2002). These issues are particularly relevant to *Carpolestes*. Statistical analysis of the primate fossil record suggests that the branching points for the origins of extant primates are significantly older than the earliest known fossil representatives currently available. Tavaré *et al.* (2002) estimated the age of primates to be approximately 82 Mya, whereas *Carpolestes* lived at the very end of the Paleocene (*c.* 56 Mya), 26 Mya later. This problem is compounded by the phylogenetic position of *Carpolestes*, which Bloch *et al.* (2001) have argued is nested deep within the carpolestids, removing the species morphologically as well as temporally from developments in the origin of primates. Indeed, from a temporal and phylogenetic perspective, the relevance of *Carpolestes* to questions surrounding primate origins is comparable (at best) to the relevance of living gibbons for hypotheses surrounding human origins. Future fossil discoveries will be needed to address these issues more directly.

36.11 Conclusions

The origin of primates of modern aspect was associated with the evolution of a suite of changes to the visual system in concert with changes in other functional systems. We contend that understanding the role of vision in primate origins and evolution requires an understanding of the integration between these systems. Changes to the visual system producing increased sensitivity to low light levels, improved fine-grained stereopsis, and increased visual acuity and motion sensitivity were accompanied by improved abilities to localize sounds or movements in space, increased dexterity, and changes to the somatosensory and somatic motor systems that provided for improved control of visually guided reaching and grasping movements. These changes were accompanied by modifications in gait and musculoskeletal anatomy of the hands and feet related to arboreal

locomotion, including leaping and grasping on fine-branch supports. These changes were manifest not only in the musculoskeletal periphery, but also throughout the central nervous system, including the origins and terminations of the CSTs, the premotor areas controlling limb movements, the visual cortex, and the primary and secondary sensorimotor areas.

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37 The Evolution of Sensory and Motor Systems in Primates

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Glossary

area or nucleus These are large, functionally significant subdivisions of neocortex and the brain stem. Each area (areas were the "organs of the brain" for Brodmann, 1909) or nucleus has a unique set of connections with other structures and uniquely con tributes to the function of the system. Sensory and motor areas and nuclei, typically, topographically represent receptors of a sensory surface (skin or deep tissues) and/or body movements.

Eutheria The mammalian clade stemming from the most recent common ancestor of placental mammals.

motor cortex Subdivisions of cortex that are specia lized to mediate and control body movements. Typically, movements can be evoked by electrically stimulating areas of motor cortex. Primary motor cortex (M1) is called agranular cortex because it has no obvious layer 4 of granule (sensory) neurons.

somatosensory cortex Areas of cortex that are more specia lized for processing sensory inputs than for motor control. While electrical sti mulation of somatosensory areas may elicit movements, these areas have a well developed layer 4 for receiving sensory inputs.

> And at last came the monkey, and anybody could see that man was not far off now. And in truth that was so. The monkey went on developing for close upon five million years, and then turned into a man to all appearances (Mark Twain *Letters from Earth*).

37.1 Introduction

Humans are known for their curiosity, and they are especially curious about themselves. Anyone who has viewed highly skilled athletes, musicians, or craftsmen must be impressed with their exceptional sensorimotor performances, which have been individualized and specialized by training and experience. These abilities depend on a massive sensorimotor network that has been gradually acquired during the course of human evolution. This network allowed our ancestors to make and use tools and weapons, craft garments, and process food. How did this system evolve and how does it work? Answers to these questions are incomplete, but we now have an outline of this system in humans and other mammals, information that allows the major steps in our evolution to be reconstructed, and a framework for understanding how the system works. This reconstruction of the course of brain evolution is informed by the fossil record, but it is largely based on comparative studies of extant primates and other mammals. Nevertheless, the fossil

record does indicate that early primates emerged as small, probably nocturnal (however, see Tan *et al.*, 2005), arboreal, small-brained mammals that likely fed on insects, fruit, small vertebrates, and buds (Ross, 1996). In some lines of primate evolution, the fossil record also indicates that brains got bigger in proportion to body size, and in the line leading to humans this transformation was remarkable, especially over the last 2 My. The extensive increase in the absolute and relative size of the brain suggests that major changes in brain organization occurred, but the fossil record provides little information about the nature of the changes.

In order to understand how sensorimotor systems evolved in primates, we need to compare the organizations of sensorimotor systems in primates and mammals most closely related to primates, and use distribution patterns across taxa to make inferences about when specific features of the human sensorimotor system emerged (see Eldredge and Cracraft, 1980; Wiley, 1981). While the focus is on the evolution of the human sensorimotor system, we also take note of a few interesting specializations that occurred in other lines of primate evolution.

37.2 The Somatosensory and Motor Systems of the Mammalian Ancestors of Primates: Inferences from the Systems of Rodents, Tree Shrews, and Other Mammals

It might seem strange to compare our nervous system with those of rats, rabbits, and tree shrews because rodents and lagomorphs seem so unlike us, and most of us know little about tree shrews. Yet these mammals turn out to be our closest living nonprimate relatives, and thereby these are the mammals to study if we want to know how the brains of our immediate nonprimate ancestors were organized.

Mammals emerged from mammal-like reptiles around 230 Mya and radiated into over 4500 surviving (extant) species of mammals. Because of recent advances in the use of molecular data to classify mammals (e.g., Murphy *et al.*, 2001, 2004), it has been possible to distinguish six major branches of mammalian evolution. Monotremes and marsupials constitute two early branches, while the placental mammals include Xenarthra (sloths, anteaters, and armadillos) and Afrotheria, Laurasiatheria, and Euarchontoglires as newly recognized clades (Figure 1). The Euarchontoglires clade emerged some 95 Mya and diverged into Glires, a line that gave rise to lagomorphs (rabbits, hares, and pikas) and rodents, and euarchontans, a line that gave rise to dermopteras (flying lemurs), scandentias (tree shrews), and primates. Of our closest living relatives – flying lemurs and tree shrews – we know very little about the brain of flying lemurs, but fortunately, tree shrew brains have been well studied, in part, because of early recognition by the great comparative anatomist Le Gros Clark that tree shrews resemble primates. Clark (1959) considered tree shrews to be primates, but this classification is no longer held tenable. In tree shrews, we look for traits that are shared by primates, rodents, and lagomorphs, as these traits may be those retained from a common ancestor of the Euarchontoglires clade. However, traits seen only in tree shrews need to be considered with caution, as they could be specializations of tree shrews rather than traits shared with the ancestors of primates (Kaas, 2002).

Euarchontoglires mammals also share a number of features of the sensorimotor system that were likely retained from early mammals. The basic features of the sensorimotor system of early mammals can be deduced by comparing components across members of the three major branches of mammalian evolution, monotremes, marsupials, and placental (eutherian) mammals (Kaas, 2004a). Some of the basic components of the somatosensory system of early mammals (Kaas, 2004b, 2004c) are illustrated in Figure 2. Starting with the inputs, low threshold cutaneous receptors project via peripheral nerves to the dorsal column–trigeminal complex in the lower brainstem, as well as to neurons in the dorsal horn of the spinal cord. Afferents terminate in the dorsal column–trigeminal complex in an orderly way so that ipsilateral tail, hindlimb, forelimb, and head are represented in a mediolateral sequence. Second-level neurons project to the ventroposterior nucleus (VP) of the opposite thalamus in a tail-to-tongue lateromedial somatotopic sequence. Third-level neurons in the VP in turn project to primary somatosensory cortex (S1) and to two smaller somatosensory areas just lateral (ventral) to S1, the second somatosensory area, S2, and the parietal ventral area, PV. Thus, VP neurons independently activate three somatosensory areas. Fourth-level neurons in S1 project to rostral (SR) and caudal (SC) somatosensory bands that border S1, and these areas and S1 all project to S2 and PV. Neurons in S2 and PV access neurons in perirhinal and parahippocampal areas that feed into the hippocampus for memory functions (Squire and Knowlton, 1994), and the amygdala for fear conditioning and other functions (LeDoux, 2000). Other

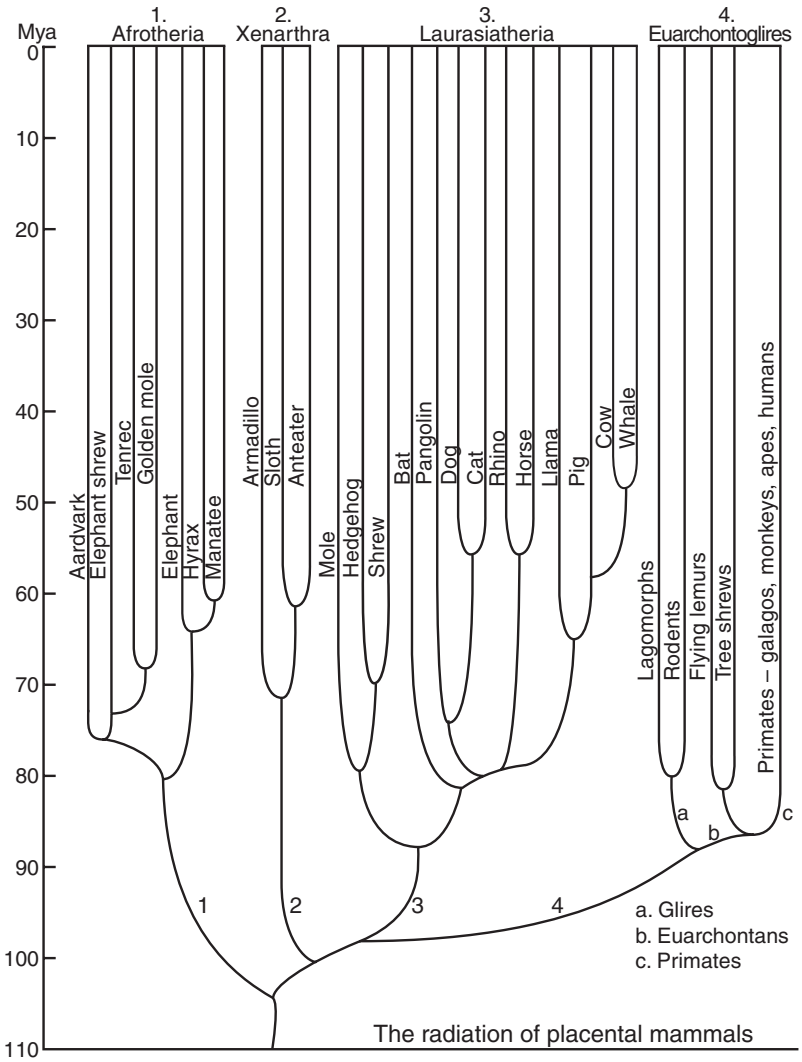

Figure 1 The emergence and radiation of the four major clades of placental (eutherian) mammals. In each clade, a few representative extant members are noted. (1) Afrotheria includes small insectivore-like mammals such as tenrecs and golden moles, as well as the very large elephants and manatees. (2) Xenarthera has the small-brained, specialized anteaters, sloths, and armadillos. (3) The diverse Laurasiatheria range from the widespread bats to carnivores, ungulates, and whales. (4) The Euarchontoglires include Glires lagomorphs (rabbits) and rodents and euarchontans primates, flying lemurs, and tree shrews. See Figure 6 for the primate radiation. Time in Mya is indicated on the left. Based on Murphy, W. J., Pevzner, P. A., and O'Brien, J. O. 2004. Mammalian phylogenomics comes of age. *Trends Genet.* 20, 631 639.

sensory inputs to the somatosensory thalamus include muscle spindle receptors for proprioception, which relay in parallel to the dorsal column pathway, first in the lower brainstem and then to the contralateral thalamus, where proprioceptive neurons in the posterior nucleus or a rostral subdivision of VP project to S1 and adjoining areas of somatosensory cortex. Muscle spindle and other somatosensory information are also sent to the cerebellum, and cerebellar deep nuclei project to the ventrolateral nucleus of the thalamus. Somatosensory information also reaches the thalamus via the spinothalamic path, which terminates in and around the VP. Early mammals apparently had no separate motor cortex, as somatosensory areas, especially SR, S1, and S2, had motor functions including those mediated by cortical projections to the brainstem and spinal cord. Other projections from the somatosensory cortex included those to the basal ganglia, and the somatosensory and motor thalamus. The zona incerta of the ventral thalamus is also an important part of this basic mammalian somatosensory system, with somatosensory inputs and inhibitory GABAergic projections to somatosensory cortex the brainstem, and the superior colliculus (Nicolelis *et al.*, 1992).

The most provocative component of the above summary of the sensorimotor system of early

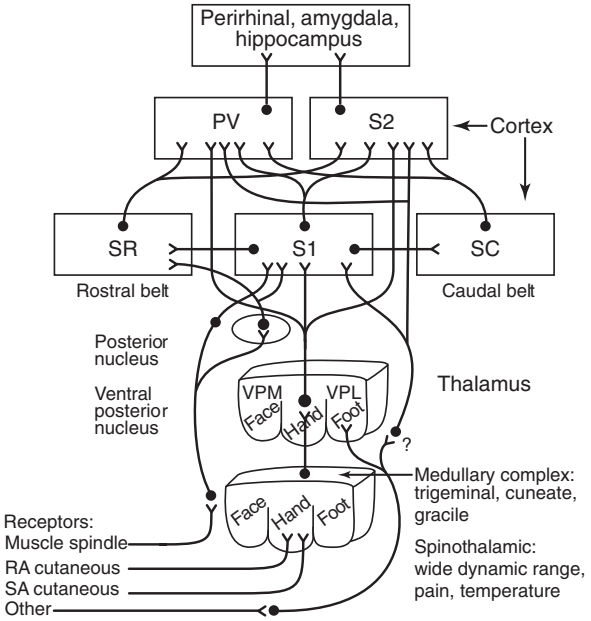

Figure 2 A block diagram of the proposed components of the somatosensory system of early mammals. Receptors and afferents in the skin, muscles, and joints projected to the brainstem and spinal cord, where branches contacted second-order neurons that projected to the contralateral thalamus, or formed fiber pathways to the medullary complex of the brainstem, where second-order neurons projected to the contralateral thalamus. Neurons in the ventral posterior nucleus (VP) received rapidly adapting (RA) and slowly adapting (SA) cutaneous receptor information and projected to primary somatosensory cortex (S1), the second somatosensory area (S2), and the parietal ventral (PV) somatosensory area. A posterior nucleus (a probable homologue of the primate ventral posterior superior nucleus) likely received a relay of muscle spindle receptor information while projecting to SR and S1. S1 projected to rostral (SR) and caudal (SC) somatosensory belt-like areas adjoining S1, and to PV and S2. All of these areas, but especially S1 and SR, were involved in motor control via subcortical projections (not shown) to the basal ganglia, the brainstem, and the spinal cord. PV and S2 interconnected with perirhinal cortex and thereby with the hippocampus and amygdala. The medullary complex, also known as the dorsal column trigeminal complex, included the principal subnucleus of the trigeminal complex, and the cuneate and gracile subnuclei, to form a representation of the body from face (trigeminal) to forelimb (cuneate) to hindlimb (gracile). The medial subnucleus of VP (VPM) represents the face and mouth, while the lateral subnucleus (VPL) represents the lower body. Based on Kaas, J. H. 2004b. The evolution of the large, complex sensorimotor systems of anthropoid primates. In: Evolution of the Vertebrate Brain and Behavior (*eds.* S. Pellis and L. Marino), *Int. J. Comp. Psychol.* vol. 17, pp. 34 52.

mammals is the claim that there was no separate motor cortex. This is based on the evidence that at least some marsupials and monotremes lack motor areas of cortex. For example, the well-studied North American opossum has the four basic areas of somatosensory cortex (Figure 3) but no motor areas (Beck *et al.*, 1996). In opossums, there is no architectonic, electrophysiological, or connectional evidence for a separate primary motor area, M1, such as those found in all studied placental mammals. Instead, the projection of cerebellum to the motor thalamus, the ventral lateral nucleus, is relayed to somatosensory cortex (Killackey and Ebner, 1973). As a note of caution on this claim, some investigators have presented evidence for a motor region rostral to S1 in monotremes (e.g., Krubitzer *et al.*, 1995) and some marsupials (see Beck *et al.*, 1996), but the more compelling evidence suggests that M1 and other motor areas did not emerge until the advent of placental (eutherian) mammals.

Lagomorphs, rodents, and tree shrews represent the closest living relatives of primates that have been available for study (Figure 1). Together they share some features of sensorimotor system organization with most other mammals. All have S1 and S2 as subdivisions of somatosensory cortex. However, these areas are specialized in different ways. In rabbits, three-fourths of S1 and S2 are devoted to tactile receptors of the head, especially those of the lips and facial vibrissae (Gould, 1986). Little cortical tissue is devoted to the forepaw and less to the hindpaw. In rats (Figure 4), most of these areas represent the facial vibrissae, the buccal pad, and other parts of the face, but the representations of the forepaw is significantly larger than in rabbits (Remple *et al.*, 2003). This difference likely corresponds to the greater use of the forepaw in manipulating food objects by rats (Whishaw, 2003). In tree shrews, there is a large representation of the glabrous nose, as well as large representations of lips and mouth parts, but the forepaw has an even larger representation than in rats, and the arrangement of the forearm representation in S1 more closely resembles that of primates (Sur *et al.*, 1980, 1981). As representational features of S1 across species closely reflect their distributions of peripheral receptors and the specialized use of parts of the sensory surface (Johnson, 1990), these differences in representation, as well as the increased representation of the forepaw in rats and especially tree shrews, likely reflect independent evolutionary trends. Yet, it is safe to propose that the immediate ancestors of primates, as semi-arboreal grasping mammals, had emphasized the forepaw in their somatosensory representations, most likely to an even greater extent than in tree shrews, their close relatives. In rats (Remple *et al.*, 2003) and tree shrews (Remple *et al.*, 2006), there is evidence for a PV somatosensory area just ventral to S2. PV may exist in rabbits and other lagomorphs as well, as this area has been found in a wide range of mammals, but there has been no attempt to identify PV

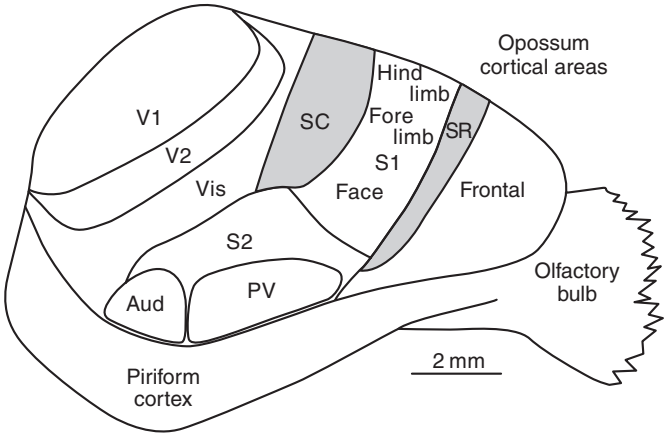

Figure 3 Subdivisions of somatosensory cortex in marsupial opossums shown on a dorsolateral view of the right cerebral hemisphere. Somatosensory areas include primary somatosensory cortex (S1), the adjoining rostral (SR) and caudal (SC) somatosensory belts, the second somatosensory area (S2) and the parietal ventral area (PV). Visual cortex (Vis) includes first (V1) and second (V2) visual areas, and auditory cortex (Aud) may include several areas. There is no evidence of a primary motor area or any premotor areas in frontal cortex. S1 represents the hindlimb to face in a mediolateral sequence, while S2, PV, and possibly SC and SR also contain topographic representations. Based on Beck, P. D., Pospichal, M. W., and Kaas, J. H. 1996. Topography, architecture, and connections of somatosensory cortex in opossums: Evidence for five somatosensory areas. *J. Comp. Neurol.* 366, 109 133.

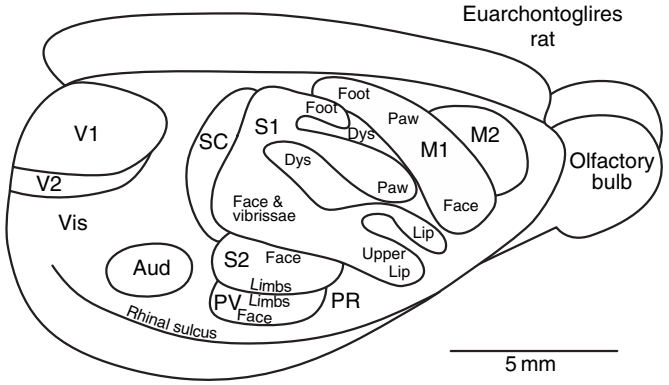

Figure 4 Subdivisions of sensorimotor cortex in rats on a dorsolateral view of the right cerebral hemisphere. The primary somatosensory area (S1), the second somatosensory area (S2), the parietal ventral somatosensory area (PV), and the caudal somatosensory (SC) belt are similar to those in other mammals. The rostral somatosensory area (SR) of other mammals (see Figure 3) forms a dysgranular (Dys) type of cortex that extends caudally to separate forepaw from face, and forepaw from foot representations of S1. A perirhinal (PR) region is indicated lateral to PV. Primary (M1) and secondary (M2) motor areas are found just rostral to S1 and Dys somatosensory cortex (SR). Auditory (Aud) cortex of the three areas, and the first and second visual areas (V1 and V2) are identified. Other visual areas (Vis) are lateral to V2. Based on Remple *et al.* (2003) and others.

in lagomorphs. As in opossums (Figure 3), rats (Figure 4), and tree shrews (Figure 5) have caudal and rostral somatosensory bands with inputs from S1. Thus, they have at least five somatosensory areas. The rostral somatosensory belt has more connections with primary motor cortex, M1, and more motor functions, as judged by the thresholds for electrically evoked movements from their cortex. In addition, there is evidence for proprioceptive inputs from the posterior nucleus of the thalamus (Chapin and Lin, 1984; Hummelsheim and Wiesendanger, 1985; Gould *et al.*, 1989). The caudal somatosensory belt may be multisensory, with visual and perhaps auditory inputs (Wallace *et al.*, 2004; Remple *et al.*, 2006). Thus, the somatosensory cortex along the rostral border of S1 has motor and sensory features that resemble area 3a of primates, and the somatosensory belt caudal to S1 has features reminiscent of areas 1 and 2 of primates. A smaller adjoining posterior region resembles posterior parietal cortex of primates.

As in most other mammals, S1, S2, and possibly PV of tree shrews receive activating inputs from the VP of the thalamus (Garraghty *et al.*, 1991). Thus, VP projects in parallel to these areas. The cortical and VP activation is completely dependent on the

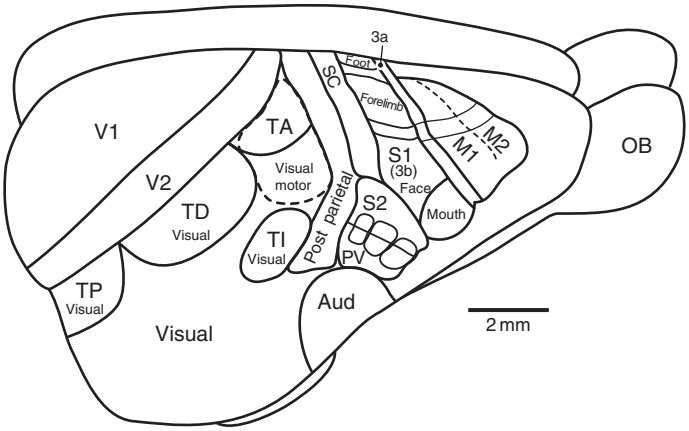

Figure 5 Subdivisions of sensorimotor cortex in tree shrews shown on a dorsolateral view of the right cerebral hemisphere. Somatosensory areas include primary somatosensory cortex (S1 or area 3b), a rostral somatosensory belt (SR in Figure 3) that is called area 3a here, a caudal somatosensory belt (SC) that has two divisions (SC1 and SC2), a second somatosensory area (S2), and a parietal ventral area (PV). In addition, a posterior parietal region has connections with somatosensory and motor areas, the anterior temporal area (TA) has visuomotor cortical connections, as does cortex just lateral to TA. The three ovals bridging the S2 PV border correspond to representations of the hindlimb, forelimb, and mouth in a caudorostral sequence. Motor cortex includes a primary motor area (M1) and a premotor area (M2). An auditory region (Aud) may contain several auditory areas, while most of the caudal half of the cerebral hemisphere is visual. The first and second visual areas (V1 and V2) are identified, as well as proposed temporal posterior (TP), temporal dorsal (TD), temporal anterior (TA), and temporal inferior (TI) visual areas. Scale bar: 2 mm. Based on Remple, M. S., Reed, J. L., Stepniewska, I., and Kaas, J. H. 2006. The organization of frontoparietal cortex in the tree shrew (*Tupaia blangeri*). I: Architecture, microelectrode maps and corticospinal connections. *J. Comp. Neurol.* 497, 133 154.

relay to VP of the dorsal column trigeminal brainstem complex via the medial lemniscus in rats (Jain *et al.*, 2003) and monkeys (Jain *et al.*, 1997), and it is not replaced after injury by the spinothalamic system.

As in other placental mammals, tree shrews (Remple *et al.*, 2006) and rats and other rodents (Wise and Donoghue, 1986) have a primary motor area, M1. In rodents, a popular proposal is that M1 partly overlaps S1, especially in the medial portion devoted to the hindlimb (Sanderson *et al.*, 1984). This premise largely stems from the observation that movements can be evoked from portions of S1 at current thresholds comparable to those for M1. This reflects the widespread role of S1 in motor functions. In rats (Li *et al.*, 1990), more of the corticospinal neurons originate in S1 than rostral somatosensory belt or M1, in marked contrast to primates (Wu *et al.*, 2000). In tree shrews (Remple *et al.*, 2006), S1 (3b), the rostral belt (3a), and M1 contribute nearly equally to corticospinal projections. M1 is an architectonically distinct area of agranular cortex in rats and tree shrews, with a complete or nearly complete representation of movements of the contralateral body. In rats, the emphasis is on facial whisker movements and in tree shrews, face and tongue movements; the evoked movements of the forelimb in these mammals are rather crude, with little evidence of individual digit control (Remple *et al.*, 2003; Sanderson *et al.*, 1984; Wise and Donoghue, 1986). As a reflection of the predominant origin of corticospinal axons from S1 rather than M1, the spinal cord terminations in most mammals are largely in the dorsal horn and intermediate zone of the spinal gray (e.g., Doetsch and Towe, 1981; Armand, 1982). The dense terminations of corticospinal axons of primates onto the motor neurons supplying distal limb muscles does not exist or is very limited in rodents and tree shrews. As an unusual feature, the spinal course of the corticospinal fibers in rodents and tree shrews is at the base of the contralateral dorsal columns rather than in the lateral spinal cord as in primates.

In addition to M1 (Neafsey *et al.*, 1986; Remple *et al.*, 2006), rodents and tree shrews have a second motor area, M2, along the rostrodorsal border of M1 (Figures 4 and 5). This second motor area has few corticospinal neurons, and higher currents are required to evoke movements than in M1. The overall pattern of somatotopy of evoked movements parallels that of M1. There are various possible interpretations of how this M2 compares to subdivisions of motor cortex in primates. As M1 in monkeys has rostral and caudal subdivisions (e.g., Stepniewska *et al.*, 1993), one possibility is that M2 is a subdivision of M1. A more likely possibility is that M2 is homologous to either the supplementary motor area (SMA) or the premotor cortex (PM) of

primates (Rouiller *et al.*, 1993). While premotor cortex has been divided into dorsal and ventral premotor areas in primates, and these areas have been further divided in monkeys (see below), the somatotopy of ventral premotor cortex, representing the head and forelimb, and the somatotopy of dorsal premotor cortex, devoted mainly to the hindlimb and forelimb, suggests that they may have differentiated out of a single premotor area bordering M1 (an area that might have resembled M2 of rats and tree shrews).

In conclusion, comparisons of the sensorimotor systems of the nearest available relatives of primates (lagomorphs, rodents, and tree shrews) suggest that the immediate ancestors of primates had a rather simple sensorimotor system. The basic subcortical components of the system were likely those found in many other mammals. In primary somatosensory cortex, a moderate enlargement and rearrangement of the forepaw representation was likely. Cortex caudal to S1 included a rostral somatosensory band (SC) bordering S1 and a more caudal zone of posterior parietal cortex with inputs from S1. The position of SC, the somatosensory region immediately caudal to S1, suggests that it subsequently differentiated into area 1 or perhaps areas 1 and 2 of primates. A band of dysgranular cortex on the rostral border of S1 had more pronounced motor functions and connections and possibly proprioceptive sensory inputs. This cortex appears to be the homologue of area 3a of primates. A separate and architectonically distinct agranular motor cortex, M1, was present as well as a premotor area, M2. However, the majority of the corticospinal projections emerged from S1, the rostral somatosensory area, RS, and M1 rather than M2. Furthermore, these corticospinal projections terminated in the dorsal and intermediate levels of the spinal grey and not in the motor neuron pools of the ventral spinal grey, thereby having a less direct effect on motor control. Finally, these cortical projections were focused in the brainstem and cervical spinal cord with little extension into the thoracic and lumbar spinal cord.

37.3 Somatosensory and Motor Systems of Early Primates: Inferences from Prosimians and Other Primates

Primates emerged as a branch of the Euarchontoglires superclade (Figure 1) over 7080 Mya (Block and Boyer, 2002; Murphy *et al.*, 2004). Subsequently, they separated into four main lines, including the plesiadapiforms, a semi-order of extinct archaic primates, and the euprimates. The stem euprimates led to the present-day lemurs, lorises, and galagos, the highly specialized tarsiers, and the greatly varied anthropoid monkeys, apes, and humans (Figure 6). Some of the lemurs and galagos closely resemble early primates in body type, brain size relative to body size, and brain shape. Most early primates were the size of cats or smaller, nocturnal, and fed in the fine branches of bushes and trees on insects, small vertebrates, fruits, and leaves (Ross, 1996; see The Role of Vision in the Origin and Evolution of Primates). This behavioral niche required exceptional sensorimotor abilities in that there was the need to stabilize the body in moving branches while reaching for food items (Block and Boyer, 2002). Whishaw (2003) suggests that visual control of hand movements is likely to be the distinguishing feature of primate behavior. To mediate improvements in visually guided reaching behaviors and other sensorimotor abilities, early primates were characterized by an increase in the complexity of visual cortex, the involvement of regions of posterior parietal cortex in visuomotor processing, and the emergence of premotor areas with visuomotor inputs.

Given that great effort is needed to reveal the organization of sensorimotor systems in any primate, and that there are over 200 species of primates (Purvis, 1995), with many of them protected or otherwise unavailable for study, research on primates has concentrated on a few useful and informative species (Kaas, 2002). Galagos are rat- to cat-sized, arboreal, nocturnal, African primates that feed on fruits, gums, and insects. They are easily bred and reared in the laboratory, and their nervous systems have been more extensively studied than those of other prosimian primates. Thus, much of what we know about the sensorimotor systems of prosimian primates is based on results of studies on galagos. In general, we assume that features of the sensorimotor system of galagos and other prosimians that are shared with other primates emerged early in primate or preprimate evolution. Traits present in prosimians and other Euarchontoglires mammals emerged early, with or before Euarchontoglires mammals.

As in other mammals, afferents from the skin and muscles of galagos and other primates enter the brainstem and spinal cord with one branch synapsing on cells in the dorsal horn or the brainstem and the other coursing to the dorsal column–trigeminal complex in the lower brainstem (Figure 2). A notable modification in primates is that the representation of the glabrous hand is expanded and differentiated in the cuneate nucleus of the complex. In galagos, this nucleus is larger than in rats

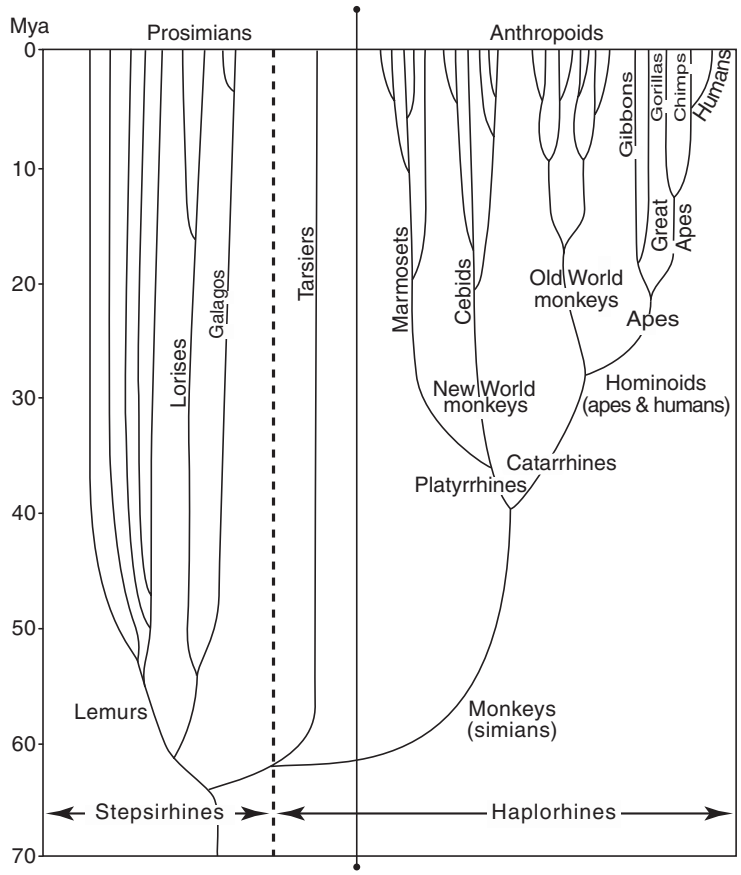

Figure 6 The evolution of extant primates. While archaic primates diverged from other Euarchontoglires over 80 Mya, the separation into prosimians, tarsiers, and anthropoids occurred between 70 and 60 Mya. Based on Purvis (1995) and others. Time in Mya is indicated on the left. Modified from Kaas, J. H. and Preuss, T. M. 2003. Human brain evolution. In: Fundamental Neuroscience (*ed.* L. R. Squire), 2nd edn., pp. 1147 1166, Academic Press.

and tree shrews, and the digits are represented from D1 (thumb) to D5 in a lateromedial sequence (Strata *et al.*, 2003). This nucleus receives the rapidly adapting and slowly adapting cutaneous afferents from the hand, while the muscle spindle (proprioceptive) receptors relay to a well-differentiated external cuneate nucleus. The dorsal column–trigeminal complex relays, as in other mammals, to the contralateral VP of the thalamus. VP is architectonically very distinct in Nissl (Figure 7), cytochrome oxidase, and other preparations (Kaas *et al.*, 2006), and the nucleus is subdivided by septa that separate subnuclei representing different body parts. Thus, a narrow lateral portion is devoted to the tail, an adjoining more medial portion represents the hindlimb, while a larger more medial sector is devoted to the forelimb, mainly the glabrous hand. An elongated medial portion has subdivisions representing various portions of the face and oral cavity (lips, tongue, teeth). VP of galagos and other primates differs from rats and tree shrews in that the nucleus is more elongated mediolaterally, with proportionally more of VP devoted to the limbs, especially the glabrous hand. Yet, the representations of the lips, tongue, and teeth are large. The VP projects to S1, S2, and PV, as in other mammals, thereby providing activating inputs in parallel to three areas of somatosensory cortex. Thus, lesions of S1 fail to deactivate S2 and PV (Garraghty *et al.*, 1991). In this way, galagos resemble their nonprimate ancestors and differ from anthropoid primates where VP no longer projects to S2 and PV, and lesions of S1 deactivate S2 (Garraghty *et al.*, 1990).

Three other nuclei of the primate somatosensory thalamus are apparent in galagos. Just ventral to VP, the ventroposterior inferior nucleus (VPI) contains small neurons that are pale-staining in Nissl preparations (Figure 7a). VPI receives inputs from the spinothalamic pathway and projects broadly to somatosensory areas of cortex, largely to the superficial layers. These VPI projections appear to modulate the activity of neurons in cortical areas, rather than provide an independent source of suprathreshold activation. VPI is not recognized as a

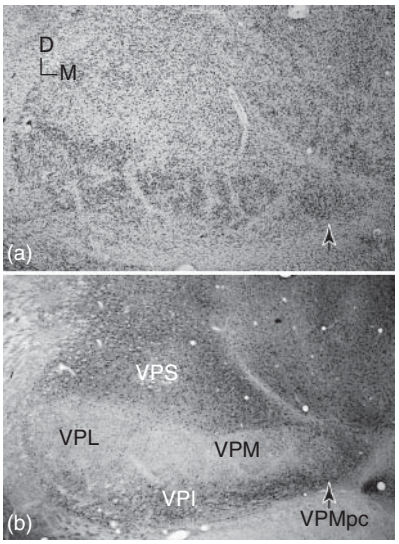

Figure 7 The somatosensory thalamus of a prosimian primate (galago). The thalamus has been cut into thin sections in the frontal (coronal) plane and processed with a Nissl stain for cell bodies (a) or for the expression of a calcium-binding protein, calbindin (b). In both sections, medial (toward the third ventricle) is right, and dorsal is up. In a Nissl preparation, the VP is apparent as a region of larger, darkly stained neurons (arrow), in contrast to the lightly stained, less densely packed neurons in the ventroposterior superior nucleus (VPS) just dorsal (superior) to VP and the ventroposterior inferior nucleus (VPI) just ventral (inferior) to VP. Medially, a 'taste' nucleus, the parvocellular ventroposterior medial nucleus (VPMpc), has more uniformly distributed, smaller cells. The VP is subdivided by a cell-poor septum into a large medial ventroposterior subnucleus (VPM) and a lateral ventroposterior subnucleus (VPL). Another septum separates VPL into a medial portion representing the hand and a lateral portion representing the foot. Other septa are apparent in VPM. In sections processed for calbindin, VP (VPL plus VPM) expresses little calbindin, while VPS, VPI, and VPMpc express more. Other histological preparations also demonstrate clear differences between these nuclei. These three somatosensory nuclei are well differentiated in primates compared to most other mammals. In many mammals (Figure 2), part or all of the region identified as the posterior nucleus may be a homologue of VPS, while a region comparable to VPI is seldom identified.

distinct nucleus in the nonprimate relatives of primates, but VPI is obvious in all primates. As spinothalamics also terminate in the septal zones of VP in primates, it is possible that VPI differentiated from a VP with both spinothalamic and medial lemniscus inputs. A VPI that closely resembles VPI of primates apparently differentiated independently in raccoons (Herron, 1983). The ventroposterior superior nucleus (VPS) is just dorsal to VP. This nucleus receives muscle spindle receptor inputs and projects to cortex termed 3a or SR (Figure 8) just rostral to S1 (area 3b). In Nissl preparations, VPS has less densely packed and less darkly stained neurons than VP, and these neurons express more calbindin and less cytochrome oxidase (Figure 7). VPS is apparent in all primates, but its homologue in nonprimates is uncertain. Possibly, the posterior nucleus of rodents or the proprioceptive rostral cap of VP is a homologue of VPS. Just medial to VPS, the anterior pulvinar (PA) projects widely to areas of somatosensory cortex, while receiving inputs from somatosensory cortex. This nucleus is more conspicuous in anthropoid primates, while having no obvious counterpart in rodents and tree shrews. Finally, a taste or gustatory relay nucleus, VPMpc, can be identified just ventromedial to VP in primates (Figure 7), as in other mammals.

In summary, the comparative evidence indicates that the somatosensory thalamus of early primates had been modified to reflect a primate pattern while retaining some primitive features. Most notably, the ventroposterior inferior and superior nuclei, together with the anterior pulvinar had differentiated to become identifiable structures. In addition, VP acquired more of a primate configuration, with a mediolaterally elongated shape and subdivisions separated by septa for the forelimb and hindlimb, as well as subdivisions of the face and oral cavity. The subdivision of VP for the forelimb, especially the glabrous hand, had become enlarged.

Somatosensory cortex of galagos has some, but not all of the features of somatosensory cortex in anthropoid primates. In some ways, anterior parietal cortex resembles that of tree shrews and rats. However, a primary somatosensory area, S1 (area 3b), is elongated mediolaterally and it contains an anthropoid-like somatosensory representation (Figure 8). The primary somatosensory area, S1, is bordered rostrally and caudally by parallel somatosensory representations. The rostral somatosensory field, SR, by position, architectonic features, responsiveness to stimuli, and inputs from VPS, seems to have most of the features of area 3a of anthropoid primates, and we use the term, area 3a, here. The caudal somatosensory area, SC, has the position, architecture, and the sparse inputs at least from the VP and the dense inputs from area 3b that characterize area 1 of anthropoid primates, but SC does not respond well to somatosensory stimuli in anesthetized galagos, and thus a detailed somatosensory representation has not been produced in microelectrode mapping studies. As such a map has been used to identify area 1 as a second systematic representation of the cutaneous receptors of the contralateral body surface in anthropoid primates (e.g., Kaas *et al.*, 1979), it remains uncertain if SC of

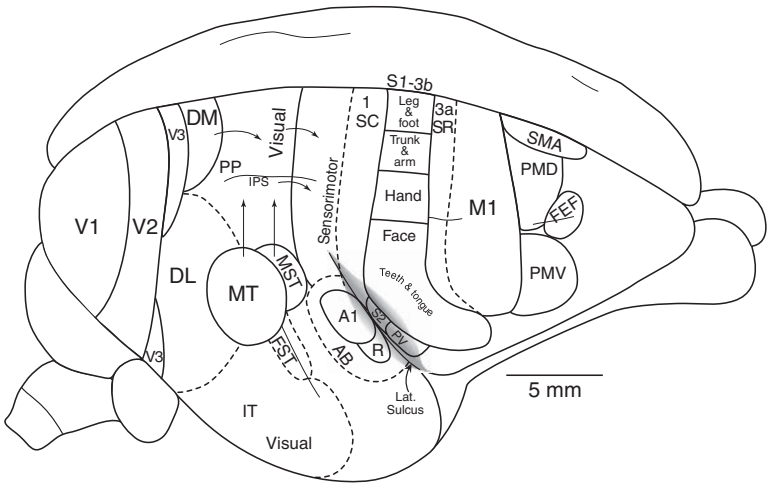

Figure 8 Sensorimotor cortex of a prosimian primate (galago) shown on a dorsolateral view of the right cerebral hemisphere. Somatosensory cortex includes S1 (area 3b), a rostral somatosensory strip (SR) that appears to be homologous to area 3a of other primates, and a caudal somatosensory strip (SC) that may be homologous to area 1 or areas 1 and 2 of other primates. On the upper bank of the lateral sulcus, adjoining S1, somatosensory areas S2 and PV are bordered deeper in the sulcus (not shown) by the parietal rostral somatosensory area (PR) and caudal and rostral divisions of the ventral somatosensory area (VSc and VSr), as well as a retroinsular somatosensory area (Ri). Posterior parietal cortex includes two major subdivisions: a rostral region with dense somatosensory inputs and projections to motor and premotor areas and a caudal region dominated by inputs from visual areas (arrows) and projections to the rostral somatosensory region of posterior parietal cortex (arrows). Both regions appear to contain several subdivisions or areas. Motor cortex includes primary motor cortex, M1, dorsal and ventral premotor areas (PMD and PMV), a frontal eye field (FEF), a supplementary motor area (SMA), a pre-SMA, and rostral and caudal cingulate motor areas (CMAr and CMAc) on the medial wall (not shown). The PMD has rostral (PMDr) and caudal (PMDc) subdivisions, differing in connections and architecture. For reference, first, second, and third (V1, V2, and V3), dorsomedial (DM), dorsolateral (DL), middle temporal (MT), medial superior temporal (MST), frontal superior temporal (FST) visual areas are shown. Auditory areas include the primary area (A1), rostral area (R), and the auditory belt (AB). IPS, intraparietal sulcus. Based on Wu *et al.* (2000), Wu and Kaas (2003), and Stepniewska *et al.* (2005).

galagos has all of the characteristics of area 1. Yet it seems reasonable to propose that SC in galagos, and perhaps other mammals, is the homologue of area 1 of anthropoid primates. Alternatively, SC could be the homologue of areas 1 and 2 combined (see below).

In cortex lateral to area 3b, galagos have the two areas, S2 and PV (Figure 8), with inputs from S1 (area 3b), SR (3a), and SC (area 1). S2 and PV in turn project to other areas located in the cortex of the lateral sulcus, including the insula (Wu and Kaas, 2003). Because these additional areas have been identified mainly by relative location, architecture, and connection patterns, their identities remain somewhat uncertain. Two of the locations appear to correspond to the recently defined caudal and rostral divisions of the ventral somatosensory complex, VSc and VSr of anthropoid primates (Coq *et al.*, 2004). Other locations with S2 and PV connections include the retroinsular cortex (Ri) and the parietal rostral area, PR, of monkeys (Krubitzer and Kaas, 1990). Thus, it appears that galagos have an array of somatosensory areas in cortex of the lateral sulcus that largely matches that described in monkeys, and includes at least two areas, PV and S2, also found in other mammals.

Posterior parietal cortex is more extensive and more complexly organized in galagos than in related nonprimates such as tree shrews and rats. The rostral half of posterior parietal cortex can clearly be identified as part of the somatosensory cortical network due to dense interconnections with somatosensory areas of anterior parietal cortex, especially area 1, and areas of the lateral sulcus, including S2, PV (Wu and Kaas, 2003), and adjoining areas (Fang *et al.*, 2005). The rostral half of posterior parietal cortex projects densely to subdivisions of motor cortex (see below). In addition, this cortex is clearly involved in motor functions. Electrical stimulation of sites throughout rostral posterior parietal cortex evokes movements of body parts, depending on the site stimulated (Stepniewska *et al.*, 2005). Overall, there is a tendency for the stimulation of medial locations in posterior parietal cortex to evoke hindlimb movements, middle locations to evoke forelimb movements, and lateral locations to evoke face and eye movements, but there is a more complex pattern imposed on this tendency. Subregions in this rostral half of posterior parietal cortex appear to relate to components of

ethologically relevant behaviors, so that there are regions where stimulation evokes defensive movements, reaching movements, and hand-to-mouth movements. All these evoked behaviors are mediated via other parts of the sensorimotor network, as movements are no longer evoked when the functions of primary motor cortex are blocked. Besides the direct somatosensory inputs to posterior parietal cortex, the rostral somatosensory half receives dense inputs from the caudal visual half, which is identified as predominantly visual in function by the presence of inputs from a number of previously defined primate visual areas (Fang *et al.*, 2005), including the middle temporal visual area (MT), the medial superior temporal area (MST), and the dorsomedial area (DM). Thus, visual and somatosensory inputs combine in posterior parietal cortex to provide guidance for the execution of reaching, retrieving, and defensive movements.

It is tempting to relate subdivisions of posterior parietal cortex in galagos to those proposed for anthropoid primates, especially the more fully studied areas of macaque monkey. Areas predominantly involved in reaching, retrieving, and eye movements have been described (Cohen and Andersen, 2002), and defensive movements have been evoked by electrical stimulation of one of these areas, the ventral intraparietal area, VIP (Cooke *et al.*, 2003). Unfortunately, not enough is known about these proposed areas in prosimians and simians to productively identify homologous areas and specify differences. Yet it is clear that galagos, as in anthropoid primates, have an extensive posterior parietal region with visual and somatosensory inputs, motor cortex outputs, and sensorimotor functions. This expanded region of cortex is larger and more complexly organized than in the extant relatives of primates.

Galagos also resemble anthropoid primates and differ from other mammals in the organization of motor cortex. In tree shrews and rats, there was evidence for only a primary motor area (M1), and a premotor field (M2). The primary motor area was not a major source of corticospinal projections, and representation of the forepaw was not proportionally larger. In galagos, M1 is not as architectonically differentiated as in simians, but a substantial sector of M1 represents forelimb movements, and this sector provides most of the corticospinal projections to the cervical spinal cord (Wu *et al.*, 2000), as in monkeys (e.g., Nudo and Masterton, 1990; Wu and Kaas, 1999). In both galagos and monkeys, additional corticospinal neurons are located in area 3a and in premotor cortex. However, M1 differs from monkeys (e.g., Stepniewska *et al.*, 1993) in that few locations in M1 of galagos evoke movements of digits. Thus, some aspects of galago M1 are intermediate between M1 of nonprimate relatives and M1 of simians.

Galagos have most of the premotor areas of anthropoid primates (Wu *et al.*, 2000). They have a dorsal premotor area, PMD, with a rostral division more densely connected to prefrontal cortex and a caudal division more densely connected to M1. They have a ventral premotor area, PMV, which is preferentially devoted to forelimb and face movements. Galagos also have a frontal eye field where electrical stimulation of sites evokes rapid, saccadic eye movements. On the medial lip of the dorsal cortex of the cerebral hemisphere, galagos have an SMA, and at least two cingulate motor areas, rostral (CMAr) and caudal (CMAc), exist in cortex of the medial wall, where there is evidence for a cingulate sensorimotor area. There is also evidence for a presupplementary motor area (pre-SMA). These motor fields are each characterized by a particular pattern of connections with other areas and thalamic nuclei in galagos and other primates (see Wu *et al.*, 2000; Fang *et al.*, 2005). Thus, PMD receives inputs from a broad band of mostly medial regions in posterior parietal cortex, PMV from more lateral and rostral parts of posterior cortex as well as from somatosensory areas, and SMA with the medial portion of posterior parietal cortex. Other connections are with frontal cortex, other motor areas including callosal connections between motor areas, basal ganglia, and the motor nuclei of the thalamus. All of these areas are also found in monkeys. As in macaques and other monkeys, these areas interconnect, and directly or indirectly influence the output of primary motor cortex, M1. In addition, most of these areas (PMV, PMDc, CSMA, CMAc, CMAr, SMA, and, of course, M1) contribute at least sparse corticospinal projections. Thus, both galagos and monkeys have highly similar systems of motor and premotor areas, as parts of a more extensive sensorimotor network.

In conclusion, it appears likely that the sensorimotor system of early primates resembled that found in present-day galagos. Many of the features of the system in galagos are shared with other primates, providing evidence that these features emerged early in primate evolution, perhaps in the immediate nonprimate ancestors of primates. To the extent that the sensorimotor system of galagos differs from that in anthropoid primates, galagos tend to resemble the nonprimate relatives of primates. Thus, M1 is less differentiated architectonically

and digit movements of the forepaw are poorly represented compared to M1 of monkeys. Posterior parietal cortex is less extensive and likely has fewer subdivisions in galagos. In addition, PMV appears to lack the rostral and caudal subdivisions that are apparent in macaque monkeys, and this would seem to be a more primitive condition. However, the important conclusion is that galagos share many features with other primates that are not found in the nonprimate relatives. The primate line emerged with an array of advances in the organization and connections of the somatosensory thalamus, anterior parietal cortex, lateral parietal cortex, posterior parietal cortex, and motor and premotor cortex.

37.4 New and Old World Monkeys: Similarities and Variations

While early primates were nocturnal visual predators in tropical rainforests, the line leading to haplorhine primates (tarsiers and anthropoids) were small, arboreal, insectivorous, but diurnal primates (Ross and Kay, 2004). The shift to diurnality led to a number of changes in the visual system (Kaas, 2003). The ancestors of tarsiers reverted back to a nocturnal niche, and specializations for nocturnal vision reemerged (Collins *et al.*, 2005). Tarsiers became the only primates who eat no vegetation. The early anthropoids modified their teeth and jaws to be able to add unripe fruits and leaves to their diet (Lockwood and Fleagle, 1999). Features of present-day platyrrhine (New World) monkeys and catarrhine (Old World) monkeys indicate that part of the anthropoid adaptation included modifications of the sensorimotor system. The radiation of New World monkeys was based on the chance immigration of early anthropoids from Africa to South America at least 30–40 Mya, probably by rafting (Flynn and Wyss, 1998). Several similar modifications of their sensorimotor systems probably occurred independently in both platyrrhine and catarrhine radiations.

In the somatosensory systems of monkeys, some modifications are apparent even at the levels of the brainstem and spinal cord. Because of an increase in the number of cutaneous receptors in the glabrous skin of the hand (forepaw) of monkeys, more of the dorsal horn of the cervical spinal cord is devoted to afferents from the digits and palm (Florence *et al.*, 1991) than in most other mammals. This selective enlargement is also apparent in the cuneate nucleus (representing the forelimb) of the dorsal column–trigeminal complex of the lower brainstem. In addition, there is at least one major variation in the somatotopy of the cuneate nucleus. In Old World macaque monkeys and humans, the distal phalanges of the digits are represented ventrally in the nucleus, and this appears to reflect the generalized mammalian pattern (Florence *et al.*, 1989). In New World squirrel monkeys, the pattern is reversed, with the representation of the digit tips dorsal in the nucleus (Florence *et al.*, 1991). This raises the possibility that different somatotopies in the cuneate nucleus characterize platyrrhine and catarrhine primates, with platyrrhines diverging from the ancestral pattern. However, more primates need to be studied to evaluate this hypothesis. While the somatotopic organizations are reversed in these two taxa, there is no obvious functional consequence of having either variation.

At the level of the thalamus, the ventroposterior (VP), ventroposterior superior (VPS), ventroposterior inferior (VPI), and anterior pulvinar (PA) nuclei are well differentiated in monkeys. However, in Old World monkeys, the marked histological differences between VP and VPS are reduced, with VPS becoming more similar to VP, suggesting an enhanced or altered role for VPS in the system. Cortical organization has also changed, as have patterns of thalamocortical connections. Most notably, in anterior parietal cortex, four strip-like, parallel somatosensory representations are found, corresponding closely to the classical architectonic fields 3a, 3b, 1, and 2 of Brodmann (1909). VP projects to layer 4 of area 3b and more superficially to area 1, and sparsely to parts of area 2 (e.g., Cusick *et al.*, 1985; Pons and Kaas, 1985; see Kaas, 2004c for review). A portion of neurons in VP, perhaps 20%, project to both area 3b and area 1. Nevertheless, evoked neural activity in area 1 depends on inputs from area 3b, as lesions of area 3b abolish the responsiveness of area 1 to cutaneous stimulation (Garraghty *et al.*, 1990). VPS projects to both area 3a and area 2, providing muscle spindle proprioceptive input, with perhaps 40% of the neurons projecting to both areas. Thus, some of the individual neurons in both VP and VPS project to areas 3a and 1, thereby providing the same information. Unlike other mammals, including prosimian galagos (Burton and Carlson, 1986), VP does not project to S2 and PV. Instead, VPI projects densely to these areas (Friedman and Murray, 1986; Krubitzer and Kaas, 1992). This VPI input apparently modulates the activity of S2 and PV neurons, as these areas depend on anterior parietal cortex for activation (Pons *et al.*, 1987). This is a modification from the ancestral condition seen in galagos and other mammals where VP activates S2 and PV independently. Thus, processing in anthropoid primates became more serial.

37.4.1 The Anterior Parietal Cortex of Simians

The representational features of the anterior parietal representations are somewhat variable across different New World and Old World monkeys. For example, the representations of the receptors of the glabrous digits are particularly large and distinct in the dexterous macaque and cebus monkeys, but the hand representation is not so pronounced in New World marmoset monkeys (Carlson *et al.*, 1986). Marmosets and other members of the family Callitrichidae are interesting as these very small monkeys have claws rather than nails on the digits of the hand. Although this has been considered a primitive feature, it appears to be a derived specialization (Sussman and Kinzey, 1984).

New World cebus monkeys and Old World macaque monkeys apparently evolved their greater hand representation and hand dexterity independently. Cebus monkeys are unusual in that they use their tail for active tactile exploration, and the ventral tip of the tail has a glabrous skin surface densely packed with cutaneous receptors. As one might expect from this arrangement, the somatosensory system devotes a large amount of tissue to these receptors in the prehensile tail, with over 10 mm^2 of area 3b activated by the tail (Felleman *et al.*, 1983). This is certainly a derived and remarkable feature of cebus and spider monkeys. Finally, certain aspects of the representation of the body in area 3b of monkeys are reversed in some taxa compared to others. In some monkeys such as cebus and squirrel monkeys, the dorsoventral dimension of the trunk is represented in a reverse order from that in other monkeys (Sur *et al.*, 1982; Felleman *et al.*, 1983). This reversal has no clear functional consequences or implications, but such features suggest that many details of brain organization will be found to vary in patterns that reflect phylogenetic relationships.

Area 1 is also variable in anthropoid primates. In most, area 1 stands out as an architectonically distinct strip of tissue along the caudal border of area 3b. In Nissl preparations, area 3b has a well-developed layer 4 of small cells (granular cells), area 1 has a notably less distinct layer 4, and area 2 has a more distinct layer 4. In addition, area 1 contains a systematic representation of cutaneous receptors, from tail to tongue in a mediolateral sequence, that forms a mirror reversal of the somatotopy of area 3b (see Merzenich *et al.*, 1978 for the first complete description). Neurons in area 1 have larger receptive fields and more complex response properties than those in area 3b. They constitute a second stage of cortical processing as they depend on area 3b projections for activation, although they also receive inputs from the VP of the thalamus. The strip of cortex caudal to area 3b in prosimian galagos also has most of these characteristics, as already discussed, but neurons in anesthetized galagos have not been responsive enough to demonstrate a second mirror image representation, as in monkeys. In addition, the cortex caudal to this strip in galagos is not architectonically very distinct from the strip. These differences suggest that some caution is needed in identifying the caudal somatosensory strip in galagos as area 1, although this does seem likely. This same need for caution applies to Callithricid monkeys, the small tamarins, and marmosets. Cortex just caudal to area 3b in the position of area 1 is not very responsive to tactile stimulation and a systematic representation in this cortex has not been demonstrated (Carlson *et al.*, 1986). Yet projection patterns from area 3b indicate that at least a crude parallel representation exists in this cortex (Krubitzer and Kaas, 1990). In this regard, the area 1 strip in marmosets resembles the SC strip in galagos. The reduced responsiveness of area 1 or SC in marmosets could reflect the retention of ancestral (prosimian) features, but more likely, a regression as the simian ancestors of marmosets evolved into the smallest of anthropoids.

Brodmann (1909) defined a strip of cortex just caudal to area 1 as area 2. In macaque monkeys, most of area 2 is highly responsive to tactile stimuli, and a representation of contralateral cutaneous receptors in area 2 roughly parallels the representation in area 1 (Pons *et al.*, 1985). Thus, the foot representation in area 2 is immediately caudal to the foot representation in area 1, and so on, to form a foot to face representation that parallels that in area 1. To some extent, this representation is a mirror reversal of the one in area 1, but the area 2 representation is not so simple, as it has further reversals of somatotopy within the field. In particular, glabrous digits and pads are represented twice. As area 2 has only been explored in detail in macaque monkeys, we do not know how or if it varies in organization across anthropoids, or even if it really exists in all anthropoids. The only microelectrode mapping study with evidence for an area 2 representation is New World monkeys provided only a brief description of a tail-to-hand representation of deep, possibly proprioceptive, receptors in a mediolateral sequence in parallel with the area 1 representation in owl monkeys (Merzenich *et al.*, 1978). More recently, Padberg *et al.* (2005) recorded from the lateral sector of the area 2 region of New World titi monkeys, and provided evidence for a forelimb representation in area 2 of New World monkeys. However, other interpretations are possible, especially in view of the lack of convincing architectonic evidence for an area 2 in New World monkeys.

Padberg *et al.* (2005) postulate that an area 2 does not exist in New World monkeys, but instead is a feature of the somatosensory system that emerged in catarrhine primates. A more conservative conclusion is that an area 2 representation of predominantly proprioceptive receptors (muscle spindle receptors) evolved in the early anthropoids and this representation became well differentiated in the early catarrhines, and perhaps independently in some of the platyrrhines such as cebus monkeys, although this is uncertain. In New World titi monkeys (Coq *et al.*, 2004), lateral area 1 has connections with the area 2 region, as expected for an area 2, and in New World squirrel monkeys (Cusick *et al.*, 1985), VPS projects to the area 2 region, as expected for area 2. Thus, there is some, but limited, evidence for the existence of an area 2 representation in New World monkeys.

37.4.2 The Posterior Parietal Cortex of Simians

The posterior parietal cortex of macaque monkeys has been subdivided in a number of ways into as many as 15–20 potential areas (Lewis and Van Essen, 2000b). The relative extent of posterior parietal cortex in some of the smaller New World monkeys is much less than in macaques, suggesting that more complexity evolved in catarrhine primates. Some of the proposed subdivisions of posterior parietal cortex of macaques are shown in Figure 9. These include the number of areas in the deep intraparietal sulcus of macaques, where the most studied region, the lateral intraparietal area (LIP), appears to be specialized for directing saccadic eye movements via connections with visual areas, the frontal eye field, and superior colliculus (Ben Hamed *et al.*, 2001). The adjacent medial intraparietal area (MIP) has been enlarged caudally by some investigators to become the parietal reach area (PRA), an area thought to be involved in the planning of visually guided reaching movements with the area via visual inputs and connections with dorsal premotor cortex (Marconi *et al.*, 2001; Cohen and Andersen, 2002). The ventral intraparietal area (VIP) receives visual and somatosensory inputs while projecting to premotor cortex (e.g., Lewis and Van Essen, 2000a). VIP appears to be important in guiding monkey locomotion, as well as defensive and avoidance movements to protect against collisions (Cooke *et al.*, 2003). The anterior intraparietal area (AIP) receives visual information from LIP, and projects to ventral premotor cortex, possibly to guide hand grasping and manipulation movements (Sakata and Taira, 1994; Nakamura *et al.*, 2001). The rostromedial bank of the intraparietal cortex, area 5ip or PEa, contains a systematic representation of cutaneous receptors of the hand and digits (Pons *et al.*, 1985) while receiving somatosensory inputs from areas 1, 2, and S2 and projecting to motor and dorsal premotor cortex (Pons and Kaas, 1986). A role in guiding reaching has been hypothesized for this region of cortex (Iwamura and Tanaka, 1996). Further subdivisions of posterior parietal cortex have been proposed for macaques, including subdivisions of large regions traditionally referred to as area 7a, 7b, 5a, and 5b. For example, the region of area 7 just lateral to the intraparietal sulcus has been recently divided into four areas with different patterns of connections to premotor cortex (Gregoriou *et al.*, 2006). At least some of these connection patterns and suggested functional roles are similar to those proposed for subdivisions of posterior parietal cortex in prosimian galagos (Stepniewska *et al.*, 2005), but too little is currently known to allow an area-by-area matching of homologous areas.

Another interpretation of the organization of the rostral half of posterior parietal cortex, including most of areas 5 and 7b, is possible. In monkeys, this broad band of cortex contains a crude somatotopic organization where medial parts relate to the shoulder, arm, trunk, and perhaps the forelimb, more lateral parts in the parietal sulcus relate to the glabrous hand, and the most lateral parts in area 7b relate to the face, lips, and oral structures (Krubitzer and Disbrow, 2006). Again, there is an overall similarity to the crude functional pattern in the rostral half of posterior parietal cortex of galagos. Thus, one could consider the complete band of cortex in both primates to be a single functional area. However, the bulk of the evidence suggests that this band of cortex contains several functionally distinct areas in both prosimians and simians.

In several parts of posterior parietal and parietal–temporal cortex of simians, both auditory and somatosensory inputs activate neurons. This includes VIP of posterior parietal cortex. More laterally, posterior parietal cortex adjoins the temporal lobe, where the caudodorsal portion of temporal cortex is subdivided into an array of three primary auditory areas, perhaps eight secondary areas, and at least two areas of a third level of cortical processing (Kaas and Hackett, 2000). At least one of the second-level areas, the caudomedial area (CM), has neurons that respond to auditory and somatosensory stimuli, with somatosensory inputs coming from somatosensory areas of the lateral sulcus and possibly the somatosensory thalamus (Schroeder *et al.*, 2003). Although cortical regions of somatosensory and auditory

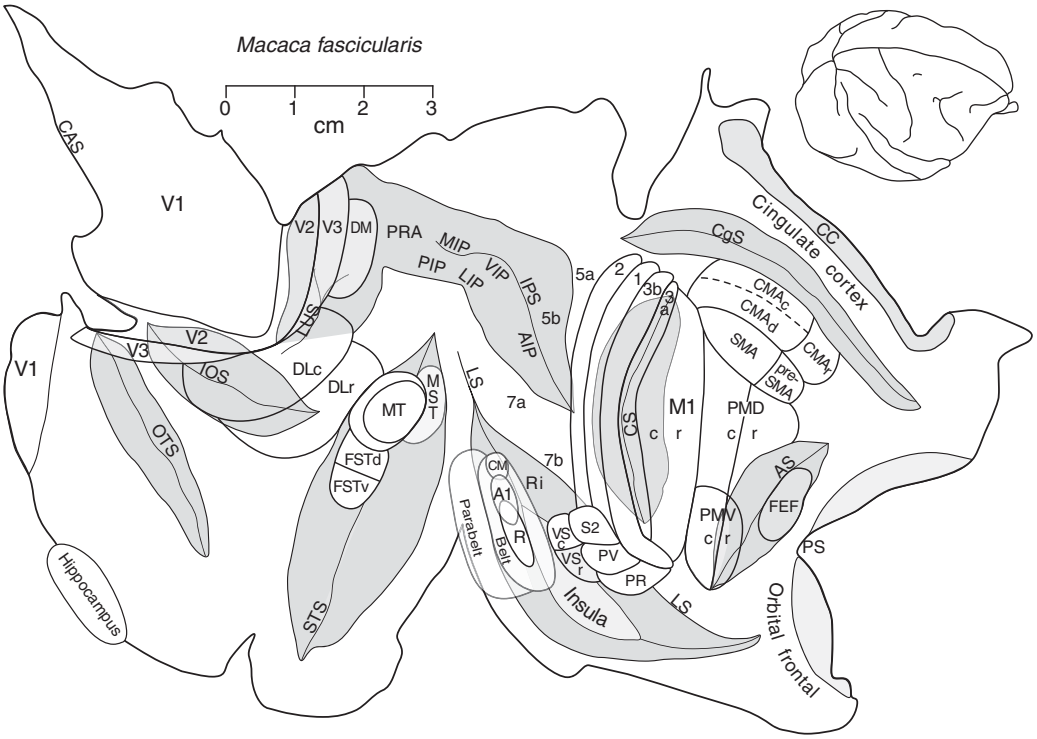

Figure 9 Some of the proposed subdivisions of neocortex of macaque monkeys. The neocortex of the macaque brain (upper right) has been separated from the rest of the brain and flattened (lower left) so that cortex buried in cortical fissures can be seen. To flatten the cortex as a single sheet, of course, requires some cuts and tears. In this view, the four large somatosensory areas of anterior parietal cortex, areas 3a, 3b, 1, and 2, are apparent as parallel, mediolateral strips. The parietal ventral (PV), second area (S2), rostral and caudal ventral somatosensory areas (VSr and VSc), the retroinsular area (Ri), and the parietal rostral area (PR) are in lateral parietal cortex, largely on the upper bank of the lateral fissure, but it is obvious that there is space for a number of other somatosensory areas. The lower bank of the lateral fissure contains auditory areas (primary, A1; rostral, R; auditory belt; and parabelt) with the caudomedial auditory area, CM, having both auditory and somatosensory functions. In posterior parietal cortex, several proposed areas are shown without boundaries, and many more areas have been proposed and may exist.

Areas of the intraparietal sulcus include anterior (AIP), lateral (LIP), medial (MIP), and posterior (PIP) intraparietal areas, along with the parietal reach area (PRA). The frontal motor areas include rostral and caudal divisions of primary motor cortex (M1r and M1c), rostral and caudal divisions of the ventral premotor areas (PMVr and PMVc), and the dorsal premotor areas (PMDr and PMDc), the frontal eye field (FEF), the supplementary motor area (SMA) with dorsal and ventral divisions, the presupplementary area (pre-SMA), and three cingulate motor areas (CMAr, CMAd, and CMAv). Several visual areas are denoted for reference: the first (V1), second (V2), third (V3), rostral and caudal dorsolateral (DLr and DLc), dorsomedial (DM), middle temporal (MT), medial superior temporal (MST), and dorsal and ventral divisions of the fundal superior temporal area (FSTd and FSTv). Cortex of the opened cingulate sulcus (CgS), the arcuate sulcus (AS), the lateral sulcus (LS), the principal sulcus (PS), the intraparietal sulcus (IPS), the lunate sulcus (LUS), the superior temporal sulcus (STS), the intraopercular sulcus (IOS), and the opercular temporal sulcus (OTS) are shaded, as is the corpus callosum (CC). See Kaas (1997) for a discussion of these visual areas.

1overlap in nonprimate mammals, there is no known homologue of CM in nonprimate mammals. Thus, CM appears to represent a primate elaboration of both auditory and somatosensory systems.

In summary, there is presently too little that is known about posterior parietal cortex in New World monkeys to allow detailed comparisons with Old World monkeys, but some overall features of shared organization are expected. Nevertheless, the large expanse of posterior parietal cortex in macaques, and the many proposed subdivisions, suggest that this region became much more complexly organized in catarrhine primates than in early anthropoids and most of their platyrrhine descendants.

37.4.3 The Motor and Premotor Cortex of Simians

Primary motor cortex varies in functional organization across taxa of simian primates in that representations of individual finger movements do not predominate in the forelimb portion of M1 in most New World monkeys (Gould *et al.*, 1986; Donoghue *et al.*, 1992; Stepniewska *et al.*, 1993), while larger parts of the forelimb region of M1 of

macaque monkeys is devoted to digit movements (e.g., Qi *et al.*, 2000). M1 of simian primates appears to have rostral and caudal subdivisions, differing in architecture and functions, with the caudal division more involved in digit movements (see Preuss *et al.*, 1996, 1997). M1 in humans may have similar subdivisions (Geyer *et al.*, 1996). Most New World monkeys, such as squirrel monkeys, differ from Old World macaques by having fewer direct projections from motor cortex to spinal motor neurons that control the movements of the hand, thereby allowing more independent movements of the digits (Nakajima *et al.*, 2000). The highly dexterous New World cebus monkeys have independently evolved similar direct cortical projections to spinal motor neurons (Bortoff and Strick, 1993).

The premotor region of cortex of macaque monkeys (Figure 9) also appears to have more functional subdivisions than premotor cortex of New World monkeys. Most notably, the ventral premotor region appears to be a single area in New World monkeys (Preuss *et al.*, 1996) and prosimian galagos (Wu *et al.*, 2000; Fang *et al.*, 2005), while ventral premotor cortex is subdivided into rostral and caudal areas (PMVr and PMVc) in Old World monkeys (Matelli *et al.*, 1985), termed PMVc or frontal motor area 4 (F4) and PMVr or frontal motor area 5 (F5) based on differences in histochemical (cytochrome oxidase) staining in the two fields. PMVc or F4 contains a motor representation of face and proximal arm movements, and neurons with tactile and visual receptive fields on (tactile) or near (visual) the face (Luppino *et al.*, 1999). PMVc (F4) appears to be involved in using information about nearby space to guide movements of the arm and face. Because PMVc resembles PMV of New World monkeys and prosimians, in that PMVc projects to primary motor cortex (M1) and to the spinal cord, PMVc of macaques likely corresponds to PMV of these other primates. PMVr (F5) has only been described in macaque monkeys, and it may be an area that is poorly differentiated or absent in New World monkeys. Stimulation of PMVr (F5) evokes movements of the hand and mouth. Neurons in PMVr (F5) respond when the monkey is performing hand and hand-to-mouth movements, and when the monkey is observing another performing such movements. Because the neurons respond both during an action or observing the same action performed by another monkey or human, they have been called mirror neurons (Gallese *et al.*, 1996). There is evidence that humans also have a mirror-neuron area, and human imitation may be based on a mirror-neuron system (Wohlschlager and Bekkering, 2002). Some have suggested that imitation is essential to language, and that PMVr (F5) is a monkey homologue of Broca's speech region. Petrides *et al.* (2005) postulate that part of dysgranular cortex just rostral to PMVr, is the monkey homologue of area 44 in humans. (Areas 44 and 45 are thought to correspond to Broca's speech region.) Electrical stimulation of this dysgranular cortex in monkeys evoked orofacial movements, and Petrides *et al.* (2005) suggested that Broca's area evolved from an area involved in controlling orofacial actions, such as area 44 of macaque monkeys (see also Preuss *et al.*, 1996). In addition, Nelissen *et al.* (2005) propose that F5 has a caudal region for the traditional mirror neurons, and an anterior region for neurons that are related to grasping, and these neurons for grasping respond to an isolated hand or a robot hand grasping, suggesting that the basics of grasping are coded in rostral F5. Thus, there is evidence for considerably more complexity in the PMV regions of macaques than in New World monkeys. Nevertheless, PMV of highly dexterous New World cebus monkeys has a well-developed representation of digits and dense connections with the hand portion of M1 (Dum and Strick, 2005), and further studies could reveal complexities of PMV organization in these New World monkeys.

In Old World monkeys, the region of the SMA has been divided into caudal SMA proper and rostral pre-SMA motor fields in macaques (reviewed by Tanji, 1994). A pre-SMA area may exist in New World monkeys, but the current evidence for such an area is limited (Sakai *et al.*, 2000). However, there is also some evidence for a pre-SMA in galagos (Wu *et al.*, 2000). Thus, pre-SMA may be an area common to primates.

37.5 Sensorimotor Systems in Apes and Humans

Compared to other primates, apes and humans are characterized by larger brains that take longer to mature and have higher energy requirements (Jablonski *et al.*, 2000). As a result, higher-quality foods are required, and reproductive rates are low. Success depends on a long life expectancy, and the ability to find and process high-quality foods. These requirements suggest that the larger brains of these primates function in many ways to allow food resources to be exploited and to extend the reproductive life span. However, only humans and some of their hominin ancestors with the largest of primate brains were able to expand their ranges from

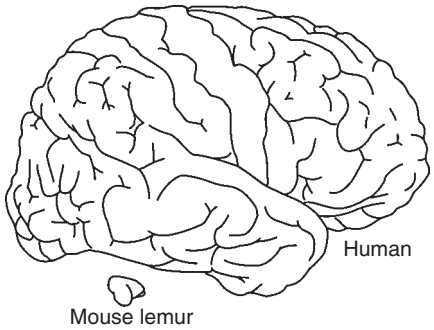

Figure 10 Primate brains vary greatly in size. The human brain is much larger than the brain of the mouse lemur, yet they share some sensorimotor areas. However, the large human brain has added a great number of additional sensorimotor and other areas, thereby becoming more modular and decreasing the problem of maintaining connections as the larger brain evolved. See text.

the favorable tropics where ripe fruit is regularly available to more challenging environments, where tool and weapon use allowed new food sources to be exploited, and resources to be defended. Changes in brain size were followed by changes in brain function, and brain organization, including alterations in the sensorimotor system (Figure 10).

As far as we know, the sensorimotor systems of apes and humans include the major components that have been proposed for their catarrhine relatives, the well-studied macaque monkeys. However, information is very limited, and there are many uncertainties. Yet early cortical stimulation (Hines, 1940; Leyton and Sherrington, 1917) and recording studies in chimpanzees (Woolsey *et al.*, 1943) indicate that at least one orderly somatotopic representation exists in anterior parietal cortex, and that an evoked movement map, M1, exists in architectonically defined area 4 (e.g., Bucy, 1935). Architectonic studies also indicate that anterior parietal cortex of apes and humans has the four fields of macaques, areas 3a, 3b, 1, and 2 (Grefkes *et al.*, 2001), and there is evidence for separate representations in areas 3a, 3b, 1, and 2 of humans (Young *et al.*, 2004). In humans, there is also evidence for areas PV and S2 of lateral parietal cortex (Disbrow *et al.*, 2000).

The organization of posterior parietal cortex in humans is generally modeled after proposals based on macaque monkeys, yet functional differences have been a focus of research. Clear evidence of the specialization of different functions in each cerebral hemisphere are evident in humans, where lesions of the right posterior parietal cortex typically produce a much more profound neglect of contralateral visual and tactile information than lesions of the left cerebral hemisphere (see Mountcastle, 2005). The general assumption, with some supporting evidence, is that the same frontal motor fields of macaques exist in humans, with, of course, specializations of ventral premotor fields of the left cerebral hemisphere to form Broca's speech region (e.g., Amunts *et al.*, 1999). Other specializations of motor fields of the left hemisphere are likely, given the greater role of the left hemisphere in motor planning and motor control (Kimura, 1993; Serrien *et al.*, 2006). Anatomical and functional asymmetries exist even in primary motor cortex. Further research is needed to determine what features of the sensorimotor cortex of humans are shared with apes and monkeys, and what features are new or perhaps lost.

For theoretical reasons, based on the major increase of brain size in humans, one would predict further evidence of differences in function between comparable regions of the two cerebral hemispheres, specialization of the larger somatosensory areas (3a, 3b, 1, and 2) and motor area (M1) for a fine-grain rather than global analysis of sensory information and motor control, and an increase in the number of cortical areas (Kaas, 2000).

Humans do have specializations of hand use that are reflected in the motor system. Humans are distinguished from other primates by their extremely dexterous use of the hands. In part, this was made possible by the evolution of upright posture, which freed the hand from a major role in locomotion, while allowing specialization that led to tool construction and use. Evidence of tool use pre-dates modern humans and extends back at least 2.5 My to our *Homo habilis* ancestors. The evidence suggests that the usual specialization of the right hand and the left sensorimotor cortex for dexterous functions began at least 2–3 Mya (Corballis, 1989, 1998; see The Evolution of Hemispheric Specializations of the Human Brain). Lethal intergroup violence, aided by the use of manufactured weapons, appears to have been an important selection factor in hominid evolution over the last 2–3 My (Kelly, 2005). Motor control for the making of tools and weapons would have likely depended on changes in the motor system. The neural basis for independent finger movements is thought to depend on the size and termination pattern of the pyramidal motor tract, which is exceptionally large in humans (Heffner and Masterton, 1983) and has the fullest monosynaptic linkage with spinal motor neurons in humans (see Wiesendanger, 1999; Lemon and Griffiths, 2005). These changes in the motor system were paralleled by changes in the hand, so a long, strong thumb could be apposed to fingertips in humans. The smaller thumb of early hominoids allowed a power grip, but possibly not a fully developed precision grip

(Napier, 1999), the hallmark of human hand use. These sorts of changes in the human motor system are well known, as they have been easy to measure, but they are likely to be only the tip of the iceberg. We expect that further research will reveal many additional hominoid and human specializations of the sensorimotor brain. Anatomical asymmetries of human cortex that are related to language functions are also expressed in the great apes (Gannon *et al.*, 2005), indicating that such hemispheric specializations initially evolved for functions other than language, and that hemispheric specializations have a long history.

37.6 Summary and Conclusions

The exceptional sensorimotor skills of humans reflect their massive and complexly organized sensorimotor system. While the details of the organization of this system are incompletely known for humans, and even more so for apes, we can deduce how the major features of the human system evolved by comparing features in extant mammals. Ideally, such an analysis would broadly survey extant mammals, consider features cladistically in a series of smaller clades leading to humans, and determine if conclusions are consistent with observations from the record of fossil brain endocasts on brain size and fissure patterns (e.g., Radinsky, 1975), as well as theoretical predictions based on brain size (Kaas, 2000). In practice, this approach is difficult to realize fully because understandings of the organizations of sensorimotor systems are fragmentary and incomplete. The sensorimotor systems of only a few taxa are well known. In addition, the fossil record is also fragmentary, and few cortical fissures, which often are apparent in endocasts, have an established significance in terms of marking functional boundaries in cortex. Finally, large brains would seem to need to be organized in specifically different ways than small brains, but comparative studies have only partially validated assumptions about how brains should change with increasing size. Nevertheless, enough is known to allow a broad outline of sensorimotor system evolution in primates, and this outline can be expanded and modified as further observations become available.

37.6.1 Early Mammals

Only a few subdivisions of the thalamus and cortex characterized the sensorimotor system of early mammals. The thalamus included a distinct VP for relaying slowly adapting and rapidly adapting cutaneous receptor information to primary (S1) and secondary (S2) somatosensory cortex, and possibly to the PV. Muscle spindle receptor information was segregated in a thalamic region dorsorostral to VP, either in the region generally referred to as the posterior nucleus, or in a cell group commonly included in rostral VP. This information relayed to the rostral belt of somatosensory cortex, SR, a region that is referred to as dysgranular cortex in rats. Spinothalamic inputs terminated in and around VP, and relayed broadly to somatosensory cortex. Just rostral to VP, the ventral lateral nucleus received input from the cerebellum and projected to somatosensory cortex. There were no separate motor and premotor areas in early mammals, and motor functions were mediated via projections from somatosensory cortex to the basal ganglia, brainstem, and spinal cord, as well as by subcortical sensorimotor circuits that functioned relatively independently of sensorimotor cortex.

37.6.2 Early Eutherian Mammals

In the line leading to eutherian mammals, a separate primary motor area of cortex, M1, emerged. M1 lacked a notable layer 4, while projecting to brainstem and spinal cord neuron pools, largely on neurons between those with sensory inputs and the motor neurons contacting muscles. Sensory inputs to M1 were from somatosensory areas S1, S2, PV, the caudal and rostral somatosensory belts (SC and SR), and an emerging but small posterior parietal cortex with somatosensory, visual, and perhaps auditory inputs. M1 also received inputs from a rostrally adjacent premotor area, M2, with posterior parietal and frontal lobe inputs, as well as from the motor thalamus (VL). M2 provided few corticospinal projections, while S1 and SR provided dense corticospinal projections. M2 may be the homologue of dorsal and ventral premotor areas, or of the SMA of primates.

37.6.3 Early Ancestors of Primates

The early members of the Euarchontoglires clade that emerged some 95 Mya did not differ significantly from other eutherian mammals, but the early archontan mammals, those that gave rise to flying lemurs, tree shrews, and primates, may have already developed some primate-like characteristics, judging from recent studies on tree shrews. Most notably, the posterior parietal region of cortex was expanded in size, and this cortex had considerable input from an array of visual areas, as well as from somatosensory areas of cortex. Posterior parietal cortex probably had several divisions projecting

differently to motor and premotor cortex. The SC belt may have had rostral and caudal subdivisions. More of S1 was devoted to sensory receptors of the forepaw, and motor cortex had more projections from a forelimb representation to the cervical spinal cord. The forepaw was used more for grasping and manipulating food items.

37.6.4 Early Primates

The most notable difference between primates and their nonprimate relatives is the presence of an array of premotor areas in the frontal lobe. Prosimian galagos and other primates have a dorsal premotor area, PMD, with a rostral division (PMDr) with connections with prefrontal cortex and sparse connections with primary motor cortex, M1, and a caudal division (PMDc) with dense connections with M1. Galagos also have a ventral premotor area, PMV, but without the distinct rostral and caudal divisions of anthropoid primates. Galagos have a frontal eye field (FEF), an SMA, a pre-SMA, and rostral and caudal cingulate motor areas (CMAr and CMAc). These are all likely features of the brains of early primates. Other changes occurred in posterior parietal cortex, which was more expansive in early primates and included a rostral zone, with subdivisions receiving different somatosensory inputs and projecting differently to motor and premotor areas, and a caudal zone with inputs from higher-order visual areas and with projections to the rostral divisions of posterior parietal cortex. The proportional representation of the glabrous hand (forepaw) was increased in somatosensory and motor fields, and the proportion of corticospinal projections from M1, compared to other fields, was increased, corresponding to an enhanced role of the forelimb in reaching and grasping small prey, fruit, and buds in the fine-branch niche of early primates.

37.6.5 Early Anthropoids

These primates gave rise to tarsiers and monkeys. As highly specialized primates, with little information about their sensorimotor system, not much can be said about tarsiers, except that VP, VPS, and VPI regions can be identified histologically in the somatosensory thalamus, and that a clear area 3b (S1) can be identified in cortex. Extant monkeys differ from prosimians by having four distinct subdivisions of anterior parietal cortex, areas 3a, 3b, 1, and 2, each with a separate representation of receptors of the contralateral body, a distinctive pattern of connections with other areas of cortex, and subcortical structures, and identifying histological features. Yet area 1 is poorly differentiated in one branch of New World monkeys, the small marmosets and other callitrichines, and the evidence for an area 2 has been questioned in these and other New World monkeys. As some features of somatosensory cortex may have regressed in the callitrichines, early anthropoids may have had a more differentiated area 1, and a poorly differentiated area 2. Area 2 became distinct in the line leading to present-day Old World monkeys, apes, and humans. In more recent anthropoids in the Old World line, posterior parietal cortex increased in size and complexity, ventral premotor cortex subdivided and motor and somatosensory areas increased their representations of the hand. Area 2 became a well-differentiated area, with inputs from VPS and interconnections with area 3a. At least some of these changes occurred independently in New World cebus monkeys, as well as their unique sensorimotor specialization for their tail.

37.6.6 Hominoids (Apes and Humans)

About 30 Mya or more, one line of Old World monkeys gave rise to apes, which are characterized by a longer gestation time, a longer time to first reproduction, and a generally larger size than monkeys. The longer gestation time, slower maturation, and larger size allowed ape brains to become bigger than monkeys', a trend that was accelerated in hominins, humans, and their bipedal ancestors. The brains of our early bipedal ancestors were about the size of those of modern-day chimpanzees (400–500 cm^3), but in the line leading to modern humans, the brain rapidly increased in size, especially over the last 1 My, to the range of 1200–1400 cm^3. This increase was accomplished by an increase in the surface area of each cerebral hemisphere from about 240 to 800 cm^2. Even the first hominids and their close ape relatives had brains much bigger than those of the well-studied Old World macaque monkeys with approximately 72 cm^2 of surface area for each cerebral hemisphere. Research and conclusions about brain organization in macaque monkeys has greatly influenced current concepts about how the human brain is organized, but because of scaling problems, it is extremely unlikely that human neocortex is simply a 10- to 15-fold enlarged version of macaque neocortex. Of course, there is clear evidence that this is not the case. Most notably, the easily identified primary sensory and motor areas are larger in the large human neocortex, but not as large as they would be if they maintained a monkey-like proportion of the total. The larger size implies that these areas

function differently than their homologue in smaller primate brains, allowing more emphasis on fine-grain discriminations and motor performances, but functioning less well on the global aspects of perception and movement control. However, if some areas of cortex did not enlarge proportionally, what happened to the rest of the neocortex? The obvious suggestion is that the number of cortical areas, the functionally distinct subdivisions of cortex, increased. The most compelling evidence for this is that humans have abilities not found in monkeys or apes, and that brain regions responsible for some of these abilities do not have symmetrical counterparts in each hemisphere. A large part of the temporal lobe and parts of the frontal lobe of the left cerebral hemisphere are specialized for language in humans, and a large part of posterior parietal cortex of the right hemisphere is specialized for spatial reasoning and functions that allow an appreciation of music and mathematics (Corballis, 1998). For purposes of reducing the connection problems of large brains alone, there should be a considerable increase in modularity, reflected in an increase in numbers of cortical areas, perhaps in the range of 150 distinct fields for human neocortex. How this impacts on the sensorimotor system is largely uncertain at this time, but the expectation is that posterior parietal cortex, lateral parietal cortex, and frontal lobe motor regions of humans have more functional divisions than Old World monkeys have or our early hominin ancestors had. The evidence for this, at least for monkey–human comparisons, is starting to emerge from ongoing fMRI studies that have great capacity and potential for revealing the functional subdivisions of human neocortex.

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38 The Evolution of Parallel Pathways in the Brains of Primates

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Glossary

analogy	Functional similarity between parts of different organisms due to parallel evo lution, without common ancestral origin.
Brodmann	Brodmann (1909) developed a com monly accepted scheme for dividing V1 into six layers (I, II, IIIA, IIIB, IVA, IVB, IVCα, IVC*β*, V, and VI).
Hässler	We have used a modification of a nomenclature devised originally by Hässler (1967). The latter allows for more appropriate cross species comparisons. This nomenclature sub divides cortex into the following layers, with Brodmann's nomenclature in parentheses: I (I), II (II), IIIA (IIIA), IIIBα (IIIB) and IIIB*β* (IVA), IIIC (IVB), IVα (IVCα), IV*β* (IVC*β*), V (V), and VI (VI).
homology	Similarity between parts of different organisms due to evolution from the same part of a common ancestor.
homoplasy	Correspondence between parts or organs as a result of evolutionary convergence.
K, M, P cells	Koniocellular (K), magnocellular (M), and parvocellular (P) cells found in dif ferent layers of the lateral geniculate nucleus of primates.
ON /OFF center cells	Retinal ganglion and lateral geniculate nucleus cells that respond with increases in response to either the onset or offset of light in the receptive field center.
ontogeny	Developmental progression of an organism from embryo to adult.

38.1 Introduction

The primate order to which we belong is quite heterogeneous in size, form, and lifestyle. Primate species range in size from some prosimians that can weigh as little as 100 g (e.g., the mouse lemur, *Microcebus murinus*) to species of great apes, whose males can weigh more than 300 kg (e.g., the gorilla; Figure 1). Such size differences can also be seen in the brain, which varies in weight from 1.73 g in the mouse lemur to 1400 g in humans (Bons *et al*., 1998; Williams, 2002).

These differences in body/brain size and lifestyle of existing primate species can make it difficult to trace the evolutionary history of brain parts and connections, particularly since big differences in brain size and lifestyle result in both addition and deletion of brain parts, and changes in connections due to scaling issues (Kaas, 2004). Moreover, the clues about brain evolution left by ancestors are limited. These clues rely on incomplete fossil records, and genes whose rate of change cannot be predicted precisely, or (in most cases) be linked to specific brain parts. Finally, relevant visual pathway data have been gathered for relatively small numbers of existing primate species. None of these clues alone, including current powerful genetic approaches, offer sufficient evidence to trace the evolutionary history of specific brain components and connections in primate evolution. The strongest evidence for evolutionary relationships between brain parts and connections of different primates is likely to be the common presence of a feature in several distantly related primates. The difficulty lies in trying to determine

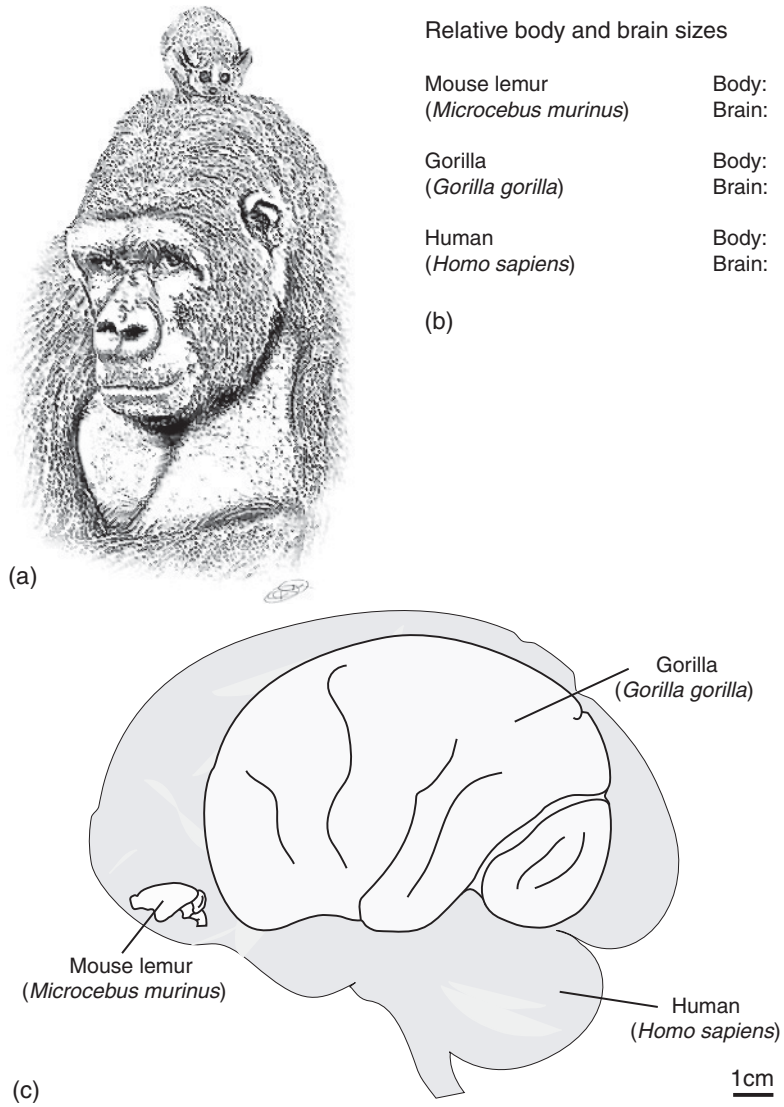

Figure 1 Relative sizes of primates and their brains. The primate order includes mammals that range widely in body and brain size from mouse lemur to gorilla and human. a, Artistic depiction of the relative size differences between a mouse lemur and a gorilla; b, comparison of body and brain weights of mouse lemurs, gorillas, and humans (Bons *et al.*, 1998; Williams, 2002); c, schematic representation of relative brain sizes of these primates. a, Reproduced by permission of David Royal.

the history of these brain parts and connections since similarities may simply reflect a form of parallel evolution (homoplasy) and not necessarily homologous relationships. Also, the fact that connections can be added, deleted, or evolve at different rates in a mosaic fashion magnifies the problem. Nevertheless, some inferences can be made by careful comparisons across existing species and by combining this information with emerging genetic maps of relationships between species.

Our goal in this article is to review relevant evidence from a variety of sources in an effort to reconstruct a reasonable scenario as to how parallel visual pathways might have evolved in primates. Given that visual system studies of living primates are limited to only a few of the many existing primate species, we must rely on work on other mammals, and even nonmammals, to construct a reasonable scenario of the evolution of the visual pathways in primates. Historically, it has been argued that the main parallel visual pathways to cortex in mammals are the retinocolliculopulvinar and retinogeniculo-V1 pathways (see Casagrande and Royal, 2004; Casagrande and Xu, 2004).

For this article, we have chosen to focus on channels passing to and through the lateral geniculate nucleus (LGN), since these pathways may have become differentially specialized in primates and

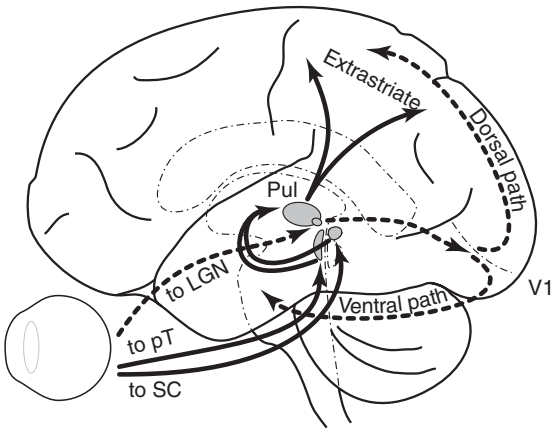

Figure 2 Parallel visual pathways from retina to cortex. In primates, visual information reaches cortex from retina via several pathways. The most studied, and important, is the pathway from the eye to the LGN to V1 (also called striate cortex or area 17), shown with dashed arrows. In V1, new pathways are constructed that enter two hierarchies of visual areas known as the dorsal and ventral paths or streams of processing, also referred to by some authors as the 'where stream' or vision for action stream and the 'what stream', respectively, in reference to their proposed function. Less studied is the pathway from retina (eye) via superior colliculus (SC) and pretectum (pT) to pulvinar (Pul). Pulvinar, in turn, sends widely distributed projections to most extrastriate visual areas to which the dorsal and ventral pathways also project.

are known to be the main pathways for conscious visual perception in primates (Figure 2). We have divided the article into eight sections, including this introduction. In Section 38.2, we define what we mean by parallel pathways and provide some other operational definitions that are used in the remaining sections. In Section 38.3, we consider whether magnocellular (M) and parvocellular (P) retinogeniculocortical pathways are homologous across primates and whether these pathways exist in nonprimates (e.g., Y and X streams in cats) as some have proposed (Casagrande and Xu, 2004). In Section 38.4, we address the controversies over whether the fine fiber system identified by Bishop (1933) in frog and rabbit optic nerve becomes the koniocellular (K) pathway in primates. Given that the K pathway is heterogeneous, we argue that the K pathway is actually made up of a number of pathways of which some are likely to have been present in the common ancestor of primates. A related issue, namely the evolution of chromatic channels and color vision in primates, is addressed separately in Section 38.5. Here we defend the position that one type of K pathway likely transmitted cone signals to the LGN even in the ancestors of primates, given that these cone signals have been found in K LGN cells in both New World and Old World primates and in some cat W cells (which share other features with primate K cells). In Section 38.6, we consider other properties that remain segregated in the LGN and cortex, such as input from the two eyes and whether it existed in the common ancestor of primates. We support the position that the laminar pattern of ocular segregation in the LGN and the columnar organization of ocular segregation in cortex show the same basic features across primates, suggesting that both were present in the common ancestor of primates. In Section 38.7, we examine the issue of whether parallel LGN pathways evolved as starting points for specific hierarchies of visual cortical areas that have been referred to as the dorsal and ventral streams of visual processing in the common ancestor of primates. In Section 38.7, we also consider the issue of whether such cortical streams are conserved across mammals or evolved separately in such species as cats. We take the position that the basic subdivisions into dorsal and ventral streams of visual processing at the cortical level can be identified in a diverse range of primates and so are likely to be homologous, but components may have been added, deleted, or modified in different primate lines. In Section 38.8, we provide a summary and also outline questions that need to be addressed in order to arrive at more definitive conclusions concerning the evolution of parallel visual pathways. We also outline some practical strategies for answering some of these questions.

38.2 Background and Some Definitions

In order to examine the issue of the evolution of parallel visual pathways we need to consider how to define the specifics of the problem. For example, how do we know if a visual pathway is homologous (derived from a common ancestor) or simply analogous (functionally similar but not inherited from a common ancestor)? Since parallel visual pathways are made up of cells at different levels of the neuraxis that differ in terms of neurochemistry, morphology, connections, and function, we need to clarify our level of analysis. For example, can we consider a pathway that carries chromatic signals from two cone types in the retina of a diurnal primate species as homologous to a pathway that appears similar in all other respects to one that carries signals from a single cone type in a monochromatic nocturnal species? We would argue that if this similarity extends to other defining features of the pathway and extends to several distantly related

species the answer should be yes. What would be useful is to understand which particular neural characters at any level in the pathway are conservative. It is likely that answers lie in the ontogeny of these pathways given that early embryological stages are quite conservative across mammals. Unfortunately, since there are almost no studies available comparing the neural development of the visual system of different primate species, we are unlikely to be able to identify such ontological characters, although some clues can be obtained by making comparisons between available primate and nonprimate developmental data. An additional related problem is that it is not clear how modifications at one level of the visual system (e.g., the retina) affect the development of more central target structures and vice versa. For example, Kaskan *et al.* (2005) have argued that major changes in retinal ganglion cell number or shifts in the proportions of rods and cones do not result in major differences in the size of the primary visual cortex (V1) which, instead, appears to scale with overall brain size. This result implies that the developmental programs for visual areas in the telencephalon and diencephalon (forebrain) are relatively independent (at least at the early stages) from changes that occur in the original out-pocketing of the forebrain, the retina. If this is the case, then using the retina as the starting point for investigating the evolution of parallel visual pathways may be the wrong approach. Careful examination of V1, however, indicates that there may be differences in relative laminar development across primates that appear to correlate with changes in the eye. Examination of the thalamus, especially the LGN, also indicates that relative laminar development varies in predictable ways with phylogeny and visual niche in primates (Figure 3). Thus, examination of detailed structure (not just gross size) may offer more insights concerning the evolution of brain parts (see Elston *et al.*, 2001).

We argue that, although the programs of neural development that establish peripheral tissues and each level of the neuraxis can differ, they are never evolutionarily divorced from each other if they are connected in the adult. After all, the entire machine needs to run reasonably well for the adult organism to survive and reproduce and this requires that connections be made appropriately. Changes at one level can never be completely divorced from changes at the next level. The latter also raises the issue of epigenetic effects. Clearly, there are a number of epigenetic mechanisms, including neural activity/experience and competition for growth factors, that must be used to match neuronal populations at different levels in large brains since the number of synapses far exceeds the number of genes available for individual specification by a large margin. For example, in humans there are about 15×10^8 synapses per mm^3 of neuropil (DeFelipe *et al.*, 1999) compared with $26–38 \times 10^3$ genes (Venter *et al.*, 2001). Still, these epigenetic mechanisms must have a genetic base and must be selected in order to ensure that brain areas wire correctly (Easter *et al.*, 1985).

Another big question that must be answered before we can even begin thinking about evolution of parallel visual pathways is the question of why these pathways arose in the first place. Parallel pathways likely arise in evolution in response to incompatibilities. A cell cannot have a large dendritic field that integrates information across many receptors and have a small dendritic field capable of discrete fine grain sampling from just one or two receptors. Such incompatibilities could also provide an evolutionary drive for parallel pathway specialization. Parallel pathways presumably also arise from the constraints on the speed of transmission, particularly in relatively large mammalian brains. It seems likely then that true parallel visual pathways originate from ganglion cells that are clearly distinct in a number of ways. As argued eloquently by Rowe and Stone (1980), dividing ganglion cells into different classes needs to be based upon a parametric approach using a variety of criteria, given that it is difficult to prove that any single characteristic defines an entire class. A true class of ganglion cells should also tile the retina without visuotopic holes, otherwise differences may simply reflect natural variation within a cell class. Presumably once the number of ganglion cell types can be established then the number of parallel pathways to the brain/LGN will be limited to that number, assuming that each ganglion cell class projects to its own unique set of cells. In the case of the LGN, the number of different ganglion cells that provide input has still not been established, but, as explained more fully below, one can make comparisons between species based upon examination of some of the established pathways. Similarly, at the level of the LGN and V1, a true visual pathway should show anatomical segregation in terms of connections even if specific functional signatures cannot be traced from level to level. Beyond the first synapse in V1, however, it appears that a separate set of parallel pathways is established that links V1 to extrastriate areas (Casagrande and Kaas, 1994). The degree to which the geniculocortical pathways are actually linked directly to the pathways leading to extrastriate areas is a matter of debate given that most signatures of early pathways disappear at the level of V1

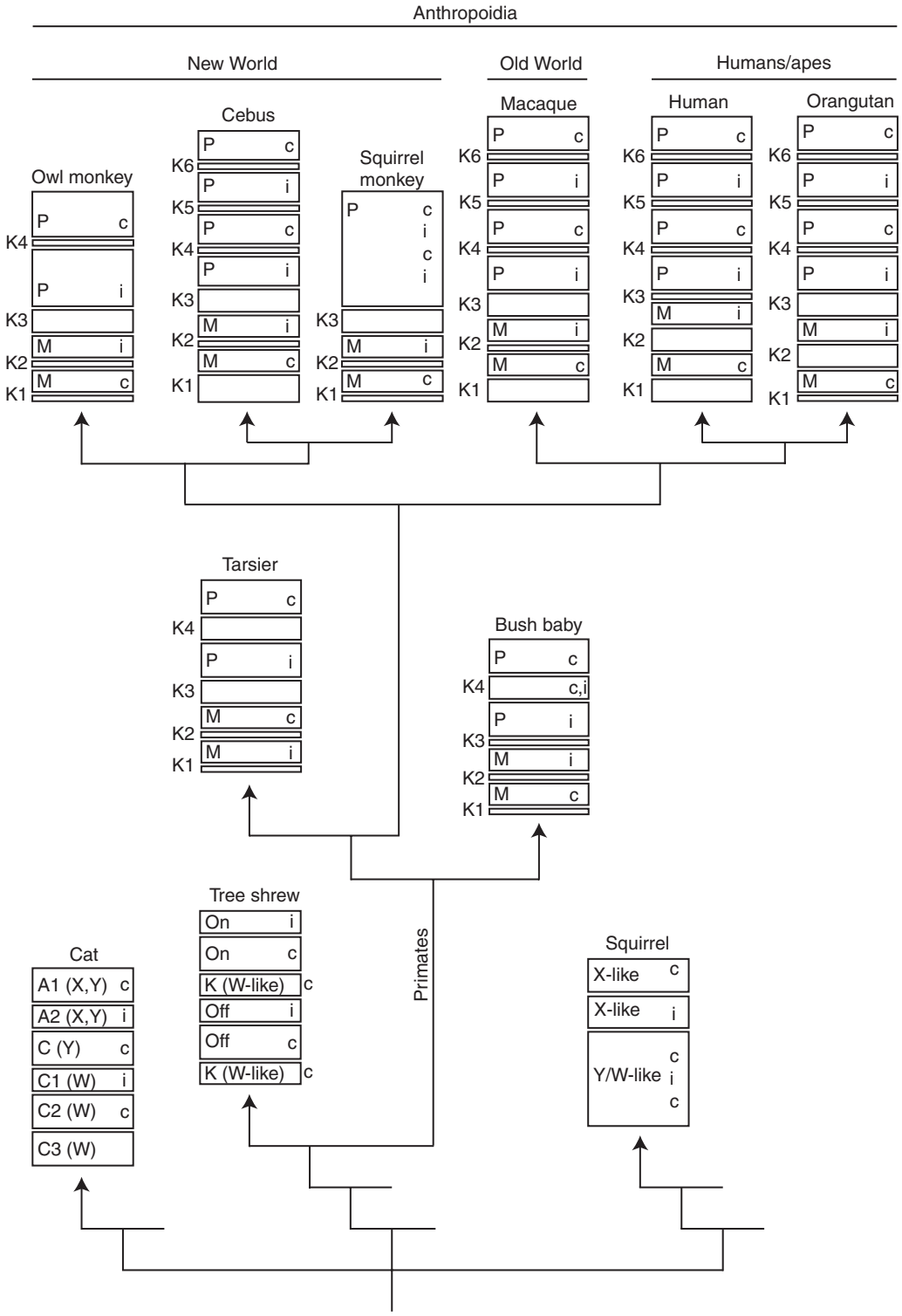

Figure 3 Laminar organization of the LGN in primates, tree shrews, squirrels, and cats. LGN cell layers for each species are indicated in boxes. The phylogenetic relationships between mammalian species are indicated by arrows, with the top branches indicating the relationships between primates. Only a few examples are shown. Note that in all primates the LGN is organized in a similar manner with two P layer and two M layers. In some primates (e.g., macaque monkeys), the P layers can split into four layers in a portion of the nucleus, and in others (squirrel monkeys) P cells exist as a cell mass where layers exist only based upon separate input from each eye. Tree shrews (Scandentia) are the closest living relatives of primates but have a very different LGN laminar organization as do squirrels (Rodentia) and cats (Carnivora). See text for details. c, contralateral retinal input; i, ipsilateral retinal input; K, koniocellular; M, magnocellular; P, parvocellular. Numerals refer to different K layers.

(Merigan and Maunsell, 1993; Casagrande and Xu, 2004). Nevertheless, similarities in the output of V1 to other cortical areas and their connections with each other allow us to ask whether similar hierarchies of visual areas are established across various primate species. As discussed in more detail below, it appears that V1 projects to the same areas in a range of primates (Casagrande and Kaas, 1994) but that, beyond V1, the evidence from connections, lesions, and behavior studies can support the idea that two major hierarchies of visual areas existed in a common ancestor of primates only in the broadest sense.

38.3 The Evolution of P and M Pathways

In almost all mammalian species so far examined, retinal and LGN cells can be physiologically classified into those that appear to convey information about higher spatial frequencies and respond in a more sustained manner, those that appear to respond better to higher temporal frequencies in a more transient manner, and those with slowly conducting axons and heterogeneous response properties (Stone, 1983; Lennie, 1993; Casagrande and Xu, 2004). In primate LGN, these classes correspond to P, M, and K neurons, respectively. In this section, we focus on the P and M pathways; the K pathway will be dealt with more fully in Section 38.4. Here, we consider the competing hypotheses that the P and M pathways (1) were present early in mammalian evolution and are thus homologous with similar pathways in nonprimates (e.g., X and Y cells in cats), (2) appeared early in primate evolution and their similarities with other mammalian species thus represent examples of parallel evolution, or (3) evolved independently in different primate lineages. Evidence for or against these hypotheses is sought from comparisons of response properties, anatomical organization, and neurochemistry in the retinogeniculocortical pathways of New World and Old World primates, cats, tree shrews, and rodents. Most of the nonprimate data come from cats, as their visual systems have been the most thoroughly studied of all nonprimate mammals.

In all primates, the M pathway originates from large retinal ganglion cells (parasol cells) which project to the M layers of the LGN, whereas the P pathway originates from smaller retinal ganglion cells (midget cells), which project to the P layers of the LGN (Figure 3). M cells in the retina and M LGN have larger receptive fields, lower preferred spatial frequencies, higher preferred temporal frequencies, and higher contrast sensitivities than their P counterparts. A similar dichotomy is found between Y and X cells in the cat retina and LGN (Table 1). Although X and Y cells in cats were first distinguished on the basis of a single criterion, linearity of spatial summation, the X versus Y classification was found to correspond to a host of other characteristics, and it is this extended sense of X and Y that is used here (Norton and Casagrande, 1982). Indeed, when W cells were described in cats, it was found that some were linear and some nonlinear, yet they were clearly a separate population based on the extended criteria that define X and Y cells (Table 1). Although it has been proposed that M and P cells are homologous to Y and X cells, respectively, an alternative hypothesis is that cat X and Y cells correspond to the linear and nonlinear subgroups of M cells, respectively, and that the P pathway is primate-specific (Kaplan and Benardete, 2001). X and Y cells, however, differ in many morphological and physiological characteristics in a similar way to M and P cells, while it is not clear that the linear and nonlinear M cells differ in characteristics other than linearity (see, however, Kaplan, 2004). It should be noted that linearity arises from a special mechanism that is added to the linear center surround mechanism present in all retinal ganglion cells, so linearity of certain

Table 1 Comparison of primate M and P cells with cat X and Y cells

Attribute	*Primate M cells*	*Cat Y cells*	*Primate P cells*	*Cat X cells*
Cell size	Large	Large	Small	Small
Conduction velocity	Fast	Fast	Slow	Slow
Response dynamics	Transient	Transient	Sustained	Sustained
Spatial resolution	Lower	Lower	Higher	Higher
Temporal resolution	Higher	Higher	Lower	Lower
Contrast sensitivity	Higher	Higher	Lower	Lower
V1 projection	Upper tier of layer 4	Upper tier of layer 4	Lower tier of layer 4	Lower tier of layer 4
Linearity of spatial summation	Most linear, some nonlinear	Nonlinear	Linear	Linear
Chromatic opponency	No	No	Yes (in trichromatic primates)	No

cell classes could be gained or lost in evolution without compromising other physiological properties.

Another physiological property that differs between primates and cats is color selectivity in that P cells have chromatic opponency, whereas X cells do not. However, this difference is affected by the fact that cats are dichromats, adapted for a nocturnal existence. As discussed more fully in the following sections, long-wavelength cones were gained (or, more likely, regained) independently in New World and Old World primates. The P cells of some dichromatic (or even monochromatic in the case of galagos and owl monkeys) primates also lack color opponency for similar reasons as cat X cells, yet they have all the other characteristics of P cells in trichromatic primates. Thus, P cells in all primate species should be considered homologous, regardless of color selectivity, because such differences can be explained by changes in single photopigment genes. By the same reasoning, lack of color opponency should not be used as evidence against homology of cat X cells and primate P cells.

Although fewer data are available, distinct physiological classes, possibly corresponding to P and M pathways, have been found in other species. In gray squirrels, P-like cells with longer latencies, sustained firing, and linear spatial summation could be distinguished from M-like cells with short latencies, transient responses, and linear or nonlinear summation (Van Hooser *et al.*, 2003). In tree shrews, although linear and nonlinear cells have been found (Sherman *et al.*, 1975), it appears that the nonlinear cells are more like K or W cells than M cells, and that nonlinear M cells are lacking (Holdefer and Norton, 1995). A clear dichotomy between transient and sustained responses is found in the tree shrew, however (Sherman *et al.*, 1975; Lu and Petry, 2003).

Within the LGN, M and P cells are segregated into different layers. The standard primate laminar pattern consists of four layers: two M layers adjacent to the optic tract, followed internally by two P layers (Casagrande and Norton, 1991; Kaas, 2004). Each of these layers receives input from one hemiretina, with the first M layer receiving crossed (nasal hemiretina) input and the second M layer receiving uncrossed (temporal hemiretina) input. The P layer closest to the second M layer also receives an uncrossed retinal input, while the most internal P layer receives a crossed retinal input. The K layers, discussed in more detail below, lie mainly between or ventral to each of the P and M layers (Casagrande, 1994; Hendry and Reid, 2000; Casagrande and Xu, 2004). In some primates, the two P layers can split into four layers, but this occurs for only a topographically limited portion of the nucleus. For example, in macaque monkeys, four P layers can be identified only within the part of the nucleus representing about 2–3° to 17° of eccentricity (Malpeli *et al.*, 1996). In some humans, P layers split into as many as eight layers in some parts of the nucleus, but in other humans only two P layers exist across the whole extent of the LGN (Hickey and Guillery, 1979). In some primates, portions of the ipsilaterally innervated M layer can split off and form an extra layer next to the optic tract within a portion of the nucleus (Casagrande and Joseph, 1980). The latter is the standard condition for M layers in the tarsier, where it has been suggested that the ipsilaterally and contralaterally innervated M layers are reversed (Rosa *et al.*, 1996). Finally, in many New World primates (e.g., squirrel monkeys), P cells exist as an unlaminated cell mass where layers can only be defined based upon segregated input from the axons from the two eyes (Tigges and O'Steen, 1974; Fitzpatrick *et al.*, 1983). All of these differences, however, can easily be recognized as modifications of the basic primate laminar pattern (Figure 3).

In most nonprimate mammals with well-developed visual systems, three main subdivisions of the LGN can be recognized, progressing internally to externally (i.e., toward the optic tract): (1) a main contralateral layer receiving X- and Y-type input, (2) a main ipsilateral layer receiving X- and Y-type input, and (3) an outermost layer comprising sublayers receiving various combinations of contralateral Y-type input and ipsilateral and contralateral W-type input. The LGN of the cat, for example, consists of paired layers A and A1 receiving mixed X and Y inputs from the contralateral and ipsilateral eyes respectively, a magnocellular C layer receiving contralateral Y cell input, and several small-celled layers receiving either contralateral or ipsilateral W cell input. The LGN of sheep and other ungulates has a similar organization (Karamanlidis and Magras, 1972; Ebinger, 1975; Karamanlidis *et al.*, 1979; Clarke *et al.*, 1988). Additionally, carnivores and ungulates possess a medial interlaminar nucleus (MIN) which receives Y and W input. Layers A and A1 are subdivided into sublayers receiving input from either ON-center or OFF-center retinal ganglion cells in such mustelid carnivores as ferrets and mink (LeVay and McConnell, 1982; Stryker and Zahs, 1983). In squirrels, contralateral layer 1 and ipsilateral layer 2 receive X- and Y-like input (referred to by some as P-like and M-like; see above), while Y-like input is found in layers 1, 2, and especially 3, and W-like input is confined to layer 3 (Kaas *et al.*, 1972; Van Hooser *et al.*, 2003).

The primate LGN thus differs from the standard mammalian plan in having complete, not partial, segregation of different cell classes. As previously pointed out (Boyd and Matsubara, 1996; Matsubara and Boyd, 2002), a simple scenario for transitioning to the primate organization involves the coalescing of ipsilateral Y cells ventrally in layer A into a separate layer. The resulting lamination pattern would have the same contra-M, ipsi-M, ipsi-P, contra-P organization as seen in primates.

Interestingly, the tree shrew, which is considered phylogenetically closer to primates than the groups considered above, has a unique LGN organization which is unlike that in primates or other mammals. The tree shrew has a six-layered LGN with two layers containing W-like cells, and the remaining four layers segregated by both eye input and contrast sign (ON center vs. OFF center). Projections from sustained and transient retinal ganglion cells do not appear to segregate into different LGN layers in the tree shrew. The tree shrew visual system thus appears to have many derived characteristics that arose independently of those in primates (Rager, 1991; Kaas, 2002).

Another criterion that has been used to determine homology in the LGN is neurochemical content. M cells (but not P cells) in the LGN of primates and Y cells (but not X cells) in the LGN of cats are selectively labeled by antibodies against a cell surface antigen, Cat-301 (Hockfield and Mckay, 1983; Hockfield *et al.*, 1983; Hendry *et al.*, 1988), or against nonphosphorylated neurofilaments (Chaudhuri *et al.*, 1996; Bickford *et al.*, 1998). These molecular markers thus support the hypothesis of homologies between LGN cell classes in different mammalian lines.

Finally, the geniculocortical projections of the different classes of relay cells provide evidence for homology between different groups. In all primates, M cells project to the upper portion of layer IV, P cells project to the lower portion of layer IV, and K cells project above layer IV (Casagrande and Norton, 1991). In cats, the laminar segregation between X and Y cells is similar (though likely not as absolute) with X-cell terminations concentrated in lower layer IV and Y cell terminations concentrated in upper layer IV. W cells project outside of layer 4 (see Section 38.4 on K pathway for further discussion). In both cats and primates, the simple laminar dichotomy between X and Y cells is likely to be complicated by subclasses of X and Y cells and M and P cells. For example, the Y cells in layer C have larger receptive fields, higher contrast sensitivity, and more pronounced nonlinearities than A-layer Y cells (Frascella and Lehmkuhle, 1984; Yeh *et al.*, 2003). Their terminations are confined to the topmost third of layer IV and, moreover, selectively target cytochrome oxidase (CO) blob columns (Boyd and Matsubara, 1996). It has been argued that a similarly defined subclass of M cells exists in primates (Hawken *et al.*, 1988; Bauer *et al.*, 1999), although the evidence for this is not as conclusive.

The sublaminar organization of geniculocortical organization in other animals is not as well described as for cats and primates, but it can be noted that the layer 3 complex in squirrels, which contains Y-like and W-like cells, projects to the upper part of layer 4 and supragranularly, and these two projections likely come from Y-like and W-like cells, respectively (Weber *et al.*, 1977; Harting and Huerta, 1983). Tree shrews have a very different geniculocortical arrangement, whereby terminations from ON-center and OFF-center cells segregate within different sublamina of layer 4 (Fitzpatrick and Raczkowski, 1990). The W-like LGN layers, however, still terminate outside of layer 4.

The data reviewed here strongly support the hypothesis that the precursors to M and P cells were present in the earliest primates, so M and P cells in all primates are homologous. Moreover, the similarities in organization of the M and P pathways in primates and similar pathways in some other mammals provide some support for the hypothesis that the M versus P dichotomy arose prior to the divergence of primates from other mammals, with the unique differences found in tree shrews representing a derived condition, not primitive characteristics representative of early primates.

38.4 Is the K Pathway Evolutionarily Old?

In Section 38.3, we focused on the parallel M and P pathways connecting the retina with V1; in this section, we focus on a third parallel pathway, currently referred to as the koniocellular, or K, pathway (for reviews see Casagrande, 1994; Hendry and Reid, 2000; Casagrande and Xu, 2004). As for the M and P pathways, the K pathway consists of a distinct class (or classes, as K cells are heterogeneous) of retinal ganglion cells that project to distinct groups of cells in the LGN, which are in turn connected to distinct layers of V1. The K pathway has a constellation of features that distinguish it from the M and P pathways and that have led some to suggest that the K pathway is phylogenetically older than the M and P pathways. In this section, we review this hypothesis, while at the same time

reviewing the data for homologues of the K pathway in other mammalian species, particularly the W pathway in the cat, the nonprimate for which the greatest amount of data on the visual system is available.

The cat W pathway was relatively well studied years before the primate K pathway was closely examined (Stone, 1983), and indeed even before the extent and importance of the K pathway in primates was widely acknowledged. There are a large number of similarities between K and W pathways. At the level of the retina, both cat W and primate K retinal ganglion cells have small cell bodies, thin but extensive dendrites, and the thinnest most slowly conducting axons in the optic tract (Casagrande and Norton, 1991). There is evidence for a similar class of retinal ganglion cells in other mammals as well, including rats, rabbits, and tree shrews.

The geniculate projections of both K and W retinal ganglion cells are to small-celled layers that are either next to the optic tract or intercalated between the main layers. Neurochemically, these small-celled layers have been identified using antibodies to the calcium-binding protein calbindin. In prosimian bush babies, and both New World and Old World simians including owl monkeys, marmosets, and macaque monkeys, calbindin is found in K layers, but not in M or P layers of the LGN (Johnson and Casagrande, 1995; Hendry and Reid, 2000; White *et al.*, 2001; Xu *et al.*, 2001). Calbindin also labels cells in the tree shrew LGN exclusively in the layers that contain W-like cells, layers 3 and 6 (Diamond *et al.*, 1993). In the cat, although W-cell layers in the LGN contain calbindin, many GABAergic (γ-aminobutryric acid, GABA) interneurons in the LGN also contain calbindin (Demeulemeester *et al.*, 1991), obscuring a possible relationship between the W-cell pathway and calbindin content.

K cells in the LGN differ in their relative laminar development in different primate lines (Hendry and Casagrande, 1996). The K pathway also appears to be physiologically and anatomically more heterogeneous than either the P or M pathways (for review, see Casagrande and Xu, 2004). For example, K cells lying ventral to the M layers in K layer 1 project mainly to layers IIIA and I of primate V1, and can be distinguished physiologically from K cells that lie close to the P layers and send axons to the CO blobs located in layer IIIBα of V1. Some K cells carry S-cone input although most K cells do not, at least in marmosets (White *et al.*, 2001). Some K cells defined by calbindin antibody labeling appear to project exclusively to the middle temporal visual area (MT) in macaque monkeys (Stepniewska *et al.*, 1999; Sincich *et al.*, 2004). This means that subdivisions of the K pathway could have been lost or added in different primate lines (Ding and Casagrande, 1998; Shostak *et al.*, 2002). In cats, W cells have similar projections: to layer 1 and to the CO blobs in layer III of V1 (Boyd and Matsubara, 1996), and to extrastriate cortex (Kawano, 1998). It is not yet clear if these different structures are targeted by different classes of W cells, or by collaterals of the same cells.

The K-cell pathway and the W-cell pathway are also similar in that they have close interconnections with the superior colliculus. Some retinal ganglion cells of the K and W classes project to the colliculus, and the colliculus makes projections to the K- and W-cell layers of primate and cat LGN. In tree shrews as well, there is a projection from the colliculus to LGN layers 3 and 6. Because the colliculus is considered by some to be phylogenically older than the LGN, being homologous with the main target of retinal axons in nonmammalian vertebrates, the optic tectum, it has been suggested that the K/W pathway is phylogenetically older than the M/X and P/Y pathways (Bishop, 1959). Other features of the K/W pathway, such as finer axons with more diffuse projections, have also been suggested to be primitive conditions. Ultimately, the question of pathway evolutionary age is extremely difficult to answer since we have no good biological markers of relative age specific for visual pathways. If anything, the K pathway in primates shows more morphological and physiological variation than the P or M pathways, so could be considered biologically (perhaps evolutionarily) less stable.

In summary, there is strong evidence from anatomy to support the conclusion that K cells in all primates are homologous and that at least some K cells have homologues in other mammals: (1) both K and W cells receive midbrain input from the parabigeminal nucleus and superior colliculus; (2) some W-like and K cells always lie adjacent to the optic tract in a large variety of mammals (Harting *et al.*, 1991); (3) K and W LGN cells send axons that terminate above layer IV in V1; (4) K cells are more likely than other LGN cell classes to project to extrastriate areas outside of V1; (5) K and W cells tend to be slowly conducting and have smaller cell bodies on average; and (6) K LGN cells in all primates and tree shrews (and perhaps W cells in cats) contain calbindin. There is also a variety of physiologically defined similarities between these cell classes, although the overlap in response properties between all relay cell classes in the LGNs of mammals and the influence of lifestyle on spatial and temporal thresholds make it difficult to make useful comparisons.

38.5 Color Vision in Primates and the Evolution of P and K Pathways

The ability to see color derives from the ability to compare wavelengths. Such color opponency is constructed at the retina from cones sensitive to short (S; e.g., blue), medium (M; e.g., green), and long (L; e.g., red) wavelengths by creating receptive fields with ON responses to one wavelength and OFF responses to an opposing wavelength or wavelengths. Thus, S cones oppose the M plus L cones to create a blue/yellow color axis, M opposes L to create a green/red axis and all three cones oppose each other to create an achromatic OFF/ON black/white axis. This simple view is complicated by the facts that some primates have only a single cone type and are therefore presumably color blind, most primates are dichromatic possessing two cone types, and some primates (such as humans) are trichomatic (Jacobs, 1996, 1998). Trichromacy, however, appears to have evolved separately in different primate lines (Jacobs, 1996). A number of articles have been written about the evolution of color vision in primates as well as the genetics of color vision (Jacobs, 1996, 1998; Nathans, 1999; Tan and Li, 1999; Dacey and Packer, 2003). A commonly held belief is that primates evolved from a nocturnal ancestor. Support for this argument has been recently reviewed (Ross, 2000) and will not be considered in detail here except where relevant to parallel pathway evolution. Relevant to the current article are proposals concerning which parallel pathways carry chromatic signals and what this might tell us about the evolution of parallel pathways in primates. At least four types of ganglion cells carrying cone signals have been identified in macaque monkeys. Of these, three carry signals from S cones, small and large bistratified ganglion cells carrying blue ON signals and large monostratified ganglion cells carrying blue OFF signals. It has been proposed that these blue pathways project to LGN K cells given that some K cells have been identified at the level of the LGN to carry S cone signals in macaque monkeys and marmosets (White *et al.*, 1998). In marmosets, approximately 20% of K cells carry S cone signals based upon studies in which immunocytochemistry for calbindin was used to directly identify K cells at the level of the LGN after single unit recording (White *et al.*, 1998). In addition, it has been argued by many that midget ganglion cells in several primates carry L/M opponent signals to the P LGN layers (see Dacey and Packer, 2003; but see, however, Calkins and Sterling, 1996). At present, it is unclear if some P cells also carry S cone signals, as was originally proposed by Wiesel and Hubel (1966), given that K cells, defined by either calbindin or CamKII immunocytochemistry, can lie below each P and M layer, can be found scattered within these layers, or can even form bridges of cells that pass directly through the P layers (Johnson and Casagrande, 1995; Hendry and Casagrande, 1996; Hendry and Calkins, 1998). Definitive data linking particular ganglion cell classes whose chromatic signature is well defined to particular visual pathways that project through the LGN to cortex is still lacking.

Evidence that does exist suggests the following. In all primates examined it has been demonstrated that K cells in the LGN defined by immunocytochemistry or laminar location send axons above layer IV (IVC of Brodmann) of V1 (Lachica and Casagrande, 1992; Ding and Casagrande, 1998). These K axons terminate in the CO blobs of V1, in cortical layer I and probably also in cortical layer IIIBβ (IVA of Brodmann) (Yazar *et al.*, 2004). With the exception of projections to layer IIIBβ this pattern of axonal projections can be demonstrated in prosimians as well as in New World and Old World simians, apes, and humans. Since, as discussed earlier, some prosimians such as the bush baby and at least one simian, the owl monkey, have only a single cone type and lack S cones entirely (Jacobs, 2002), it could be argued that the K pathway evolved before the evolution of color vision in primates. This would have to be the case if the prosimian bush baby represents the ancestral original nocturnal condition of primates.

An alternative proposal is that ancestral primates were actually dichromatic (Tan and Li, 1999). The evidence to support this view is as follows. First, S cones are considered to be of ancient origin genetically and are present in many mammalian groups, including carnivores, ungulates, and primates (Calkins, 2001). More important is the fact that both prosimian bush babies and simian owl monkeys appear to have the gene for S cones but this gene is not expressed in either species due to defects in the gene (Jacobs, 2002). The presence of the gene strongly suggests that functional S cones existed in their ancestors. Support for this view comes from studies that have examined for the S opsin gene in a relative of the bush baby, the slow loris (*Nycticebus coucang*) (Kawamura and Kubotera, 2004) and found evidence to support the view that this gene was disrupted in the common ancestor of galagiods (e.g., bush babies) and lorisids. Second, S and M cones are both present in at least one nocturnal prosimian, the mouse lemur (*M. murinus*), as well as in several diurnal lemurs and in the tarsier (Dkhissi-Benyahya *et al.*, 2001). Third, S and M cones have been identified in cat W cells (Wilson *et al.*, 1976). Cat W cells also project to the CO

blobs in V1 (area 17) just as K cells do in primates. Cat W cells also share many other characteristics in common with primate K cells as reviewed above and earlier (Casagrande and Norton, 1991). Taken together, these data support the view that the common ancestor of primates may actually have been diurnal with S and M cone signals passing to a population of K cells. Since all LGN cells receive input from cones, this does not inform us about the evolution of color vision relative to the parallel pathways. If this hypothesis is correct, it does predict that, as in cats, S cone signals should be confined to K cells in those prosimians that have functional S cones, a hypothesis that could be tested directly by examining for S cone input to LGN K cells in the mouse lemur. It also predicts that in dichromatic lemurs (perhaps all dichromatic primates) wavelength discrimination would depend upon K cells since P and M cells would only receive from a single M cone.

The issue of whether some primate ancestors were trichromatic is more complicated given the different ways prosimians, Old World primates, and New World primates construct color vision. All Old World primates have two separate opsin genes (M and L) on the X chromosome in addition to the single S autosomal gene. New World simians, and prosimian lorises, and lemurs have only one opsin gene on the X chromosome, but polymorphism of this gene allows females with different versions of the opsin gene on each of their two X chromosomes to achieve trichromacy. Males, with only one X chromosome, can never be trichromats in species that rely on polymorphism. Tan and Li (1999) have argued that the phylogenetic distribution of the M and L opsins across strepsirhine primates (lemurs, lorisids, and tarsiers) supports the idea that the X-linked polymorphism and primate trichromacy arose early in primate evolution. It is interesting that, regardless of whether trichromacy is achieved via polymorphism of a single gene on the X chromosome as in New World simians or on two separate genes on the same chromosome as in Old World simians, it would appear that the M/L (green/red) opponency can be identified electrophysiologically in some P cells in both cases but not so far in K cells (White *et al.*, 1998). This would support the view that M cone input to P cells via midget ganglion cells was the default condition in dichromatic primates with L cone opponency added later. Whether the P pathway further specializes when trichromacy is the norm as in Old World simians, one branch of New World primates, as well as apes and humans, remains unclear (Jacobs, 2002).

One aspect of the chromatic pathway to V1 that appears to show species-specific differences concerns the S cone input to V1 layer IIIBβ (IVA of Brodmann). Callaway and colleagues (Chatterjee and Callaway, 2003) have shown that, in macaque monkeys, cells in layer IIIBβ respond to S cone input in the form of blue ON- and blue OFF-center cells. Since thalamic axons project to layer IIIBβ in many diurnal simians but not in the nocturnal owl monkey, the prosimian bush baby, or in some apes (chimpanzee), or in humans, this pathway appears to be a specialization of some primates and not others (Preuss and Coleman, 2002). These findings indicate that apes and humans may have diverged from a primate ancestor in which the K pathway carrying S cone input did not innervate layer IIIBβ. In macaque monkeys, there is no physiological evidence for a direct S cone input via the thalamus to the CO blobs based upon recording from LGN axons in V1 where cell responses were silenced with the $GABA_A$-receptor agonist muscimol (Chatterjee and Callaway, 2003). Presumably, S cone input reaches cortex via another pathway in apes and humans. Taken together, these observations support the hypothesis that components of the K pathway may either have been lost in the evolution of apes and humans or that their common ancestor showed a parallel pathway organization more like that of present-day prosimians where the thalamus does not project to layer IIIBβ.

38.6 Ocular Dominance and Other Properties

At the level of the LGN in primates, retinal ganglion cells within the left and right eyes send input to separate layers. Additionally, ganglion cells with either ON-center or OFF-center responses innervate separate sets of cells at the level of the LGN. These parallel pathway features from retina to LGN appear to generalize across placental mammals. At the level of V1, however, the degree to which these properties remain segregated at the first synapse varies widely among mammals (see Casagrande and Norton, 1991, for review). For example, although close relatives of primates (e.g., tree shrews) show both ocular segregation and segregation of ON- and OFF-center responses to separate cortical layers, primates do not. Instead ON- and OFF-center responses appear combined at the first synapse in all primates examined to date, even

Table 2 Ocular dominance columns in striate cortex

Columns present	*Columns present*	*Columns absent*
Macaque (Hubel and Wiesel, 1969)	Talapoin monkey (Florence and Kaas, 1992)	Rat (Hubel and Wiesel, 1977)
Human (Hitchcock and Hickey, 1980; Horton and Hedley-Whyte, 1984)	Capuchin monkey (Hess and Edwards, 1987; Rosa *et al.*, 1988)	Mouse (Drager, 1974)
Owl monkey (Rowe *et al.*, 1978; Diamond *et al.*, 1985)	White-faced saki (Florence and Kaas, 1992)	Tree shrew (Casagrande and Harting, 1975; Hubel, 1975)
Marmoset (DeBruyn and Casagrande, 1981; Spatz, 1989)	Chimpanzee (Tigges and Tigges, 1979)	Gray squirrel (Weber *et al.*, 1977)
Green vervet (Hendrickson *et al.*, 1978)	Cat (Shatz *et al.*, 1977)	Brushtailed possum (Sanderson *et al.*, 1980)
Red monkey (Hendrickson *et al.*, 1978)	Ferret (Law *et al.*, 1988)	Rabbit (Hollander and Halbig, 1980)
Baboon (Hendrickson *et al.*, 1978)	Mink (McConnell and LeVay, 1986)	Sheep (Pettigrew *et al.*, 1984)
Spider monkey (Florence *et al.*, 1986)	Bush baby (Glendenning *et al.*, 1976; Hubel and Wiesel, 1977; Diamond *et al.*, 1985)	Goat (Pettigrew *et al.*, 1984)

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though ON- and OFF-center cells have been reported by some to be segregated to separate layers in the macaque LGN (Schiller and Colby, 1983). The finding that ferrets, but not cats, show segregation of ON and OFF pathways through the LGN to V1, suggests that parallel ON and OFF pathways that extend to cortex evolved several times in different mammalian lines of descent (Zahs and Stryker, 1988). The advantage of maintaining separability of ON and OFF pathways to cortex in diurnal tree shrews and nocturnal ferrets remains unclear given that these species have very different lifestyles and evolutionary histories.

Similarly, although eye input remains segregated at the first cortical synapse in cortex in tree shrews and many other mammals including some primates, the variability in both the pattern and degree of segregation of ocular inputs suggests that the organization of ocular dominance pathways from LGN to cortex evolved independently in primates and other mammalian species. In tree shrews, left and right eye input to cortex is segregated into sublayers within layer IV of V1 (Casagrande and Harting, 1975), whereas in all primates ocular input segregation (if present) occurs in the form of columns not layers. Among primates, examples of well-developed cortical ocular dominance columns can be found in some members of a number of distantly related groups including prosimian bush babies, New World simian spider monkeys, and all Old World simians and apes thus far examined, including humans (Florence *et al.*, 1986; Florence and Kaas, 1992; Preuss and Coleman, 2002; see Table 2). Even in primates in which ocular dominance columns show high interindividual variability, such as New World squirrel monkeys, or show very weak segregation, as in New World owl monkeys and marmosets, segregation occurs in the form of columns and not in the form of layers as in tree shrews (Florence *et al.*, 1986). These findings suggest that the tendency to segregate ocular information into columnar dominance columns in V1 was present already in the common ancestor of primates but not in the ancestor of tree shrews and primates.

Examination of the different patterns of ocular dominance columns in different primate species, however, indicates that well-developed ocular dominance columns either evolved several times in different lines of descent or regressed in different lines of descent from a well-developed pattern (Florence *et al.*, 1986; Florence and Kaas, 1992). Distinguishing between these different scenarios is difficult given that we do not understand the functional significance of ocular dominance columns since they appear to occur in both small nocturnal primates with no color vision and in large diurnal primates with good color vision, and appear variable across simians (Florence *et al.*, 1986). It may also be the case that such segregation is simply a byproduct of the degree of synchrony between active ganglion cells in the two eyes during a critical phase of development, especially since the expression pattern shows such a high degree of interindividual variation in squirrel monkeys (Horton and Hocking, 1996).

38.7 The Evolution of Dorsal and Ventral Cortical Streams

It has been proposed that there are basically two cortical streams for processing visual information

in primates – a ventral stream to the temporal lobe and a dorsal stream to the parietal lobe – the first being involved with object vision and the second with spatial vision or vision for action (Mishkin *et al.*, 1983). The dorsal and ventral streams both start with the intracortical circuitry in V1, which processes the three main classes of LGN inputs described earlier (M, P, and K) to create multiple distinct outputs originating from separate classes of projection neurons in cortical layer III and projecting to several different extrastriate areas. Two hierarchical chains of connections, one to the temporal lobe and one to the parietal lobe (albeit with some connections between areas and compartments belonging to the different streams), can be traced through the multiple (more than 30) extrastriate areas found in primates (DeYoe *et al.*, 1994).

The ventral stream, in order, consists of layer IIIBα blobs and layer IIIBα interblobs in V1, thin stripes and interstripes in the secondary visual cortex (V2), DL/V4, and various inferotemporal areas. The temporal areas at the top of this hierarchy are physically close to and interconnected with perirhinal cortex and hippocampus, structures involved with object recognition and encoding visual memories. The dorsal stream consists of layer IIIC and layer IIIBα interblobs, which give rise, respectively, to a direct and an indirect pathway via V2 to V5/MT, CO thick strips in V2, MT/V5, and surrounding superior temporal areas, and, finally, parietal cortex. These parietal areas are close to, and interconnected with, premotor cortical areas involved with programming eye movements and other visually guided behaviors. In this section, we examine the evolution of these processing streams. We will first consider the early stages of processing through V1 and V2, and then the later stages through specialized extrastriate areas.

To review, in primates, M LGN afferents terminate in upper layer IV, P afferents in lower layer IV, and K afferents in the blobs in layer IIIBα and in layer I. As mentioned in previous sections, these pathways likely have homologues in other mammals, so the building blocks for the two streams will at least be homologous structures. Immediately above the M input layer is found a population of projection neurons that are an important early part of the dorsal pathway, receiving M input and projecting directly to V5/MT. These cells are found in all primates, where they may be pyramidal (prosimians) or stellate (Old World monkeys) or both (New World monkeys). In cats, large pyramidal cells at the base of layer III receive Y-cell input and project to lateral suprasylvian cortex (Matsubara and Boyd, 2002), which is, like V5/MT, an area that processes motion (see below). These projections are probably homologous. In cats, prosimians, and New World primates, these projections are robust, and are concentrated directly below CO blobs. In macaque monkeys, there are far fewer MT-projecting cells in V1 and it has been debated as to whether or not these are concentrated beneath CO blobs (Boyd and Casagrande, 1999; Boyd and Matsubara, 1999; Sincich and Horton, 2003). This could represent a gradual evolutionary reduction of the fast direct pathway to V5/MT in primate evolution, and perhaps of the entire dorsal stream, as increased emphasis is placed on slower indirect pathways passing through upper layer III, which is proportionately thicker and more differentiated in primates (especially simians) then in other mammals. This scenerio suggests a change in visual processing, with more emphasis on analysis of visual form and less emphasis on reaction to movement.

The indirect dorsal stream through V2 originates from neurons in layer IIIBα interblobs that probably receive both M and P input via intralaminar projections from layer IV. The neurons of the ventral stream to V2 are in layer IIIBα blobs and interblobs, and so receive K input in addition to M and P. In species with color vision, the K input may carry color information (see previous sections), an important cue for object recognition but not for visuomotor tasks. Within V2, recent evidence points to the existence of four stripe compartments, two which stain darkly for CO (the CO thick and thin stripes) and receive input from dorsal and ventral stream neurons in V1, and two interstripe compartments that may also receive from both streams but definitely get input from ventral stream neurons in V1 (Xu *et al.*, 2004). In some prosimians, CO stripes are faint or absent in V2, but there still is evidence that projections from blobs and interblobs are segregated into different compartments within V2, so the striped architecture likely is homologous across all primates. There is no clear example yet of a similar striped architecture (with or without accompanying CO staining) in nonprimates. There is some evidence for segregation of blob and interblob projections to extrastriate areas in the cat, but this occurs not in V2, but in area 19. The cortical hierarchy in primates continues through V2 into V5/MT for the dorsal stream, and into V4/DL for the ventral stream. Again, although the prosimian galago does not have distinct CO stripes or functional compartments with low orientation selectivity as in simians (Xu *et al.*, 2005), neurons projecting from V2 to V5/MT and to V4/DL form interdigitated stripes (Krubitzer and Kaas, 1986), showing that the underlying architecture (albeit perhaps less complex) is the same in prosimian V2 as for other primates.

Thus, the earliest levels of the dorsal and ventral streams can be recognized in all primates. Can the same be said of the higher levels? This is an important question because an increase in neocortex size and an increase in the number of sulci and gyri have occurred independently in the evolution of different mammalian lines, and even in the evolution of different primate lines. Both Old World primates and sheep, for example, have large, gyrencephalic brains, but examination of fossil endocasts suggests that their last common ancestor had a small lissencephalic brain (Radinsky, 1967, 1975, 1981). Not surprisingly, sheep and primate neocortex, while superficially similar, show important differences, as, for example, the relative development of the temporal lobe, which is proportionately less prominent in sheep brains than in primate brains, and the olfactory cortex, which in sheep is proportionately enormous by primate standards. Also, the obvious occipital development and landmarks that characterize the primate visual cortex such as the calcarine fissure are not obvious in sheep (nor in other nonprimate mammals) in spite of the fact that other fissures are well developed and the sheep brain is larger and more fissured than many primate brains. In summary, primitive mammals had small brains and likely possessed only a few cortical areas for each sensory modality, perhaps only V1 and V2 for vision (Northcutt and Kaas, 1995). The number of extrastriate visual areas has increased independently in different mammalian lines, so it might be impossible to define homologies across mammalian groups for many extrastriate areas.

Even within the primate lineage, the patterns of sulci and gyri vary between New World and Old World monkeys, apes, and prosimians, and brain size has increased independently in these lines. It is therefore important to determine which of the multitude of visual areas can be unambiguously identified in all primates and are thus likely to be homologous. Homologies among visual areas in different primate lines are recognized on the criteria of size, shape, and position in the cortex with respect to other cortical areas, layout of the visual field map, physiological response properties, patterns of connections with other cortical areas and subcortical structures, and cortical architecture. For example, the V1 can be recognized, not just in primates but also in all mammals, by its position in the occipital lobe, by receiving strong projections from the LGN, by the complete map of the visual field it contains, and by its distinctive histological architecture.

In all primates (and likely all mammals), V2 forms a narrow strip immediately lateral to V1. In addition to its position, it can be recognized by its visual field organization, sharing a representation of the vertical meridian with V1, and by its distinctive mosaic pattern of connections with V1, which are related to the CO architecture (Casagrande and Kaas, 1994). In all primates, an important dorsal stream area, called MT (sometimes referred to as V5) in Old World primates, New World primates and prosimians, occupies a densely myelinated oval-shaped area in the dorsal temporal lobe. This area contains many motion-sensitive neurons, most selective for the direction of stimulus motion. MT/V5 is also identified by its distinctive patterns of projections from V1 and V2, and by its projections to parietal cortex. In all primates, an important ventral stream area, called V4 in Old World primates and DL in New World primates and prosimians, occupies cortex caudal to V5/MT and receives inputs from compartments in V2 not projecting to V5/MT. The homology, however, of this region is less well established, perhaps due to uncertainties in the extent and possible subdivisions of this region of cortex, as it does not have a distinctive architecture, and its visual field map is not as regular as that of MT. Proposed homologies of primate cortical areas higher in the hierarchy are even more tenuous, for similar reasons. It is possible that more homologies will become apparent when the cortical organization of different primates becomes better understood. (This presupposes that regions of cortex outside of primary areas and certain easily identifiable areas such as V5/MT are, in fact, best described as collections of discrete areas with sharply defined borders, and not as larger fields of loosely graded response properties and connections.) With presently available information, then, only areas on the lower levels of the visual-processing hierarchy can be homologized across different primate species, suggesting that areas higher in the hierarchy were added independently in different primate lines. Even so, the dorsal and ventral streams in different primate lineages can be identified without concomitantly identifying homologues for all of the visual areas involved.

Is it possible to identify dorsal and ventral streams in other mammals, given that so few extrastriate areas are likely to be homologous between primates and other mammals? As suggested above, processing in the dorsal and ventral streams prepares visual information for the ultimate use by motor cortex and limbic cortex, respectively, structures that are likely homologous in all mammals. Even if the primitive mammalian visual system consisted of a single area, V1 (although V2 at least was likely also present in the earliest mammals), separate

dorsal and ventral streams could still exist, consisting of separate populations of V1 neurons projecting directly to motor and limbic cortex, respectively. As was suggested to be the case for different primate groups, extra areas could be inserted to form processing hierarchies independently in different mammalian lineages. Inserting areas between V1 and limbic cortex will route the ventral stream through the temporal lobe based on simple proximity to the hippocampus. Similarly, inserting areas between V1 and motor cortex will result in a dorsal stream through parietal cortex.

There is evidence for dorsal and ventral streams in mammalian lineages as different as carnivores and rodents, both of which have multiple extrastriate visual areas that are unlikely to be homologous with any primate areas. The cat has about 15 different extrastriate areas and, as a model species, has the rare advantage that many of these areas have been extensively investigated (Payne, 1993). Evidence for dorsal and ventral streams in cats comes from studies of connections, physiological response properties, and behavioral deficits. Similar to V5/MT in primates, an area in the lateral suprasylvian (LS) sulcus of the cat receives a direct input from V1, projects to parietal and visuomotor areas, and displays motion selectivity. Inactivating this area leads to visual orienting and motion processing deficits (Lomber, 2001), as would be expected from a dorsal stream area. The cat also possesses a temporal visual stream consisting of multiple areas progressing through the temporal lobe to the hippocampus. As would be expected for the ventral stream, inactivation of the temporal lobe areas does not impair visual orienting behavior (Lomber, 2001).

The similarities between V5/MT and LS cortex are strong enough that it has been proposed that these areas are homologous (Payne, 1993). If V5/MT was present in the last common ancestor of cats and primates (more than 65 Mya), one would expect it to also be present in all mammalian lines that share a common ancestor with either cats or primates that is more recent than their last common ancestor (Northcutt and Kaas, 1995). Current mammalian classifications place primates in the superorder Euarchontoglires along with Glires (rodents and rabbits), flying lemurs, and tree shrews. As carnivores, cats are members of the superorder Laurasiatheria, which also includes insectivores, bats, ungulates, and whales (Madsen *et al.*, 2001; Murphy *et al.*, 2001; Waddell *et al.*, 2001; Amrine-Madsen *et al.*, 2003). Thus, if V5/MT and LS are homologous, a similar area should be identifiable in other members of these two superorders; such identifications are currently hampered by lack of data from relevant species.

For Euarchontoglires, at least partial data on extrastriate cortical organization are available from tree shrews and some rodents. Tree shrews have a series of visual areas adjoining V2, one of which, the temporal dorsal area (TD), has been proposed as a possible homologue for MT. Like MT, TD contains a complete representation of the visual field (Sesma *et al.*, 1984), stains more strongly than surrounding cortex for myelin and the Cat-301 antibody (Jain *et al.*, 1994), and receives inputs from V1 (Lyon *et al.*, 1998). However, TD in tree shrews is adjacent to V2, unlike MT, which is separated from V2 by DL/V4, and TD appears to lack connections with visuomotor areas of frontal cortex (Lyon *et al.*, 1998), which is part of the connectional signature of MT in at least some primates (Krubitzer and Kaas, 1990). No data on the detailed response properties in TD are currently available, so it is not yet known if this area contains direction-selective neurons.

The organization of extrastriate visual cortex in rodents is not completely clear, and appears to show substantial species variability (Rosa and Krubitzer, 1999). Germane to the present discussion is that rodents are thought to be monophyletic, and that mice and rats share a more recent common ancestor than either do with squirrels (Reyes *et al.*, 2004). In squirrels, V2 forms the lateral border of V1, with at least two tiers of multiple extrastriate areas lateral to it (Kaas *et al.*, 1972, 1989). In the rat, microelectrode mapping studies suggest that V1 is bordered laterally, not by a single area V2, but by multiple small retinotopically defined extrastriate visual areas named topographically (rostromedial, anterolateral, lateromedial, posterolateral, etc.) and corresponding to regions free of callosal connections (Espinoza *et al.*, 1992; Montero, 1993). Injections of tracers in different retinotopic locations in V1 lead to changes in the location of patches of label within these extrastriate areas that is consistent with the electrophysiological maps (Coogan and Burkhalter, 1993; Montero, 1993), mitigating against the argument that these projections correspond to multiple modules within a traditional retinotopically mapped V2 which, similar to other mammalian groups, extends along the entire lateral border of V1 (Malach, 1989). In mouse, microelectrode mapping shows a single V2 bordering V1 laterally, with at least one other area lateral to that. However, corticocortical projections from mouse V1 had a similar pattern as in the rat (Olavarria and Montero, 1989), suggesting that multiple visual areas adjacent to V1 were common at least to mice and rats. In order to resolve the

differences in cortical organization between different rodent species, it has been assumed that the largest of the areas bordering V1 laterally in rats (the lateromedial area, LM) is homologous to V2 in other species (Rosa and Krubitzer, 1999). According to this hypothesis, either new areas adjoining V1 were added in the mouse/rat lineage, or regressive events caused more lateral visual areas (perhaps homologous to the lateral visual areas in squirrels) to be shifted toward V1, at the expense of V2. A recent optical imaging study of mouse visual cortex (Kalatsky and Stryker, 2003), however, not only found evidence for multiple retinotopically defined extrastriate areas, but also suggested a narrow V2 with only a central visual field representation; detailed optical imaging maps of rat extrastriate cortex have not yet been published. The many patches following a V1 injection, and the tendency of visual fields to be congruent across borders, means that V1 projections to a narrow V2 could be continuous with a patch of labeling in an adjacent area, and thus overlooked in the anatomical mapping studies. The coarse sampling of microelectrode mapping, combined with the large receptive fields, may also have made it possible to have missed a narrow V2. Projections from V1 need to be combined with functional mapping and histological verification of the extent of V1 to determine if there really is a narrow V2 interposed between V1 and the lateral extrastriate areas in rats and mice.

Returning to the original question of functional streams, areas responding preferentially to moving stimuli can be found in both squirrels and mice/rats. In rats, the anterolateral area (AL) appears to have cells selective for movement (Montero and Jian, 1995), while, in mice, AL and another area (LM) bordering V1 laterally give rise to different connectional streams, AL preferentially connecting with dorsal and medial regions of cortex, LM with ventral regions of cortex (Wang and Burkhalter, 2004). In ground squirrels, an area (ML) with large receptive fields and direction-selective cells was found lateral to V2 (Paolini and Sereno, 1998), and thus in the right position to be homologous with MT. Both AL in rats and mice and LM in squirrels receive direct projections from V1, which is another similarity with V5/MT, although neither area appears to have the extensive myelination, an anatomical signature of V5/MT.

On the cat (Laurasiatheria) side, there is even less evidence from which to draw conclusions. It does appear that LS cortex, at least, has homologues in fellow carnivores, the mustelid ferrets (Manger *et al.*, 2002). Another laurasiatherian animal whose extrastriate cortex has been mapped is the megachirpopteran flying fox (*Pteropus*). Although once thought to be more closely related to primates than to michrochipoteran bats, all bats are now thought to comprise a single group within the Laurasiatheria (Van Den Bussche *et al.*, 2002). The occipitotemporal visual area (OT) was proposed as a possible megachiropteran homologue to LS/V5/MT based on its location lateral from V2, and its receptive field organization (Rosa, 1999). Microbats, relying on echolocation for navigation, have an enlarged auditory cortex, and very little extrastriate visual cortex. If this is a primitive condition for bats, it would mitigate against any proposed homologies of megachiropteran visual areas, given that bats are likely monophyletic. It is also possible that extrastriate cortex may have been reduced during microbat evolution.

In conclusion, specialized extrastriate areas belonging to dorsal and ventral cortical streams can be recognized in a wide range of mammals. Only the earliest stages of these streams and the last stages in motor and limbic cortex are likely to be homologous across mammalian lines, however. Even within primates, only a few areas can be unequivocally identified as homologues. Different lineages have added areas to the middle levels of these cortical streams independently. The constraint of proximity of the inserted areas to limbic cortex or motor cortex keeps the temporal stream temporal and the dorsal stream dorsal.

38.8 Conclusions, Questions, and Future Strategies

What can we usefully conclude about the evolution of parallel pathways in primates? We need to constantly remind ourselves that without specific definitions of what we are comparing and at what level (genes, molecules, cells, or pathways), we cannot develop definite or testable hypotheses. In this article we have focused on pathways originating with distinct classes of retinal ganglion cells and asked whether homologues of these visual pathways can be found across different primate species or between primates and nonprimates. We hypothesized that examining for similarities across distantly related species is the most important initial step in arguing for homology given the lack of genetic and fossil signatures of visual pathways. Nevertheless, we remain cognizant of the fact that different regions of the nervous system (e.g., retina, thalamus, and cortex) have different patterns of gene expression controlling their cellular composition and distribution. Therefore, we cannot simultaneously address the issue of homology at different levels of comparison (i.e., proteins, cells,

pathways, or brain regions). It is even more difficult to determine if similarities result from homology or homoplasy given that the developmental programs that establish visual cells and pathways are conservative and presumably have a restricted set of viable functional solutions for species to survive using the visible portion of the energy spectrum here on earth. Therefore, a useful future approach would be to compare the ontogeny (both early and late) of distantly related primates (e.g., a prosimian with a New World simian and an Old World simian) and primates and nonprimates (e.g., macaque monkey with rodent) examining for similarities at both the genetic and systems levels. A fuller understanding of commonalities in the ontogeny of different species would aid enormously in examining for homology in visual pathways.

Our examination of P, M, and K pathways leads to the hypothesis that these pathways are homologous across primates in spite of vast differences in the lifestyles and retinal organization in different primate species. It is also likely that what we call the P, M, and K pathways have general counterparts in other mammals since cats certainly appear to have pathways that specialize in spatial versus temporal resolution (i.e., X vs. Y cells) in a similar way to P and M cells in primates; W cells also resemble K cells anatomically and physiologically. Nevertheless, details of these pathways in nonprimates (even close relatives like the tree shrew) differ significantly; so significant changes have occurred independently in the P, M, and K pathways of different lineages.

We have also argued that the K pathway may be made up of more than one pathway so its evolutionary history is more difficult to try to define. Nevertheless, it does appear that cells in this pathway across a range of species can be recognized by the presence of calbindin. Other similarities to W cells in cats and other mammals suggest that a K-like pathway may have originated early in mammalian evolution. This does not necessarily make the primate K-cell pathway phylogenically older or newer than the P and M pathways since the K pathway shows enormous variability in the relative numbers of cells present in different LGN layers (identified neurochemically) across different primate species. What would be useful to know is which ganglion cells actually project to K layers in different primates and in close primate relatives such as tree shrews. For example, do bistratified ganglion cells project uniquely to K layers in tree shrews as would be predicted from work in macaque monkeys? This easily tested question would reinforce the view that some K cells evolved prior to the split between tree shrews and primates. Examining the same issue in cat W cells would extend the evolution of this component of the K pathway to other mammals.

A closely related issue concerns the evolution of chromatic pathways in different primates. Since some K cells receive input from S cones in some New World (marmosets) and Old World (macaque monkeys) primates, and K cells carrying S cone signals project to cortical layer IIIBβ in macaque monkeys, it will be important to understand how S cone signals are transmitted to V1 in primates such as apes and humans that lack an LGN projection to cortical layer IIIBβ. Such information could potentially inform us about the evolutionary split between monkeys and apes. Similarly, it would be informative to know if tarsiers or any diurnal lemurs that have functional S cones send these signals via K cells to cortical layer IIIBβ.

We have argued that, since nocturnal prosimians, such as bush babies, have the S cone gene (even though it is not functional) in addition to functional M cones, and that other prosimians (and tarsiers) also have both M and S cones, it is likely that earliest ancestors of primates were dichromatic like present-day tree shrews. If all nocturnal prosimians, however, show the same defect in the S cone gene, this would argue in favor of a nocturnal bottleneck. Alternatively, if distantly related nocturnal primates, such as galagos and owl monkeys, show that S cone genes were disabled in different ways, this would argue that the lack of functional S cones evolved secondarily when species moved from a diurnal to a nocturnal niche.

We reviewed also the evidence that segregation of ON and OFF pathways and segregation of left and right eye inputs (ocular dominance columns) evolved independently in different lines. ON and OFF pathways are combined at the first level in all primates examined, and the tendency to segregate ocular inputs into columns, although variable across primate species, exists in distantly related primates. These observations support the presence of at least weak ocular dominance segregation into columns in the common ancestor of primates and support the view that the ON and OFF pathways were not segregated to columns or layers in a primate ancestor. Why ocular dominance columns exist in primates remains a mystery. Given the high inter-animal variability of ocular dominance columns in squirrel monkeys, it might be useful to examine both the genetics and visual experience of animals that do with those that do not appear to show clear columns. It would also be useful to examine for ocular segregation in a wider range of primates.

Finally, we examined the most difficult issue, namely the evolution of dorsal and ventral cortical pathways originating in V1. Given that there is

disagreement even about the definitions of cortical areas that receive input from V1, we cannot provide solid conclusions about the homologies of dorsal and ventral streams beyond the statement that there is evidence for sets of projections to similar hierarchies of areas in all primates thus far examined. There is also evidence that the general cortical design for such streams may exist in nonprimate mammals even if specific cortical areas within each hierarchy are not homologous. Clearly, much more evidence concerning the number of visual areas in a range of primates and other mammals will need to be examined before more definitive statements can be made. Perhaps with the advent of high-resolution functional magnetic resonance imaging we will be in a position to more rapidly map visual areas in a variety of species.

Acknowledgments

We are grateful to David Royal for the artwork in Figure 1 and comments on the manuscript, Xin Chen for comments on the manuscript, and Julia Mavity-Hudson for help with illustrations and comments on the manuscript. Supported by EY01778, IBN-0234646, and core grants EY08126 and HD15052.

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39 The Evolution of the Primate and Human Auditory System

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Glossary

architectonic features	Refer to the anatomical features that comprise brain tissue. These features typically vary among distinct areas of the cerebral cortex. Specific architectonic features include the number, arrangement, and types of neurons in a particular area of cortex. Other features include the differential expression of proteins or density of myelinated axons within an area. Comparison of architectonic profiles derived from multiple architectonic features facilitates identification of individual areas, as well as the grouping of areas with similar architectonic profiles into regions.
auditory cortex	Refers specifically to those areas of the cerebral cortex for which the primary sources of thalamic input are the principal nuclei of the medial geniculate complex.
auditory related cortex	Refers specifically to those areas of the cerebral cortex for which the primary source of auditory input is the auditory cortex, with relatively little, if any, input from the principal nuclei of the medial geniculate complex.
cortical area	A subdivision of cerebral cortex defined by a unique set of anatomical and physiological features. An area may be considered a functional module with unique patterns of connections with other cortical areas.
cortical region	A group of cortical areas that share a defined set of features. In auditory cortex, for example, the core region includes three areas that occupy the first stage of auditory cortical processing, as defined by their patterns of connections with the medial geniculate complex and other areas of cortex.

39.1 Introduction

The identification and characterization of areas that contribute to auditory processing in the mammalian cerebral cortex has been the subject of sporadic investigation for over 130 years. The first insights were derived from lesion studies and detailed descriptions of anatomical features during the late 1800s and early 1900s (Beck, 1928, 1929; Broca, 1865; Brodmann, 1905, 1909; Clark,1936; Ferrier, 1875; Poljak, 1932; von Bonin, 1938; von Economo and Horn, 1930; von Economo and Koskinas, 1925; Walker, 1937; Wernicke, 1874). These studies, which often provided detailed parcellations of the superior temporal region, comprise the classical descriptions of human and nonhuman primate auditory cortex and remain influential to this day. In the mid-1900s, electrophysiological studies confirmed the location of auditory-responsive cortex on the superior temporal plane of monkeys and chimpanzees, and produced evidence that a representation of the basilar membrane was preserved in the organization of the primary auditory region (Ades and Felder, 1942; Bailey *et al.*, 1943; Celesia and Puletti, 1969; Gross *et al.*, 1967; Hind *et al.*, 1958; Katsuki *et al.*, 1962; Licklider and Kryter, 1942; Pribram *et al.*, 1954). These landmark studies were accompanied by others in which the connections of the superior temporal region were explored (Nauta, 1957; Pandya *et al.*, 1969; van Buren and Yakovlev, 1959). Interest in the organization of primate auditory cortex increased during the 1970s, marked by studies that related patterns of thalamic and cortical connections to architectonic subdivisions and tonotopic maps (Imig *et al.*, 1977; Merzenich and Brugge, 1973; Mesulam and Pandya, 1973; Pandya and Sanides, 1973; Sanides, 1975). Studies of the auditory cortex of nonhuman

primates and humans since that time have been characterized by stepwise refinements of earlier findings, culminating in a working model of the primate auditory cortex that is the subject of ongoing testing and modification (Kaas and Hackett, 1998, 2000; Kaas *et al.*, 1999). The development of the primate model has paralleled that of other mammalian models, yet the extent to which findings from one species can be generalized to another remains uncertain. The number of auditory areas identified varies between species, and presently only the primary auditory area, A1, is considered to be homologous. Additional homologies are likely, and may eventually be established on the bases of relative location, receptive field organization, and other anatomical and physiological features. The extension of findings from research animals to humans is especially problematic because experimental constraints greatly limit direct comparisons. Nevertheless, the model of auditory cortex established in nonhuman primates shares a number of key features with humans, and therefore provides a useful foundation for comparative study that will improve our understanding of audition in primates (Hackett, 2002).

39.2 Gross Anatomical Features of the Superior Temporal Lobe

Allometric measurements of surface area and volume indicate that the superior temporal gyrus (STG) increases in size successively by a factor of nearly three times each between squirrel monkeys, macaque monkeys, chimpanzees, and humans (Rilling and Seligman, 2002). The threefold expansion of the STG is proportional to overall temporal lobe expansion for all of these primates, except humans, where temporal lobe volume is about four times that of the chimpanzee (Figure 1). This appears to reflect greater expansion of temporal fields beyond the STG in humans.

In all primates, the auditory cortex is located on the dorsal surface of the temporal lobe where it occupies a large portion of the STG and lower bank of the lateral sulcus (LS). The relative size, location, and orientation of the auditory fields varies between species, reflecting differences in the morphology of the temporal lobe (Sanides, 1975). The length and depth of the LS increases with brain size, as does the prominence of the circular sulcus and insular region (Figure 1). In prosimians (e.g., lemur, galago), there are no major sulci on the lateral surface of the brain other than the LS; therefore, there is no obvious division between the superior and inferior temporal regions. In some New World monkeys (e.g., marmoset), a shallow superior temporal sulcus (STS) extends for a short distance, partly dividing the temporal lobe into superior and inferior gyri. STS depth in the marmoset ranges from 2.5 mm to little more than a shallow depression (de la Mothe *et al.*, 2006). In other New World monkeys (e.g., squirrel monkey), the STS is deeper and longer, clearly demarcating the superior and inferior gyri. In Old World macaque monkeys and great apes, the STS completely divides the temporal lobe into superior and inferior gyri, except at its rostral pole. Caudally, the LS and STS merge in the temporoparietal junction of these primates. In humans, gyrification of the ventral temporal lobe is more elaborate, but the STG remains bounded by the LS and STS.

39.3 Location of the Auditory Cortex in the Superior Temporal Lobe

'Auditory cortex' is defined as the array of cortical areas that receives its principal thalamic input from either the ventral (MGv) or dorsal (MGd) divisions of the medial geniculate complex (MGC) (see Shared and Convergent Features of the Auditory System of Vertebrates). Accordingly, the auditory cortex occupies a large portion of the ventral (lower) bank of the LS and STG in nonhuman primates (Figures 1 and 2). Cortical areas that receive inputs from auditory cortex, but not the MGv or MGd, are referred to as 'auditory related'. These areas are located in the temporal pole, and portions of the STS, intraparietal sulcus, and prefrontal cortex. The precise boundaries of the auditory cortex are not entirely known for any primate species, but are most certain in marmoset, owl, and macaque monkeys. In these primates, the medial boundary lies within the LS, where auditory areas adjoin the insula (rostrally) and parietal operculum (caudally). The caudal boundary is located near the junction of the LS and STS, bordering the temporal parietotemporal (Tpt) area, an auditory-related multisensory area that occupies the caudal terminus of the STG (de la Mothe *et al.*, 2006; Galaburda and Pandya, 1983; Hackett *et al.*, 1998; Leinonen *et al.*, 1980; Pandya and Sanides, 1973). The rostral boundary has not been defined with certainty, but thalamic and cortical connections indicate that auditory cortex does not extend all the way to the temporal pole (Galaburda and Pandya, 1983; Hackett *et al.*, 1998; Kosmal *et al.*, 1997; Pandya and Sanides, 1973). The ventral (lateral) boundary has not been defined, but the connections of the STG and STS suggest that

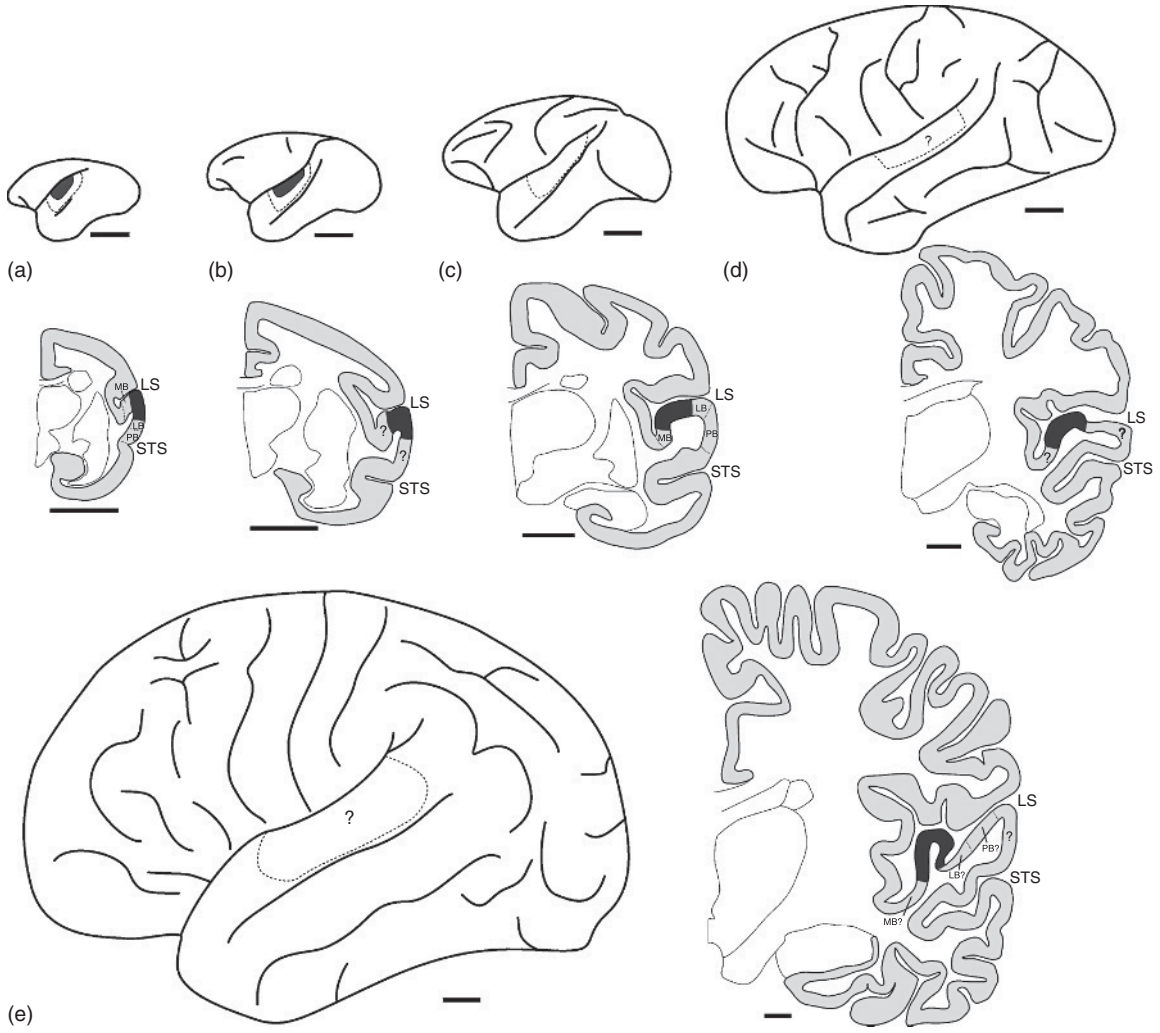

Figure 1 Schematic drawings of the cerebral cortex and location of auditory cortex in several primates. A lateral view of the left hemisphere and a coronal section through auditory cortex is illustrated in each panel. a, Marmoset monkey (*Callithrix jacchus jacchus*); b, squirrel monkey (*Saimiri sciureus*); c, macaque monkey (*Macaca mulatta*); d, chimpanzee (*Pan troglodytes*); e, Human (*Homo sapiens*). Dark shading, core region; MB, medial belt region; LB, lateral belt region; PB, parabelt region; ?, region not defined. Scale bars: coronal sections, 5mm; lateral views, 10mm.

auditory cortex covers most of the STG, but does not extend far onto the dorsal (upper) bank of the STS (de la Mothe *et al.*, 2006; Hackett *et al.*, 1998).

Classic and modern studies of the human temporal lobe have localized the auditory cortex to a region encompassing the posterior STG, transverse temporal gyri (TTG) of Heschl (HG), and the planum temporale (Hackett, 2002). This corresponds generally to areas 41, 42, 52, and 22 of Brodmann (1909). Compared to monkeys, however, the precise location and extent of auditory cortex in great apes and humans is much less certain, because the thalamic or cortical connections of individual areas cannot be determined experimentally. Therefore, areas must be identified from other anatomical or functional characteristics. This is problematic for several reasons. First, auditory areas cannot be distinguished from auditory-related or nonauditory fields purely on the basis of architectonic features (e.g., cytoarchitecture, myeloarchitecture, chemoarchitecture), as there is no distinctive set of traits that defines a cortical area as auditory. Second, functional imaging and noninvasive electrophysiological techniques currently lack either the spatial resolution or functional specificity to distinguish one area of auditory cortex from another. In general, auditory stimulation activates numerous auditory and auditory-related cortical areas within and beyond the superior temporal cortex (Hall *et al.*, 2003; Scott and Johnsrude, 2003). The sources of this activity are largely indistinguishable, because the 'physiological signatures' of the auditory areas involved are not known. These uncertainties highlight the need for studies directed at the

identification and characterization of auditory areas in the human brain. To some extent, this can be achieved through comparative studies involving nonhuman primates, for which the organization of the auditory cortex is more certain. As reviewed below, there is both direct and indirect evidence that major features of monkey auditory cortex organization are conserved in humans.

39.4 Organization of the Auditory Cortex of Monkeys

Models of auditory cortex organization in mammalian species other than humans are the subject of ongoing testing and refinement. Currently, most of this work is being accomplished in bats, cats, ferrets, mice, rats, New World monkeys, and Old World monkeys and apes. A common theme across models is that a central primary, or 'core region', containing one or more areas, is adjoined by a variable number of secondary areas comprising a 'belt region' (Figure 2). The core and belt regions are strongly interconnected, but the main source of thalamic inputs to the core areas is the MGv, whereas the MGd is the principal source of inputs to the belt areas. Species differ with respect to the number of areas present, their relative position and arrangement, connections (input/output), and tonotopic organization. For example, the number of areas identified ranges from 5 to 6 in rodents, 6 to 9 for cats and ferrets, 10 to 12 in monkeys, and over 30 in humans. Thus, there is a tendency to identify more auditory areas in larger brains. In nonhuman primates, the core–belt scheme has been extended to include a third region, known as the 'parabelt region' (de la Mothe *et al.*, 2006; Hackett *et al.*, 1998; Morel *et al.*, 1993; Morel and Kaas, 1992). The parabelt region receives thalamic inputs from

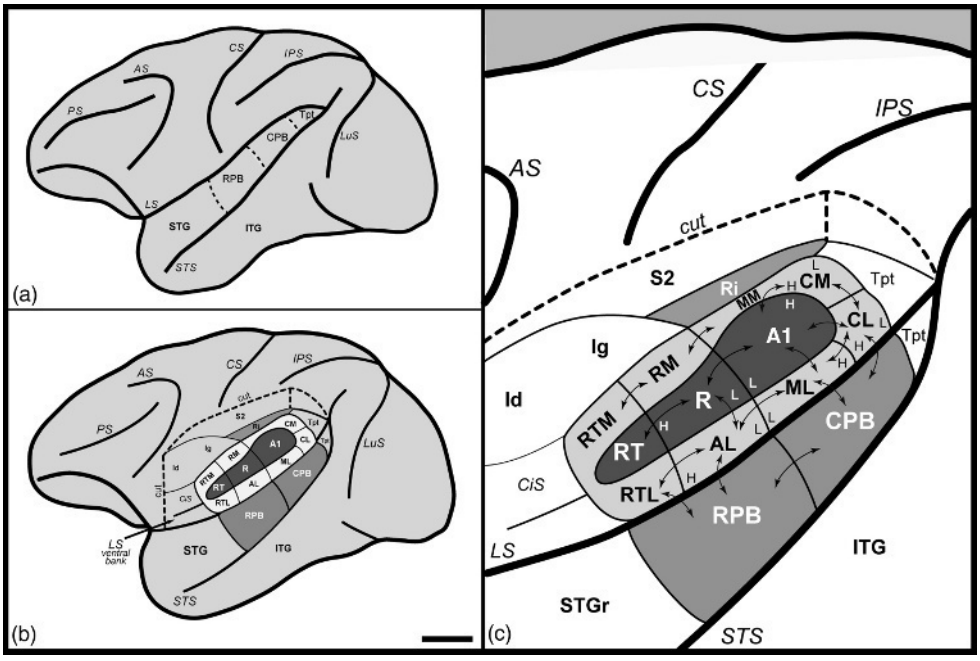

Figure 2 Schematic diagram of primate auditory cortex model illustrated for the macaque monkey. a, Lateral view of the left hemisphere showing locations of the rostral (RPB) and caudal (CPB) parabelt areas on the surface of the superior temporal gyrus (STG); b, the lateral sulcus of the left hemisphere was graphically opened (cut) to reveal the locations of auditory cortical areas on the lower bank of the lateral sulcus (LS); c, expanded view of auditory cortex, showing major connections between areas (arrows). The circular sulcus (CiS) has been flattened to show the rostromedial (RM) and rostrotemporal medial (RTM) areas that occupy its lateral wall. The upper bank of the LS was partly opened to show the locations of areas adjoining auditory cortex: the retroinsular area (Ri) in the fundus, second somatosensory area (S2) on the upper bank, granular insula (Ig), and dysgranular insula (Id). The three areas that comprise the core region of auditory cortex (dark shading) are located on the lower bank (A1, auditory area 1; R, rostral; RT, rostrotemporal). The core is surrounded by eight areas that belong to the belt region (light shading) (CM, caudomedial; CL, caudolateral; MM, middle medial; ML, middle lateral; RM, rostromedial; AL, anterolateral; RTM, rostrotemporal medial; RTL, rostrotemporal lateral). The core and belt regions are mostly contained within the LS. On the surface of the STG are the two areas that make up the parabelt region (medium shading) (RPB and CPB). The rostral part of the STG (STGr) extends to the temporal pole. The temporal parietotemporal (Tpt) area occupies the caudal end of the STG and extends onto the supratemporal plane within the LS. Tonotopic gradients within areas are indicated by H (high frequency) and L (low frequency). Other sulci and gyri shown include the arcuate sulcus (AS), central sulcus (CS), intraparietal sulcus (IPS), superior temporal sulcus (STS), lunate sulcus (LuS), and inferior temporal gyrus (ITG). Scale bar: 10 mm.

the MGd, but is distinguished from the belt region in that it does not receive direct inputs from the core (Figure 2c) (Hackett *et al.*, 1998). Information reaching the parabelt region from the core is mediated by connections with the belt region. Accordingly, a processing hierarchy (core–belt–parabelt) is formed across these three regions (Kaas and Hackett, 1998).

Each of the three major regions of the monkey auditory cortex contains two or more areas, or subdivisions (Figure 2). In our working model, there are three subdivisions of the core (Al, R, and RT), seven areas within the belt (CM, RM, RTM, CL, ML, AL, and RTL), and two divisions of the parabelt (RPB and CPB). Each subdivision receives inputs from lower and higher stages of processing, and subdivisions within a region appear to process inputs from multiple sources in parallel. Within the core, for example, each subdivision receives parallel inputs from the MGv (not illustrated), and has reciprocal connections with more than one belt area. In the belt region, all subdivisions receive inputs from the MGd and have reciprocal connections with one or more subdivisions of the core and parabelt. Thus, the connections between regions and areas within regions indicate that both serial and parallel processing is accomplished within the auditory cortical network (Kaas and Hackett, 1998; Kaas *et al.*, 1999; Rauschecker, 1998; Rauschecker *et al.*, 1997).

The establishment of individual subdivisions depends on the identification of unique subsets of anatomical and physiological features. Confidence in the delineation of an area is increased when its profile is based on multiple anatomical and physiological criteria; thus, some areas are defined better than others. The greatest differences are found between areas located in different regions (e.g., core vs. belt). By contrast, areas within a region share several key features, and since adjacent areas tend to share more features than nonadjacent areas, they are the most difficult to delineate. Within the core, for example, areas AI and R have similar architecture and connections. At present, the most reliable distinction between them is receptive field topography. The frequency organization of the cochlea is represented in both A1 and R, but the tonotopic gradients run in opposite directions from a common low-frequency border (see Figure 2) (Aitkin *et al.*, 1986; Brugge, 1982; Cheung *et al.*, 2001; Imig *et al.*, 1977; Kosaki *et al.*, 1997; Luethke *et al.*, 1989; Merzenich and Brugge, 1973; Morel *et al.*, 1993; Morel and Kaas, 1992; Rauschecker *et al.*, 1995, 1997; Recanzone *et al.*, 2000; Tian *et al.*, 2001). A reversal in the tonotopic gradient also distinguishes areas R and RT within the core (Bendor and Wang, 2005; Morel *et al.*, 1993; Morel and Kaas, 1992). Microelectrode recordings have also revealed tonotopic gradients in the belt region, supporting the existence of subdivisions that were previously distinguished on the basis of anatomical features alone. In these experiments, pure tones and narrowband noise stimuli were used to demonstrate complementary tonotopic gradients among the lateral belt areas (AL, ML, CL) (Kosaki *et al.*, 1997; Rauschecker *et al.*, 1995). In areas AL and ML, the gradients match the adjacent core areas (A1, R), while the tonotopic gradients in CM and CL mirror that of AI (Kajikawa *et al.*, 2005; Recanzone, 2000). Thus, an independent representation of the cochlea, or absence thereof, is an important criterion in the establishment of auditory cortical areas, especially when anatomical features are similar.

39.5 Organization of the Auditory Cortex of Great Apes and Humans

Compared to monkeys, much less is known about the organization of the auditory cortex in the great apes and humans. Due to the absence of information about connections and near-field electrophysiology, extending findings from monkeys to these primates is primarily limited to descriptions of architectonic features and noninvasive neurophysiology. Detailed parcelations have been produced by multiple investigators spanning about 100 years (Hackett, 2002). Most of these were derived from the analyses of cytoarchitecture and/or myeloarchitecture (Beck, 1928, 1929; Brodmann, 1909; Campbell, 1905; Flechsig, 1920; Galaburda and Sanides, 1980; Hopf, 1954; Morosan *et al.*, 2001; Pandya and Sanides, 1973; Poljak, 1932; Rademacher *et al.*, 1993; Seldon, 1981a, 1981b, 1982; Vogt and Vogt, 1919; von Economo and Horn, 1930; von Economo and Koskinas, 1925). The most recent studies also used the distribution of various markers (e.g., acetylcholinesterase, cytochrome oxidase, parvalbumin, receptor autoradiography) to identify or characterize auditory-related cortical fields (Clarke and Rivier, 1998; Hackett *et al.*, 2001; Hutsler and Gazzaniga, 1996; Morosan *et al.*, 2005; Nakahara *et al.*, 2000; Ong and Garey, 1991; Rivier and Clarke, 1997; Sweet *et al.*, 2005; Wallace *et al.*, 2002). Despite substantial variations in conclusions and nomenclature across studies, a common finding has been the identification of a central core region with primary, or primary-like, architectonic features surrounded by belts of several nonprimary

fields (Figure 3). The correspondence between the core region of monkeys, apes, and humans is rather certain, whereas the homology of areas located beyond the core is not.

In monkeys, the core region is elongated along the anterior–posterior axis of the temporal lobe. In apes and humans, the core is mainly confined to the posteromedial two-thirds of the TTG, which is oriented from posteromedial to anterolateral across the superior temporal plane (Figure 3; Hackett, 2002; Hackett *et al.*, 2001). This region most closely corresponds to area 41 of Brodmann (1908, 1909), as well as to comparable territory identified by other investigators (Table 1). The distinctive cytoarchitecture of the core is commonly referred to as 'granulous' or 'koniocellular', named for the dense concentration of small cells in layers II and IV. Other cytoarchitectonic features include a conspicuous absence of large pyramidal cells in layer III, and a relatively sparse population of pyramidal cells in layer V. Myelin density is higher in the core, compared to most of the surrounding fields. The myelination pattern of the core is characterized by a matrix of small to large caliber fibers of such high density that the inner and outer striae of Baillarger in layers IV and Vb are difficult to resolve (Hackett *et al.*, 2001; Hopf, 1954; Pandya and Sanides, 1973; Sanides, 1972). Other studies have added that the expression of the acetylcholinesterase, cytochrome oxidase, and parvalbumin is greater in layers IIIc and IV of the core than in the belt or parabelt regions (Hackett *et al.*, 1998, 2001; Jones *et al.*, 1995; Kosaki *et al.*, 1997; Morel *et al.*, 1993; Morel and Kaas, 1992; Nakahara *et al.*, 2000; Rivier and Clarke, 1997; Sweet *et al.*, 2005; Wallace *et al.*, 2002). The consistency of this architectonic profile is such that the core can be easily identified in monkeys, apes, and humans. Thus, there is significant conservation of these features among these primates.

Despite the architectonic similarities, there is greater variability in the gross morphological features of the superior temporal cortex of apes and humans, compared to monkeys. In chimpanzees and humans, the number of TTG varies between individuals and sometimes between hemispheres (Hackett *et al.*, 2001; Leonard *et al.*, 1998; Rademacher *et al.*, 1993). The position of the core region varies relative to sulcal and gyral landmarks; therefore, precise localization depends on the detailed architectonic analyses of individual specimens. In humans, the most common configurations are a single or paired TTG, also referred to as a posterior duplication or bifid HG. In humans with a single HG, the core occupies most of the gyrus and usually does not extend beyond its anterior and posterior sulcal boundaries. When the HG is divided by an intermediate transverse sulcus (i.e., bifid HG), the core occupies portions of both gyri and spans the intermediate sulcus. Most chimpanzees have a single HG, but some lack a definitive HG. In these cases, the core is situated deep in the LS and elongated along the medial edge of the superior temporal plane. In chimpanzees with a prominent HG, the orientation and appearance of the core is more similar to that found in humans (Hackett *et al.*, 2001). The core in these cases is confined to the HG. These

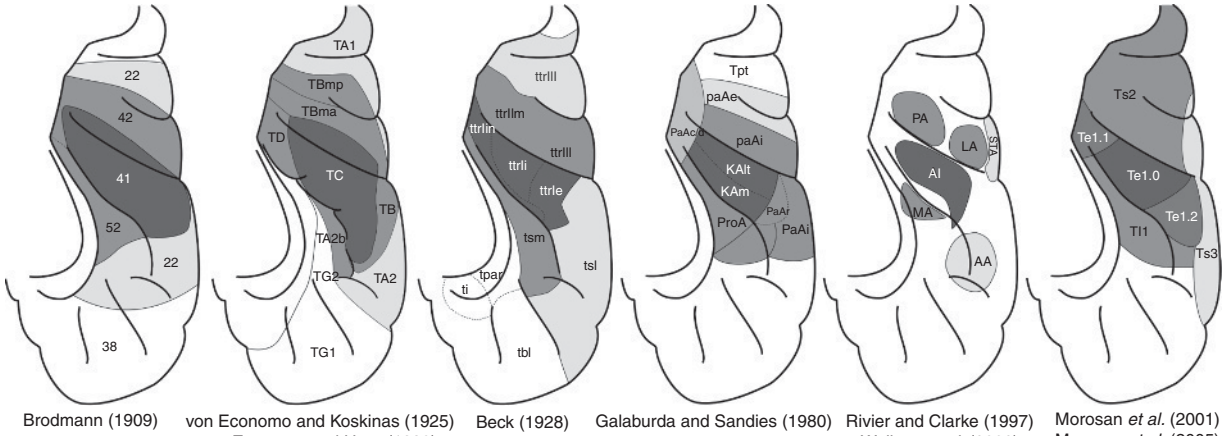

Figure 3 Parcelations of the human superior temporal cortex by different investigators. For each panel, the locations of major auditory cortical regions are drawn on a standardized schematic of the superior temporal plane. The STG is not visible. Dark shading, core region; medium shading, belt region; light shading, parabelt and possibly other regions. Posterior is up, lateral is right. Modified from Hackett, T. 2002. The comparative anatomy of the primate auditory cortex. In: Primate Audition: Behavior and Neurobiology (*ed.* A. Ghazanfar). CRC Press with permission from Taylor & Francis Group LLC.

Table 1 Proposed homologous auditory cortical regions in great apes and humans, with reference to the model of auditory cortex established in monkeys. For each study cited, the species is listed, along with the corresponding region or areas, and the anatomical methods used to identify the region. Note that the size and location of areas varies widely between species and corresponding regions are approximate

Study	*Species*	*Core*	*Medial belt*	*Lateral belt*	*Parabelt*	*Methods*
Bailey *et al.* (1950)	Chimpanzee	TC	TC	TB	TA	C
Bailey and von Bonin (1951)	Human	TC, koniosus supratemporalis	TB, 42	TB, 42	TA, 22	C
Beck (1928, 1929)	Human, chimpanzee	Ttrli/e	Tsm	TtrII	ND	M
Braak (1978)	Human	Temporalis granulosa	Temporalis progranulosa	Temporalis paragranulosa	Temoralis magnopyramidalis	L, C
Brodmann (1908, 1909)	Human	41	52	42	22	C
Campbell (1905)	Human, chimpanzee, orangutan	Auditosensory	ND	ND	Auditopsychic	C, M
Von Economo and Koskinas (1925)	Human	TC, supratemporalis granulosa	TB, TD	TB	TA_1, TA_2?	C
Von Economo and Horn (1930)	Human	TC, supratemporalis granulosa	TD, (TA_2a)	TBma	TA_1, TA_2?	C
Flechsig (1876)	Human	7	14?	14?	14	M
Flechsig (1920)	Human	10	18?	19?	19?	M
Galaburda and Sanides (1980)	Human	Kam, kalt	ProA, PaAc/d	PaAi	PaAe	C, M
Hackett *et al.* (2001)	Human, chimpanzee, macaque	Core	Medial belt	Lateral belt	ND	C, M, A
Hopf (1954)	Human	Ttr1	Tsep	Ttr2	Tpart, Tmag	M
Mauss (1911)	Orangutan, gibbon	40				M
Morosan *et al.* (2001, 2005)	Human	Te1.0	TI1	Te2	Te3	C; RA
Nakahara *et al.* (2000)	Human	Zone 1	Zone 2	Zone 2	ND	PV, C
Ong and Garey (1990)	Human	41	ND	42	22	C, G, M
Rademacher *et al.* (1993)	Human	41	ND	ND	ND	C
Rivier and Clarke (1997)	Human	AI	MA	LA, PA	PA, STA	A, CO, N
Smith (1907)	Human	No. 27	21, postcentral insular	26, temporalis superior	26, temporalis superior	G
Sweet *et al.* (2005)	Human, macaque	Core	ND	Lateral belt	Internal and external parabelt	C, A, PV
Vogt and Vogt (1919)	Human, macaque	41, temporalis transversa interna	ND	42, temporalis transversa externa	22aB	M, C
Wallace *et al.* (2002)	Human	AI, LP?	MA, AA?	PA, LA, ALA	STA	C, A, M, CO, PV, N

A, acetylcholinesterase; C, cytoarchitecture; CO, cytochrome oxidase; G, Golgi; L, lipofuscin (pigment architecture); M, myeloarchitecture; NADPH, NADPH-diaphorase; PV, parvalbumin; RA, receptor autoradiography.

findings highlight the variable relationship of the core region to the surface landmarks. The anatomical variability is more problematic for functional studies in which delineation of the core and belt regions is important for interpretation of experimental results. Functional imaging and electrophysiological approaches have been broadly used to study activity in auditory cortex (for reviews, see Hall *et al.*, 2003; Scott and Johnsrude, 2003). However, since auditory stimulation tends to activate core and belt regions, it has been difficult to precisely dissociate their respective contributions, especially given the anatomical variability between subjects (Seifritz *et al.*, 2002; Lehmann *et al.*, 2006).

Flanking the core region on the anteromedial and posterolateral sides of the TTG are two distinct regions that are likely to comprise part of the auditory cortex of humans and apes. The anterior region, interposed between the core and circular sulcus of the insula, is a region with distinctive architecture that most closely corresponds to the medial belt region of monkeys. Although variably named by different investigators (e.g., area 52 of Brodmann) (Figure 3), the various architectonic descriptions of this region are remarkably similar. The planum temporale (PT) occupies the superior temporal plane posterolateral to the TTG. Aside from the TTG, the expansion of the PT is perhaps the clearest differences in the gross morphology of the superior temporal lobe among primates. Compared to monkeys, the PT and STG appear to be greatly expanded in apes and humans. Accurately accounting for this expansion poses a significant challenge, because comparative studies are lacking. With respect to the PT, most descriptions have noted significant architectonic heterogeneity, consistent with the presence of at least two subdivisions in apes and humans (Figure 3). Brodmann (1908, 1909) identified area 42 and part of area 22 on the human PT, and while details vary between investigators, subsequent reports have generally not departed greatly from this interpretation (Figure 3, Table 1).

The rotation of the core in apes and humans suggests that the PT may contain parts of the core and parabelt regions of monkeys (Figure 3). Anatomical support for this hypothesis includes evidence that belt areas corresponding to CM and CL cap the core region posteromedially in both chimpanzees and humans (Hackett *et al.*, 2001). Most recently, areas possibly corresponding to the lateral belt and parabelt of monkeys were identified in the human PT adjacent to the core on the TTG (Sweet *et al.*, 2005). These assignments were made on the basis of position, relative to the core region, and architectonic similarities between species. While further comparative studies are needed to validate and extend these findings, it follows that a significant portion of the STG (area 22) would have no clear homologue among monkeys, apes, and humans. That is, if the PT contains most of the lateral belt and parabelt regions identified in monkeys, then most of the STG of apes and humans is comprised of cortex with no clear homologue in monkeys. Since there have been no comparative studies of the STG among these primates, correspondence remains an open question.

39.6 Conclusions and Directions for Future Research

Anatomical and functional studies suggest that certain elements of the monkey model of auditory cortex are directly applicable to apes and humans. The homology of the core region is the most well established at present, and homologies among the medial and lateral belt regions appear likely. Undoubtedly, further comparative studies will be needed to identify other similarities and differences in the organization of auditory cortex across taxonomic groups. For architectonic studies, progress will depend on the establishment of anatomical profiles to distinguish cortical fields. Functional studies may help to validate the anatomical predictions through the discovery of common physiological features, such as tonotopic organization. While it is not expected that the anatomical and functional features will be identical among areas identified as homologous, it is likely that many corresponding areas will be revealed, with the possibility that additional areas have been added in apes and humans. The differences in auditory cortex organization are expected to contribute to well-known differences in the perception and production of speech and music among primates, and may also reveal clues about the evolution of these abilities.

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40 The Evolution of Language Systems in the Human Brain

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Glossary

acheulean The stone tool technology associated with the hominid species *Homo erectus*.

akinetic mutism Immobility and nonresponsiveness due to dorsal frontal midline cortical damage involving the anterior cingulated cortex, that includes verbal nonresponsiveness.

allometry The nonisometric scaling of anatomical structures with growth and comparative size.

arcuate fasciculus The fiber tract extending from the temporal and inferior parietal lobes to the inferior frontal lobes on the human brain passing beneath the supramarginal gyrus somatic and motor areas just dorsal to the Sylvian fissure.

Baldwin effect A theoretical evolutionary mechanism proposed independently by James Mark Baldwin, Conwy Lloyd Morgan, and Henry Osborne in 1896 arguing that physiological and behavioral plasticity could shield a lineage from elimination by natural selection long enough for new variations to accumulate (e.g., by chance mutation) that could supplement or replace the plastically acquired adaptatation.

Broca's aphasia (area) The language disorder first described by Paul Broca in 1861 and elaborated in 1865 that was produced by damage involving the posterior part of the inferior third frontal convolution on the left side of the brain (commonly referred to as Broca's speech area). This aphasia syndrome is typically associated with nonfluent, telegraphic speech and often agrammatism (a difficulty constructing or assessing syntactic structures).

co evolution An evolutionary dynamic that is a consequence of two interacting selection processes, typically occurring between species whose survival is linked or between organizational levels of the biological hierarchy. In this context, it is

used to describe the complex interactions that have likely characterized the evolution of the human brain and the evolution like processes of language transmission and change which may have imposed novel selection pressures on human brain evolution as an artificial niche.

diffusion tensor weighted MRI — A structural MRI technique that uses oriented diffusion processes (constrained by fiber tract orientation) to visualize three dimensional organization of major axonal pathways.

FOXP2 — A highly conserved transcription factor gene of the fork head family of genes that is associated with an inherited disorder of speech articulation and syntactic regularization.

generativity (generative grammar) — The capacity for indefinite novelty of combinatorial uses of words provided by the grammatical and syntactic apparatus of a language. Generative grammars are theoretical rule governed grammars that by recursive application of these rules enable generativity of sentential forms.

genetic drift — The random mixing, accumulation of genetic variants, and elimination of alleles due to the relaxation of the effects of natural selection or the greater effect of probabilistic factors in small breeding populations.

Homo erectus — A long persisting fossil precursor species to modern humans (from approximately 1.6 Mya to 350 kya, depending on which specimens are included) with roughly modern stature and postcranial skeletal structure, and a brain size average in the range of 950 cm^3 (at the very low end of the modern range). *H. erectus* is found in Africa and also throughout Eurasia, extending into Europe, central Asia, China, and Indonesia.

Homo habilis — One of the two earliest identified fossil members of our genus *Homo* dating to approximately 1.8 Mya in northeast Africa and associated with early stone tools. This species is characterized by reduced dentition and a slightly larger brain case in comparison to earlier australopithecine hominids.

Homo rudolfensis — One of the two earliest identified fossil members of our genus *Homo* dating to approximately 1.8 Mya in northeast Africa and associated with early stone tools. This species is characterized by a significantly larger brain case but minimally reduced dendition in comparison to earlier australopithecine hominids.

hopeful monster — A hypothetical significantly deviant member of a species produced by a mutation that radically alters some phenotypic character which just happens to be better suited to the current environment and thereby manages to outreproduce and eventually replace the more typical phenotypes of the population. This theory was championed by Goldschmidt in the early 1900s and is often tacitly assumed by proponents of a saltational origin of language abilities.

index (and indexicality) — The mode of reference that works by virtue of correlational relationships, as in pointings, symptoms, samples, and simple learned associations.

Lamarckian inheritance — The mode of trait inheritance proposed by Jean Baptiste de Lamarck in the early 1800s in which physical and behavioral traits acquired by effort, exercise, or exposure to demanding conditions were presumed to be passed directly to offspring during reproduction.

Mousterian — The stone tool industry associated with Neanderthals and early modern humans prior to the upper Paleolithic period beginning somewhere between about 60 and 75 kya.

niche construction — The effect that a species has in altering its immediate environment so that the influences of natural selection are significantly affected by this modification, as in the way that beaver dam construction has played a significant role in providing selection favoring the evolution of aquatic adaptations.

symbol (symbolic) — The mode of reference that picks out objects of reference by virtue of a system of sign sign relationships (correlational relationships,

	as in pointings, symptoms, samples, and simple learned associations).
universal grammar (UG)	The hypothetical common core of grammatical rules that all human languages share. The theory was championed by the linguist Noam Chomsky and is argued by many linguists to be the innate endowment of all humans from which the specific grammars of existing languages are derived.
upper Paleolithic	An archeologically delineated period of human prehistory beginning roughly between 60 and 75 kya (most notably in Europe, but with more ancient precursors appearing in Africa) in which stone tool technologies begin to exhibit significant regional varieties and the first unambiguous representational forms (i.e., carvings and cave paintings) appear.
Wernicke's aphasia (area)	The language disorder first described by Wernicke (1874) that is produced by damage involving the posterior part of the superior temporal lobe on the left side of the brain (commonly referred to as Wernicke's speech area). This aphasia syndrome is typically associated with fluent speech that includes inappropriate, phonologically deviant, and/or semantically deviant word choice, and typically a deficit in comprehending sentence meaning and in naming objects.

40.1 Introduction: Human Neural Language Adaptations

40.1.1 Language Uniqueness and Nonhuman Communication

The comparative uniqueness of language is probably its most important and troubling feature. Besides being vastly more complex, language is substantially different in referential function, behavioral organization, and neural control than any other known animal communication system. Although a number of features are shared in common with the communication systems of some species, for example, vocal–auditory medium, social transmission, its most distinguishing characteristics – symbolic reference, grammar, open-ended generativity, and combinatorial patterning – are unprecedented. The lack of clear behavioral homologies in other species renders the comparative method problematic. There are no other species with various grades of language to provide clues about the contexts that support language evolution (though there are species that exhibit the ability to acquire aspects of language; see below), or the range of brain systems that can be involved. There is only one exemplar, *Homo sapiens*, and if there were intermediate levels of these abilities in our ancestry they have all been eliminated. This noncomparability is made all the more enigmatic when we consider the apparent absence of neurological dishomologies with respect to major neuroanatomical structures that might be expected to correlate with such a significant cognitive-behavioral discontinuity. Generations of comparative neuroanatomists have failed to identify even one major novel brain structure in humans. This suggests that our special adaptations for language are the result of using previously evolved primate brain structures in new ways and in new combinations.

40.1.2 Linguistic Context of Language Adaptation

Because of this unusual status of language, it has long been regarded as one of the defining features of human distinctiveness. Historical efforts to explain its origins have consequently been confounded with efforts to define the essence of humanness. This tendency is well exemplified by linguistic debates about the origin and basis for language. Under the influence of persuasive arguments by the linguist Chomsky (1972) and the psychologist Lenneberg (1967), it became popular to argue that language depended on elaborate innate capabilities unique to humans. Chomsky has been particularly influential in articulating what this might entail. At the center of this theory is the claim that all humans have inherited a common innate universal grammar (UG). This innate faculty is presumed to make the acquisition of language possible even at a stage in the life when other forms of learning are undeveloped, and makes effortless the unconscious deployment of a vast set of syntactic rules. These rules are thought to underlie the real-time capacity to interpret or generate a nearly infinite number of grammatical sentences (generativity). Despite the fact that Chomsky, and other colleagues, locate this language capacity in the brain, he maintains the view that it cannot be explained as an adaptation, whereas other linguists (e.g., Jackendoff, 1994; Pinker, 1994) argue that it is an evolved adaptation (see Section 40.5). The formal tools developed by generative linguists over the past four decades have

provided unparalleled rigor for the analysis of morphology and sentence structure; however, despite their theoretical commitment to an inherited biological substrate for linguistic capacities, these methods have yielded relatively little in the way of verified neurological predictions, and it is also not clear that they could be substantiated or falsified by brain research. More than a generation has elapsed since this view achieved ascendancy; neither a discrete neural locus for grammatical processes or a neurological lesion that selectively disrupts core features of UG nor a genetic defect that produces systematically divergent forms of grammar have been identified (though neural and genetic impairments of certain features of morphological or syntactic processing have been identified; see below). One major reason for this may be the difficulty of translating highly abstract linguistic formalisms into concrete anatomical predictions. At least superficially, language appears to be generated according to symbolic principles that are very different from the phylogenetic and epigenetic principles that determine functional organization within brains. Nevertheless, linguistic lists of the necessary and sufficient capacities for language remain highly influential, and linguists have been the staunchest proponents of a radical discontinuity between humans and other animals with respect to language.

40.1.3 Animal Exceptions and the Significance of Animal Language Experiments

Efforts to identify analogues to human language features in nonhuman species' naturalistic communication have demonstrated only limited behavioral and functional overlap. The most influential examples include the vocal learning of parrots and songbirds, the socially transmitted songs of humpback whales, and the referential alarm calls of numerous species, but most notably vervet monkeys (see Section 40.3). Vocal learning is deemed significant because the vast majority of terrestrial mammals to not exhibit any significant capability to learn or mimic noninnate species-typical vocalizations. The examples of complex socially transmitted vocalizations in many bird lineages and in humpback whale pods thus exhibit a deviation from the norm that parallels a key characteristic of language. The referential function of alarm calls to pick out distinctive classes of predators has been demonstrated in primates, birds, and even rodents. Classic theories of animal calls had caricatured them as merely extrinsic symptoms of emotional states, so demonstrations of specific extrinsic reference linked to specific innate calls also suggested parallels with the ubiquitous referential function of words and sentences. However, these superficial similarities are to be contrasted with many unprecedented language features.

Despite a failure to demonstrate any naturally occurring language-like systems outside of humans, partially successful efforts to train nonhuman species to perform certain limited language tasks have helped focus attention on the specific cognitive differences that separate them from humans. Studies of ape, dolphin, and parrot abilities to acquire language-like systems tailored for their different propensities and sensory-motor capacities have variously demonstrated the simple use of symbolic reference and a very basic understanding of syntactic operations, even if not anywhere near the level of interpretive and generative competence observed in a 3-year-old human child. Significantly, these three animal groups represent considerably different brain structures, since dolphin and especially bird brains (see below; and see Do Birds and Reptiles Possess Homologues of Mammalian Visual, Somatosensory, and Motor Cortices?, The Evolution of Vocal Learning Systems in Birds and Humans) are organized quite differently than are human brains. One possible implication is that at least rudimentary language-like capacities are not dependent on primate (or even mammalian) brain architecture, and so may be achievable in diverse ways.

40.1.4 Gestural Language: Neural Correlates and Evolutionary Scenarios

A counterpart to this diversity of potential neural substrates for language-like behaviors is demonstrated by the modality independence of language in humans. The manual languages that have developed in numerous deaf communities throughout the world show that fully complex languages can be acquired independent of the aural–vocal modality. However, despite this significant modality difference, there is also considerable overlap in neural representation with spoken language (Neville *et al.*, 1997). This may indicate that the critical neural adaptations supporting language are not, as is often suggested, merely specializations in motor or auditory processing, though these are also likely. Many scenarios for language origins have suggested that manual or gestural language preceded spoken language in evolution (see, e.g., Hewes, 1973; Corballis, 2002). Neurological support for this view comes from the absence of voluntary articulate control of laryngeal musculature in most terrestrial

mammals that have been studied, including all great apes (anatomy discussed below). Humans are the sole exception. It is therefore likely that the common ancestor of humans and chimpanzees lacked this control and that articulate vocal control is a derived trait that arose at some point in hominid evolution, possibly after the emergence of the genus *Homo*. Although most face-to-face speech is accompanied by gesture for emphasis and indexicality, language develops almost exclusively in the vocal channel, not manually, if speech and hearing are possible. Also, the locations of the major cortical systems critical for language processing are similar in both speakers and deaf signers. So support for a separate prior specialization of the brain for gestural language is weak. More likely, spoken and gestured symbolic communication were employed in linked fashion for a significant part of the evolution of language, with vocal capacities lagging behind but eventually becoming the more prominent modality.

40.2 Human Neuronatomical Features Associated with Language

40.2.1 Gross Neuroanatomical Homologies

Generations of comparative neuroanatomists have explored the possibility that human brains contain species-unique large-scale structures (e.g., distinct nuclei, cortical areas, fiber tracts) that might correlate with our species-unique form of communication. However, since the famous nineteenth century debate between Thomas Huxley and Richard Owen in which Huxley disproved the existence of a uniquely human hippocampal structure, there has been widespread confirmation of the extensive homologies linking human and great ape brains and no verified claims of any phyletically unprecedented macroscopic structure in the human brain. Nevertheless, claims of the evolutional functional divergence of brain structures are widespread in the literature. Two cortical regions have been consistently implicated in these claims: Broca's region in the inferior frontal lobes and the angular gyrus region at the temporal-parietal–occipital junction. The uniqueness of speech has led to hypotheses that Broca's region is uniquely developed in human brains, and the supramodal nature of semantic associations has led to hypotheses that a cross-modal association area is uniquely developed in the region of the angular gyrus. The hypothesis that Broca's region was uniquely developed in the hominid lineage led to investigations of fossil skull endocasts (see critical discussion below) and reports that the distinguishing sulci of this region could first be detected on an endocast of a *Homo rudolfensis* specimen, KMNER 1470 (then identified as *Homo habilis*) (Falk, 1983; Tobias, 1987). Phyletic novelty of this structure has since been cast in doubt by the evidence of both cytoarchitectonic and connectional homologies of this region with corresponding regions in ape and even monkey brains (Deacon, 1992b; see Section 40.2.3). The hypothesis that the human angular gyrus region is an unprecedented cross-modal association area critical for language was first articulated by the neurologist Geschwind (1964). Early claims of poor cross-modal transfer of information in monkeys were subsequently disproven (Wegener, 1965; Blakeslee and Gunter, 1966), and subsequent studies have since identified polymodal function in homologous cortical regions as well as other inferior parietal and middle temporal areas (e.g., Ettlinger and Wilson, 1990). In response to this failure to find unprecedented brain structures relevant to human language facility, most attention has turned to quantitative, connectional, and peripheral dishomologies that may be relevant.

40.2.2 Allometric Deviations Potentially Associated with Language Adaptation

That the human brain has been subject to quantitative deviation from ape brain proportions is indisputable. Human brains are both absolutely and comparatively larger than expected for an anthropoid primate or even a great ape. For this reason, much attention has been focused on the plausible link between this deviation in brain proportions and the deviant features of human language. Both brain/body proportions and the internal scaling of brain structures with respect to each other and the gross brain size are highly correlated (Sacher, 1970; Gould, 1975; Finlay and Darlington, 1995), but most brain structures do not scale up or down isometrically with respect to total brain size across species. Allometric scaling patterns are also exhibited at every level of brain structure. For example, larger brains tend to have higher proportions of telencephalon to diencephalon, more neocortex to limbic cortex, more eulaminate cortex to specialized agranular and sensory koniocortex, more white matter to gray matter, more glia per neurons, and so on.

Generally, it is argued (on theoretical, not empirical grounds) that structural proportions that are predictable from allometric scaling (e.g., with respect to the trend exhibited by large interspecific sample as a background) indicate nondeviant function as well. Consequently, interest has mostly focused on quantitative findings of allometric deviation of human brain

structures with respect to apes or to anthropoid primates in general (e.g., Stephan, 1969; Stephan *et al.*, 1981). These investigations are complicated by disparities of results obtained using different statistical approaches, different methods of structural measurement, and disagreements about the significance of deviations that these analyses suggest. At present, there is no agreed upon theoretical basis (and limited empirical data) for predicting the functional correlates of either the allometric or deviant changes in relative proportions of brain regions. Comparative studies showing quantitative structural correlations with peripheral specialization offer the most useful comparisons. Examples of regional enlargements with respect to manipulative forelimbs (e.g., large forelimb tactile representation in primates and raccoons), elaborated or degenerate sensory organs (e.g., specialized tactile representation in the star-nose mole or elimination of visual cortical responses in the blind mole rat, respectively), or highly modified and hypertrophied organs (e.g., cerebellum in electric fish) offer support to the phrenological null hypothesis that increase in relative size equals functional augmentation as well. Perhaps those most relevant to the cognitive-behavioral specialization of language are the size correlations between song complexity in songbirds and the relative sizes of forebrain nuclei involved in singing (e.g., DeVoogd *et al.*, 1993). It is likely, however, that there are other possible correlates of allometric deviation that have yet to be explored (other alternative possibilities are explored in Deacon, 1990b).

Despite this uncertainty about the significance of allometric scaling and deviations in brain structure proportion, there has long been an interest in searching for possible correlations between allometric deviations of human brain structure and language. Different studies have, for example, provided analyses that suggest that human brains have divergent enlargement of cerebral and cerebellar cortices, prefrontal cortex, and certain thalamic nuclei, and divergent reductions of primary visual cortex, primary motor cortex, and olfactory bulbs. Similarly, quantitative studies have found hemispheric asymmetries in language-related areas of cortex, though such asymmetries have also been reported for nonhuman apes. Many of these findings must be considered preliminary, however, since most have been contradicted by studies using different methods that have come to different conclusions. There is also a considerable variation in the size of cytoarchitectonically identified language areas to contend with (e.g., Amunts *et al.*, 1999). One illustrative example of a quantitative dispute with implications for language concerns the allometric predicatability or deviation of the human prefrontal cortex. Studies based on histological analyses of the cytoarchitectonic distinction between granular prefrontal and agranular premotor cortex have reported that human prefrontal cortex is allometrically larger than predicted with respect to other anthropoids (e.g., Deacon, 1997). In contrast, MRI-based studies using major sulci and fissures as morphological markers to discern frontal from parietal and temporal cortex suggest no deviation (Semendeferi *et al.*, 1997). Claims of disconfirmation of one or the other result are, however, clouded by the use of these different anatomical methods, different definitions of frontal and prefrontal cortex, comparison of nonhomologous structures, including different primate species as a comparison set, and employing different statistical tests for deviance (Deacon, 1997, 2004).

Despite these unresolved methodological issues, most studies have concluded that human brains deviate from allometric predictions in a number of internal relationships that might be relevant for language. Probably the most consistently reported finding is that human cerebral cortex is larger than allometrically expected with respect to the two major forebrain nuclear complexes that are most intimately related to it: the thalamus and the basal ganglia (Deacon, 1988, 1990a; Dunbar, 1993; Rilling and Insel, 1999). Additionally, there is evidence for allometric deviation of kiniocortex and agranular cortex to eulaminate cortex (Deacon, 1990a), visual cortex (e.g., Holloway, 1979), temporal lobe morphology (Rilling and Seligman, 2002), and prefrontal cortex (Deacon, 1988, 1997; Rilling and Insel, 1999). It is hard to believe that significant deviations in these major forebrain relationships would not have an impact on language. For example, Deacon (1997) argues that this disproportion may have aided invasion of cortical efferents into brainstem vocalization nuclei (see below), as well as biasing developmental competition among cortical afferents affecting parcelation of functional cortical areas. Finally, difficulties of discerning comparable boundaries of cortical areas across species of widely differing sizes have made more fine-grained allometric studies of the scaling of individual cortical areas even more problematic.

Efforts to link allometric deviations of some of these structures with language adaptation have mostly focused on two findings, the expansion of prefrontal cortex (e.g., Aboitiz and Garcia, 1997; Deacon, 1997) and quantitative deviations and asymmetries of Broca's and Wernicke's language areas compared to their homologues in chimpazees

(e.g., Gannon *et al*., 1998). For example, prefrontal expansion may provide working memory support for symbol learning, visual cortex reduction may reflect parietal cortex expansion and an augmentation of cross-modal cognition, and asymmetries of language cortex may provide the substrate for hemispheric specialization for language.

40.2.3 Connectional Homologies and Dishomologies Relevant to Language

Neither the lack of novel human brain structures nor the uncertainties about the existence or relevance of allometric deviations precludes the possibility of evolutionary changes in neural circuitry. Unfortunately, methods used to accurately trace axonal connections between brain structures require lethal experiments and so are only available for study of nonhuman species, and indeed are not even applicable to apes because of their endangered status and the ethical issues involved. Thus, information concerning human neural connections is mostly lacking and must be extrapolated from nonhuman data. However, indirect evidence from human functional differences, clinical studies, and *in vivo* imaging can be compared to connectional data derived from nonhuman primates to support a handful of fairly robust connectional claims.

Probably the most robust behavioral distinction between the vocal abilities of humans and other primates (and in general with respect to all terrestrial mammals) is the ability for humans to produce a wide range of vocal sounds that can be freely organized into diverse combinations. In addition, we also have an unprecedented ability (for a terrestrial mammal) to mimic vocal sound combinations that we hear others produce. In contrast, the vast majority of terrestrial mammals, including primates, has relatively fixed vocal repertoires, for which sound mimicry learning plays almost no role. Associated with our lack of constraint on productive sound combination, we experience a relative freedom from specific correlations between vocalizations, emotional states, and stereotypic referential contexts, unlike what is characteristic of the other primates (Deacon, 1997). This is a critical requirement for language, as it allows socially transmitted patterns of sound production (e.g., words) to be learned in association with any given reference. Though humans still exhibit a small repertoire of innate stereotypic species-specific vocal 'calls' such as laughter and sobbing, which do have fixed structure and are associated with highly constrained emotional contexts, this call repertoire is both small compared to that in chimpanzees and atypical in form and context (Deacon, 1997; Provine, 2000).

These differences are probably in part attributable to a change in the central innervation of the laryngeal control nucleus of the brainstem, the nucleus ambiguus. Tracer studies in nonhuman primates have demonstrated that this nucleus is almost entirely innervated by subcortical structures from midbrain and adjacent brainstem regions (Jürgens *et al*., 1982). This is an expected pattern given that the nucleus ambiguus is a visceral motor nucleus that is segregated from significant influence from volitional systems in order to provide reliable automatic responses for a system that is associated with life-and-death consequences. Though electrical stimulation of ventral motor cortex regions in the macaque monkey brain can result in vocal muscle movement, there is little evidence that this is mediated by a direct projection. In addition, bilateral ventral frontal motor cortex damage in monkeys does not appear to block their ability to vocalize. In contrast, unilateral damage to left inferior motor cortex in humans (even just in the left hemisphere) can produce significantly impaired vocal ability, and even mutism. This clinical evidence is supported by experimental studies that also suggest that the human nucleus ambiguus is directly innervated from motor cortex (Jürgens *et al*., 1982). Taken together, this makes it likely that this connection constitutes a uniquely human feature, by virtue of which precise control of pitch and vocal timing is achieved in speech and song. This would also explain the remarkable coordination of vocalization with the other cortically controlled tongue, jaw, and facial muscles that are necessary for articulate speech. In this regard, humans have dual control of vocalization, as is exhibited in the tendency for speech to be interrupted with impulses to laugh or sob in response to intense emotional states (Provine 2000; see Figure 1).

The left inferior frontal cortical region that likely includes Broca's speech area has been subject to conflicting claims concerning (1) which components of this region are responsible for the deficits associated with Broca's aphasia, (2) how they are functionally connected with other cortical and subcortical regions also associated with language processing, (3) whether the cortical area itself or its connections with other areas are more important for its language role, and (4) whether or not its connectivity with other structures is typical of other primate brains. The clinical literature is even still split on what Brodmann's areas (Brodmann, 1909) are the substrates for Broca's area language

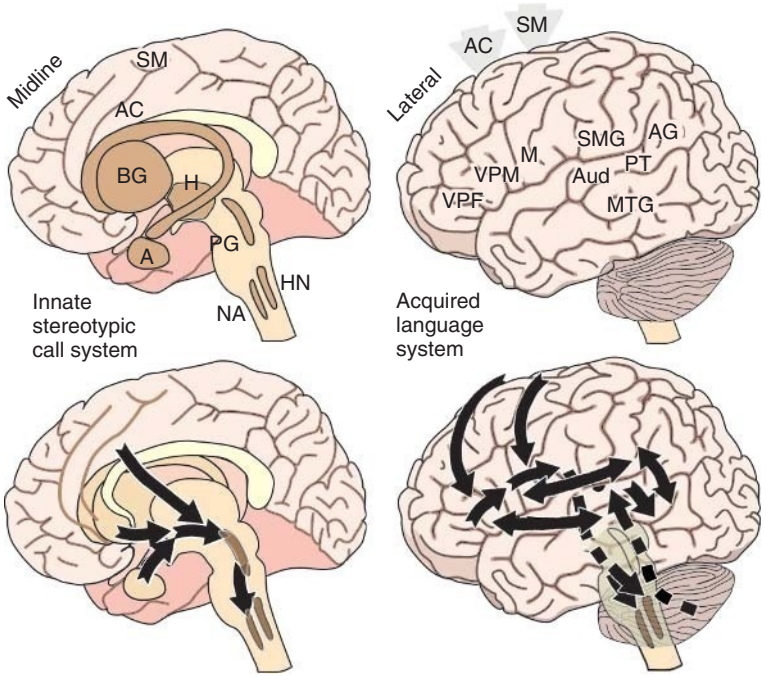

Figure 1 Highly simplified schematic comparison of brain structures (above) and major connections (below) comprising the mammalian innate call production system and the human spoken language system. Many connections and relevant structures are not shown, including notably the involvement of a cortical basal ganglia thalamic cortical loop and the cerebellum in language production. The forebrain structures of the innate call system are almost exclusively associated with arousal control, whereas those supporting language are almost exclusively sensorimotor and 'association' cortical structures. So these two vocal communication systems of the brain are largely nonoverlapping in terms of structures and connections, with the exception of final brainstem output systems controlling oral, vocal, and respiratory muscles, and the anterior cingulate cortex. Projections from motor cortex extend directly to brainstem vocal motor nuclei, whereas forebrain output controlling innate calls is mediated by the periaqueductal gray area of the midbrain. In humans, both systems operate in parallel, and may compete for the control of vocal output. The differential involvement of numerous interconnected forebrain systems in language as compared to the few involved in innate vocalizations is superficially similar to the neural differences between birds that learn complex variable songs and those with innate stereotypic songs (see Figure 2). A, amygdala; AC, anterior cingulate cortex; AG, angular gyrus; Aud, auditory area; BG, basal ganglia; H, hypothalamus; HN, hypoglossal nucleus; M, motor cortex; MTG, middle temporal gyrus; NA, nucleus ambiguus; PG, periaqueductal gray; PT, planum temporale; SM, supplementary motor area; SMG, supramarginal gyrus; VPM, ventral premotor; VPF, ventral prefrontal.

functions (Dronkers *et al*., 1992), whether multiple frontal cortical areas subsume component language functions (Paulesu *et al*., 1997; Deacon, 2004), and whether these cortical areas are the primary locus, rather than the underlying white matter and striatal structures (D'Esposito and Alexander, 1995; Lieberman, 2002). Nevertheless, claims that this area is in some way uniquely organized in humans are reinforced by the common incidence of agrammatism in Broca's aphasics and by the belief that it is grammar that sets humans apart from the other species.

One of the long-standing assumptions about Broca's area is that it is a convergence zone where auditory input contributes to the formulation of speech. Could this connection pattern be a unique feature of human brains supporting speech? Combining connection data from primates with new *in vivo* functional image data on language processing, it is possible to settle some of these long-standing questions. Tracer studies of connections of the macaque inferior frontal cortex demonstrate linkages with other cortical and subcortical sites that include both parietal and temporal cortical areas. But these tracer results delineate at least three quite different connection patterns associated with different subregions of the monkey ventral frontal cortex (Deacon, 1992b). Motor and premotor cortical areas are primarily interconnected with inferior parietal and superior insular regions of cortex, and premotor cortex is connected with dorsal midline supplementary motor cortex. In comparison, the rostrally adjacent ventral prefrontal area is primarily interconnected with superior temporal and middle temporal gyrus areas, as well as with dorsal prefrontal areas and anterior cingulate cortex, but lacks connections with motor areas. So primate connection data do not support a simple convergence of auditory and tactile motor

functions in their anatomical homologue to Broca's region, and instead exhibit a tier-like organization with caudal–rostral segregation of parietal from temporal input zones. Physiological confirmation of an auditory projection zone in this macaque ventral prefrontal region has been provided by single cell recording (Romanski *et al.*, 1999). But is this segregation of auditory and motor functions in the primate homologue to Broca's area evidence that monkeys and humans differ in this respect? The corresponding connection patterns in the human brain have recently been traced using diffusion tensor weighted MRI techniques, which enable the visualization of fiber tracks (Catani *et al.*, 2005). This study mapped the course of the components of the fiber bundle known as the arcuate fasciculus, which in humans carries fibers presumed to interconnect Wernicke's area with Broca's area. The findings are consistent with the monkey brain connection pattern, not with a simple convergence zone logic. Inferior parietal projections terminate in ventral motor and premotor areas and superior and middle temporal gyrus projections terminate more rostrally in ventral prefrontal areas. Inferior parietal areas and superior temporal areas are also interconnected, but not superior temporal areas and ventral motor or premotor areas. As in the macaque brain, auditory information is relayed to the frontal areas by way of a prefrontal cortical area in front of and separate from the premotor–motor areas involved in speech production.

This evidence for fractionation of the contributions to language processing in ventral frontal cortex is also consistent with the accumulation of *in vivo* imaging data that show slightly different localizations in this region for heightened activity during language tasks that differentially involve auditory and motor processing. For example, word association and linguistically mediated mnemonic tasks preferentially activate the ventral prefrontal component, while tasks involving motor analysis preferentially activate premotor and motor areas located more caudally. The implications are first that Broca's area is not a single functional unit, but comprises two or more adjacent regions, second that only the prefrontal component utilizes temporal auditory input, and third that the language specialization of this region did not depend on any major restructuring of connectivity. In addition, if as in macaques this same auditory recipient ventral prefrontal area is linked to the anterior cingulate cortex, it would also represent a bridge between a language-specialized area and the one cortical area known to be involved in primate call production.

40.3 Comparative Functional Analyses

40.3.1 Functional Dissociation of Call and Speech Motor Control

The discovery of predator-specific alarm calls in vervet monkeys (Seyfarth *et al.*, 1980) suggested that the functional dichotomy between language and primate call systems might not be so great as once believed. The existence of distinct calls given to leopards, eagles, and snakes suggested that the origins of language might be envisioned as a gradual elaboration of a larger and larger specific repertoire eventually requiring more complex production and combination mechanisms. However, evolutionary continuity is difficult to support when the difference in neural substrates between calls and language is considered. Electrical stimulation and lesion experiments established that primate calls could be elicited by stimulation of midbrain and limbic forebrain structures, including basal forebrain, ventral striatum, amygdala, hypothalamic, and anterior cingulate cortex, but not by cerebral cortical areas (e.g., Jürgens, 1979). Correspondingly, damage to monkey's cerebral cortical areas homologous to those involved in language processing in humans do not interfere with call production. Conversely, damage to limbic and telencephalic structures homologous to structures supporting primate calls do not produce language deficits in humans. There are some exceptions that prove this rule. One is the anterior cingulate cortex, which if bilaterally damaged in humans, may result in akinetic mutism (immobility that includes vocalization). But this is arguably an impairment of the arousal to speak and move rather than a disturbance of language processing. There have also been reports that stimulation of the amygdala in human subjects can sometimes produce spontaneous curses as well as emotional cries. And in patients with global aphasia, cursing is sometimes spared or even facilitated. Expletives are, however, an interesting intermediate; an acquired vocalization that has become relatively automatic and stereotypically associated with specific intense emotional experience. Taken together, these data demonstrate that the neural substrates for language functions and innate calls derive from almost completely dissociated brain systems. Along with evidence that speech depends on direct cortical projection to oral–vocal motor nuclei, independent of the older

limbic-midbrain–brainstem pathway, and the fact that speech and human innate calls exist side by side in our vocal repertoires, we can confidently conclude that the language system is not an elaboration of the call production system. The only significant overlap of these two systems is a final common output pathway.

40.3.2 Songbird Comparisons

Despite the fact that telencephalic organization in birds and mammals is radically different, there are useful analogies that can be drawn from comparison to birdsong control and its differences in different species (Jarvis, 2004). Research into the organization of song acquisition and control in different bird species demonstrates a consistent pattern that distinguishes song learners able to produce complex songs from nonlearners with simple songs. Comparisons between songbirds, parrots, and hummingbirds also demonstrate that complex singing abilities have evolved independently at least three times in the course of bird evolution and that in each of these lineages motor control of song output is mediated by slightly different forebrain systems. Species with highly stereotypical innate songs utilize only one or two forebrain motor nuclei for song production. In both songbirds and birds with stereotypic songs, a primary motor output nucleus in the caudal telencephalon (RA in songbirds) projects to the common vocal output pathway in central midbrain, and from there to brainstem motor nuclei. In addition, species that learn significant aspects of their songs and produce complex variable songs may require the coordinated contributions from as many as a dozen forebrain structures. In this regard, the difference between birds that sing complex sounds and those that sing stereotypic songs is crudely analogous to the human/nonhuman primate difference. So understanding the differences between the alternative complex song control strategies in different bird lineages and the difference between complex singers and stereotypic singers may provide useful comparisons (see Figure 2).

Two major classes of forebrain systems are integrated with the forebrain motor output nuclei to enable song learning and song complexity: auditory and striatal motor systems. These are necessary for learning from auditory experience. In addition, a higher-order premotor nucleus (high vocal center (HVC) in songbirds) is also critical for song complexity and flexibility. The differences between different lineages where vocal learning evolved are also interesting as a comparison. Some of the most sophisticated learners, such as parrots, have a different motor output pathway from the forebrain than do oscine songbirds. Although it is not clear from current research whether these differences are more than variations on a theme, differences in the midbrain/brainstem output targets of these nuclei are suggestive. For example, in songbirds, a midbrain region (probably homologous to the mammalian periaqueductal gray area which mediates call production) mediates between forebrain and brainstem motor control nuclei, but in parrots and possibly hummingbirds there is also a direct projection from forebrain motor nuclei (not homologous to RA) to brainstem motor systems. It is tempting to speculate that this latter pattern is analogous to the direct forebrain (cortical) projections to the vocal motor centers that distinguishes humans. Whether these differences account for the remarkable vocal mimicry of parrots and their kin is unknown.

So, although a good deal is still to be learned about the evolution of vocal learning and vocal skill in birds, exploring this more experimentally accessible parallel to the human case may provide important clues about how vocal flexibility evolves. An intriguing example is discussed below.

40.3.3 Lateralization of Language Functions

One of the more enigmatic features of language representation in the brain is the fact that the two hemispheres play very different and unequal roles in controlling speech and comprehension. This is not because there are different cortical areas on the two sides. Ever since the French surgeon Paul Broca first catalogued cases of speech impairment associated with localized brain lesions (Broca, 1865), it has been known that the left-hemisphere damage is far more debilitating for language functions than the right-hemisphere damage. The brain regions in the inferior frontal gyrus and superior temporal gyrus that are the loci most likely damaged in Broca's and Wernicke's aphasia, respectively, are identified for the left hemisphere, but their right-hemisphere counterparts can often suffer damage with no obvious speech impairment. This left 'dominance' for language, as it is often described, is not universal, with just a few percent of people exhibiting complete right-sided language bias. The exceptions also correlate strongly with left handedness, suggesting a link between the asymmetrical biases. Beginning in the 1960s, a series of surgical interventions to limit the spread of epilepsy susceptibility from one hemisphere to the other cut the corpus callosum, and other forebrain commissures, severing the two hemispheres so that they could not

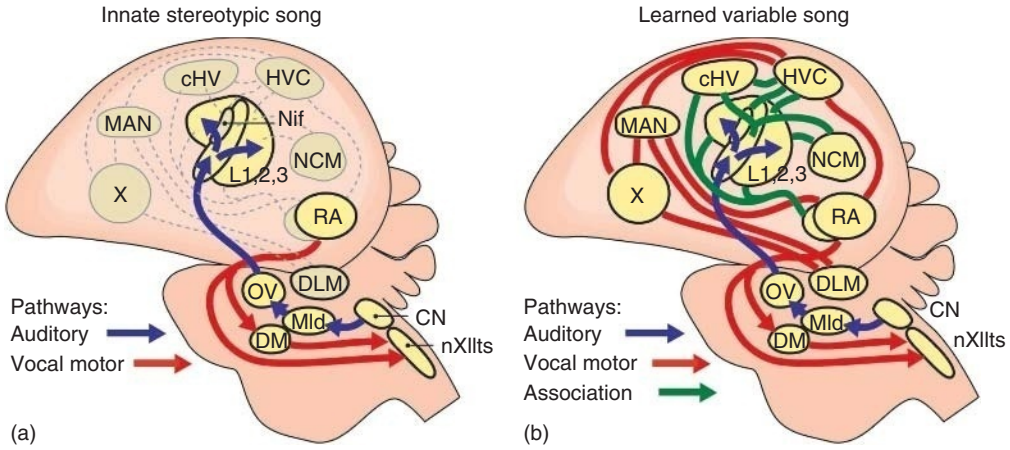

Figure 2 Comparison of major structures and connections in brains of (a) songbirds that do not learn their song and (b) those that do. Dashed connections and structures not connected by heavy lines are minimally involved in song (many structures and connections are not shown for simplicity). Innate stereotypic birdsong is relatively insensitive to damage to most forebrain structures (a) excepting a primary motor nucleus (RA) which projects to the central midbrain and to vocal motor nuclei, such as the hypoglossal nucleus (nXIIts). In contrast, striatal structures (e.g., areas X and MAN), auditory centers (e.g., L1, L2, and L3), and numerous pathways linking these with premotor (e.g., HVC) and motor (RA) nuclei are all involved in various aspects of learning a song 'dialect' from adult singers in early life. In these birds damage to any of these structures or their interconnections can disturb learning, complexity, or flexibility of song. Other bird orders exhibiting learned vocalizations, such as parrots and hummingbirds, are distinguished by differences in final motor output pathways (not shown). The distribution of song control to a diverse system of forebrain structures in song learners is loosely analogous to the shift in control from limbic structures to the diverse system of interconnected sensory, motor, and association cortical areas and striatal nuclei that evolved to control language (see Figure 1). In both systems the involvement of a diverse constellation of interconnected forebrain structures appears to be correlated with complex, flexible, context-sensitive, socially transmitted, learned vocal skills. For a more detailed account of the comparative neurology of bird vocalizations and human language see Jarvis (2004). cHV, caudal region of the hyperstriatum ventrale; CN, cochlear nucleus; DM, dorsal medial nucleus of the midbrain; DLM, medial nucleus of the dorsolateral thalamus; MAN, magnocellular nucleus of the nidopallium; HVC, high vocal center; L1 3, primary auditory fields; Mld, mesencephalic lateral dorsal nucleus; NCM, caudal medial nidopallium; Nif, interfacial nucleus of the nidopallium; nXIIts, hypoglossal nucleus (nucleus XIIth cranial nerve, tracheal syringeal division); OV, nucleus ovoidalis; RA, robust nucleus of the arcopallium; X, area X of the striatum.

exchange signals. The results were startling. If information was carefully provided to only one of the isolated hemispheres, patients could use language to describe the stimulus only if presented to the left hemisphere (input from the right side). This suggested that the right hemisphere was essentially mute.

There are a few clues to how and why this functional asymmetry evolved to be so robustly associated with language functions. The first clue comes from understanding what language-related functions, if any, are contributed by the contralateral counterparts to Broca's and Wernicke's areas in the right hemisphere. Two lines of evidence suggest that, contrary to earlier views, the right hemisphere does indeed contribute to language processes. Both kinds of evidence come from cases of right-hemisphere brain damage. First, there is a higher incidence of aprosodia with right-hemisphere damage (Ross, 1981). Aprosodia is an impairment of the ability to produce or accurately comprehend the changes in tonality and rhythmicity that is used to convey emotional tone, emphasis, or differential focus in speech. This suggests that the right hemisphere is involved in regulating the nonreferential social–emotional context of spoken conversation in parallel with the left-hemisphere production and comprehension of the syntactical and semantic content of speech. Second, right-hemisphere damage appears to impair the ability to keep track of the larger semantic and pragmatic frames of speech. Right-hemisphere-damaged patients have difficulty understanding what makes one joke funny and another lame, and also have difficulty following the theme of a story, often failing to recognize the insertion of incongruous elements (Gardner *et al*., 1983). So although the right hemisphere appears to be minimally, if at all, involved in immediate semantic and syntactic processing of words and sentences, it appears to be carrying out important supportive background tasks in parallel.

This parallelism may help to explain another curious feature of language lateralization: its

development in childhood. Studies of very young children who have had their left hemispheres surgically removed show a remarkable sparing of language abilities, with minimal obvious impairment in adulthood (see review in Kolb, 1995; and recently Boatman *et al*., 1999). So, although left lateralization of the semantic, syntactic, and phonological processing of language is highly predictable and probably reflects an innate bias, it is only a bias and not a fixed and inflexible adaptation.

Why should there be laterally asymmetric distributions of language functions in an otherwise bilaterally symmetric brain? Although there is no certain answer to this question, considering the functional characteristics that are lateralized, the fact of their progressive differentiation during maturation, and other correlates of lateralization (e.g., handedness) some plausible hypotheses can be formulated. First, there are consistent structural asymmetries in human brains (discussed already). The relative sizes of cortical areas and morphological structures associated with Broca's and Wernicke's language areas indicate that the right side counterparts are on average smaller. This could be a source of developmental bias or also a consequence of developmental differentiation, but other left–right asymmetries in neonatal brains support the possibility that anatomical bias contributes. Second, the language functions that appear to segregate to opposite hemispheres seem to divide according to both rate of processing (rapid processing on the left) and extent of conscious online monitoring required (also left). This can probably be understood in terms of segregating functions that need to run in parallel but would likely interfere with one another because of their very different processing parameters (Deacon, 1997). Third, the correlation with asymmetric manual skill suggests a possible linkage, perhaps with tool use (Kimura, 1993). Finally, the unilateral representation (also to the left) of vocal skill learning is also characteristic of songbirds (Nottebohm and Nottebohm, 1976). Since vocalization involves muscle systems that are aligned along the midline of the body and are bilaterally controlled, it may be necessary to strongly bias control to one hemisphere in order to avoid functional conflict. In summary, lateralization may not be a requirement for the evolution of language, but it is likely a bias built in to aid functional segregation of processes that are best run in parallel systems and thus avoiding mutual interference.

40.3.4 The Mirror System

Although the class of cells called 'mirror neurons' are discussed elsewhere in this volume, including their possible roles in language processing, this class of neural responses has also been implicated in many language origins theories (Rizzolatti and Arbib, 1998) Recording from single neurons in a ventral premotor subregion (designated F5) of the macaque monkey brain, Rizzolati and colleagues identified a subset of neurons that preferentially spiked when the subject observed himself picking up an object and also when observing an experimenter picking up the same object in the same way. This responsiveness to the general form of the action, irrespective of the role of agency and perspective, suggested the name 'mirror neuron'. The relevance to the evolution of the language capacity is twofold: first it suggests the possibility that this kind of neural responsiveness could play a role in the ability to mimic others, and second the location of these cells in the macaque brain is in a region generally considered adjacent to the monkey brain region deemed homologous to the premotor division of Broca's area (see Section 40.2.1), and possibly overlapping. *In vivo* imaging data have further suggested that there is a similarly responsive premotor region in the human brain. Although it can be debated whether this response characteristic is specifically found in the same premotor region in human brains as the one that plays a critical role in language, these coincidences make it reasonable to entertain the hypothesis that this might help support the vocal mimicry necessary for word learning in language. If so, what might be the implications for language evolution? First, it must be noted that the presence of mirror neurons in macaque brains is sufficient to exclude these cells or their connections from being the difference in human brains that makes language possible; however, it could be argued that their presence predisposed this premotor zone for a later role in language processing. Second, speech mimicry demands that a parallel class of auditory–vocal mirror neurons be identified (visual–manual mirror neurons might be more important for mimicry in gestural language), though some of these neurons exhibit responses to the sound of an object being manipulated as well. In the monkey brain, mirror neurons receive input from neurons in inferior parietal cortex that are also responsive to visuomanual stimuli, but if there are corresponding auditory–vocal mirror neurons we might rather expect them to receive input from superior or

middle temporal sources. Until such a parallel class of neurons is identified it is probably premature to assume that mirror neurons are critical to language functions, but looking for them is thus a relevant enterprise. One plausible scenario – assuming that mirror neurons are indeed critical for mimicry – is that they played a role in an early, more gestural phase of language evolution, and possibly paved the way for the evolution of these hypothetical auditory counterparts.

40.4 Genetic Correlates of Language Adaptation

40.4.1 Hopeful Monsters and Megamutation Scenarios

Probably the most popularly accepted scenario for language evolution is what has sometimes derisively and sometimes seriously been referred to as the 'big bang' scenario. On the analogy to the birth of the universe, this scenario suggests that language was made possible as a result of one or just a few major mutation events that resulted in the significant reorganization of brain functions. This resonates well with assumptions about an innate UG (see Section 40.1.2) or a 'language acquisition device' constituting the difference between human and nonhuman brains. It also resonates with paleoarcheological theories for explaining the sudden burst of cultural artifacts (such as diverse tools types, representational cave paintings, and carvings) that arose within the last 50 000 years. Since *H. sapiens* has been around for greater than 100 000 years, and hominids with comparably large brains and complex stone tools have been around for roughly half a million years, this transition appears quite recently in human evolution. But the idea that what distinguishes speaking humans from other species and from our recent ancestors can be explained by a couple of very lucky genetic accidents seems both counterintuitive in terms of what is known about the genetics of the developing brain and what is known about the complexity of language control. But more generally, it also leaves almost the entire explanation of this adaptation to an incredibly lucky accident. This kind of evolutionary scenario is often described as a hopeful monster story, because it imagines that a mutation producing a major phenotypic distortion becomes so enormously successful that it replaces all alternatives. Though one cannot argue that it is impossible, it is a claim about evolution that is little better than invoking a miracle. Nevertheless, there is at least one serious proposal for just such a critical genetic change.

40.4.2 Genes Affecting Language Processing

In the mid-1980s, when excitement about the plausibility of innate UG was at its peak, a surprisingly specific inherited language disorder was described. Called specific language impairment (SLI) by researchers, it was expressed in a family (identified as the KE family) in which many members exhibited specific difficulty with regularized aspects of English syntax (Gopnik, 1990; Gopnik and Crago, 1991). Most notably, this was manifested in a problem learning to use the regular past tense ending '-ed'. Subsequent study of this deficit showed it to also be accompanied by significant oral motor apraxia (pronunciation and fluency problems) and neurological reduction of motor areas and basal ganglia (Vargha-Khadem *et al.*, 1998). Chromosomal damage to a common locus was subsequently correlated with expression of this trait, and in 2002 a transcription factor gene, FOXP2, was identified as the critical damaged gene in this disorder (Enard *et al.*, 2002), expressed in structures affected in the KE family (Lai *et al.*, 2003). It is the first single gene to be correlated with a known neurological disturbance of language function. It is not a 'new' gene unique to the human lineage, since it is present and plays a critical role in development of the brain in all mammals (a homologue is also found in birds and fruit flies), and it is a highly conserved gene in terms of sequence variations across species, and the KE family variant is damaged at a site conserved in all known species (and so likely critical).

Important with respect to its plausible role in the evolution of language are two point mutations in the human variant that distinguish it from the chimpanzee version (and basically from all other mammals in which it is highly conserved). Linkage information even suggests that these human deviations are relatively recent – possibly within the last 100 000 years – and are likely universal or nearly so in living humans. This does not prove, however, that these human-specific differences contribute to a crucial change in function (though the evidence is highly suggestive), and the alterations do not correspond to the damaged locus in the KE family. At the present time, we cannot even say for certain that having the chimpanzee gene would result in diminished language function, or whether a chimp with a human version of the gene would have improved oral motor capacity. But damage to a regulatory gene that is critical for early brain development (as it is in all

mammals) will almost certainly result in significant disruption of function, since it likely controls the expression of many other genes. So although the gene did not evolve for language, the neural features it controls during development have clearly been recruited by language, and it seems likely that the mutations that occurred in it in human evolution played some role in the evolution of speech.

Assuming that the point mutations of FOXP2 that are unique to the human lineage do play a role in our language adaptation, we next need to ask what kind of effect. And this requires considering its contribution to development of specific brain structures. Comparative and clinical data suggest that it plays a role in the development of the basal telencephalon, which will become ventral basal ganglia and basal forebrain in the adult. Though these basal ganglia structures are not classically identified as language structures *per se*, there are many reasons to think that basal ganglia structures could be important contributors to language learning and use (Lieberman, 2002), particularly of those processes that become relatively automatic. This is consistent with the critical role played by basal ganglia in skill learning, the automatization of many routine behavioral functions, and the establishment of procedural memories. Since there is an extensive interdependence between the anterior cortical areas and basal ganglia, via a recurrent circuit through the pallidum and thalamus, it should be no surprise that functions associated with frontal language cortex might also be affected by basal ganglia disturbances, especially motor functions. As a comparison, disruption of fluency, pronunciation, and syntactic processing have all been shown in Parkinson's disease patients, who also have reduced basal ganglia function (Lieberman, 2002). So if recent human-specific point mutations in the gene FOXP2 do reflect an adaptation for language processing, it is most likely with respect to aiding speech automatization. This role in learned vocalization is also supported by two additional comparative findings. First, the bird homologue to FOXP2 is found to be expressed in striatal nuclei associated with song learning (particularly in area X), and is more extensively expressed in species that learn their songs (Haesler *et al.*, 2004). Second, damaging one copy of FOXP2 in mice produces an impairment of their ultrasonic vocalization, and damaging both causes severe motor impairments, elimination of ultrasonic vocalizations, and premature death (Shua *et al.*, 2005). So although it is not specifically a gene for language, nor did it evolve only in humans, it has clearly been critical for neural systems underlying vocal motor functions in terrestrial vertebrates for a very long time, and it may have been tweaked in recent human evolution.

40.5 Evolutionary Processes and Brain–Language Co-Evolution

40.5.1 Evolutionary Scenarios

Estimates of the age of language date from as little as 50 000 years to more than 2 million years. Some of this difference reflects different definitions of language, some reflects different notions about the tempo of evolution (i.e., whether the change was sudden or gradual), and some takes different views about the number of mutational changes that were necessary. In general, those who argue that the language faculty is a highly specialized modular capacity tend to favor a recent date of origin and a saltational transition, whereas those who favor a more generalized conception of the language faculty supported by a constellation of adaptations tend to favor more ancient dates.

Exactly how the processes of natural and sexual selection might have contributed to the evolution of the human language adaptation is also contentious. Darwin (1871) argued that language might have evolved from something like courtship song under the influence of sexual selection. Modern theories that appeal to sexual selection have also focused on the use of language for social manipulation. The most common scenarios, however, focus on the role of language as a tool for social coordination and maintenance of social groups.

Two extreme language selection scenarios are commonly opposed in the literature to predict what changes in brain structure might be relevant: scenarios assuming that language is a consequence (or late-stage tweak) of a more prolonged trend toward increasing general intelligence (exemplified by a 2-million-year expansion of brain size) and scenarios assuming that language is the consequence of domain-specific neural modifications and is independent of general intelligence. These are not mutually exclusive options, but they do make different predictions with respect to neural structural and functional consequences, as well as evolutionary timing. These functional implications can be used retroductively to probe the plausibility of each. If language has an ancient origin, it would follow that it is likely supported by a significant and extended natural selection history, including the contributions of many genetic changes affecting

the brain. If, on the other hand, language is of recent origin and largely without precedent, it would follow that little time has elapsed for significant effects of natural selection to accumulate. As a result, ancient origin hypotheses predict that language functions will be more thoroughly integrated into other cognitive functions, will likely have distributed representation in the brain, and should be highly plastic with respect to both minor brain disorders and genetic variation. Recent origin hypotheses, on the other hand, are more consistent with language processes being highly modular and domain specific, localized to one or a very few neural systems, fragile with respect to brain damage and genetic variation, and possibly radically altered in grammatical organization by genetic abnormalities. With the exception of claims for domain specificity, which are controversial, the neuropsychological evidence argues against a recent rapid transition to language capacity. But archeological evidence is also brought to bear on this question. The paleoarcheological record is surprisingly stable from about 1.6 Mya to roughly 350 kya, with the transition from Acheulean to Mousterian tool culture, but does not begin to show signs of regional tool styles, decorative artifacts, and representational forms (e.g., carvings and cave paintings) until roughly 60 kya, with the dawn of what is called the Upper Paleolithic culture. This recent transition to technological diversity and representational artifacts has been attributed to a major change in cognitive abilities, which many archeologists speculate reflects the appearance of language. Fossil crania, however, provide no hint of a major neuroanatomical reorganization, and the genetic diversity of modern human populations indicates that there are some modern human lineages who have been reproductively separated from one another for at least twice this period and yet all have roughly equivalent language abilities. These considerations weigh in favor of a protracted evolution of language abilities and for the convergence of many diverse neural adaptations to support language (Johansson, 2005).

An adaptive convergence logic also helps to resolve some of the mysteries concerning the absence of direct neuroanatomical or functional homologies between language and nonhuman communication adaptations. The novelty of language can be understood in terms of the combined effects of systems which individually may have served quite different functions in ancestral species but which collectively interact in novel ways to produce emergent consequences. If the human language adaptation reflects the combined contribution of many diverse systems whose parallel evolutionary paths have come together to provide an unprecedented functional synergy, we should not expect to find highly divergent local changes in brain structure, but rather global reorganization in which most structures participate in some respect or other. But considering language functions to be emergent adaptations, in this sense, poses new questions about the evolutionary process. Specifically, we must explain how such functional synergies among diverse systems can be explored and recruited by the process of natural selection. In general, this reflects a common challenge posed to evolutionary theory since the time of Darwin, and can be generally answered the way he explained the probable evolution of the eye. He argued that even quite minimal non-image-forming light-sensitive proto-eyes would, none the less, provide an adaptive advantage over the absence of any light sensitivity, and that any minor modifications to adjacent structures that improved on this in any way would likewise be advantageous and selectively retained. As more comparative anatomical and genetic information has come to light concerning the evolution of eyes, in the century and a half since Darwin's time, his speculation has found ample justification. However, language differs from this sort of complex adaptation in one important respect: much of the detail of a language's functional architecture is transmitted socially.

40.5.2 Co-Evolutionary Scenarios

In contemporary behavioral biology, the concept of instinct no longer comes with the connotation of learning playing no role. Many species-typical behaviors from the social learning of birdsongs to the hunting behavior of wolf packs involve the interaction of behavioral and learning biases with socially transmitted habits and variable environmental contexts. Darwin recognized the relevance of this environmental conditionality when he described the language adaptation as "an instinct to acquire an art." Language is, of course, special with regards to the relatively massive contribution of extrinsic factors, and also with respect to the likely combinatorial and emergent character of its supporting neurology. So its emergent character is unusually dependent on interactions between diverse neural and social mechanisms producing specific outcomes. This combinatorial co-dependence provides a challenge to simple caricatures of language evolution on the analogy of other physiological adaptations.

Recognition of this co-dependency has given rise to evolutionary scenarios that incorporate this interactional logic. Most develop from an evolutionary logic that has come to be called the Baldwin effect, after Baldwin (1896) who described how behavioral plasticity enabling the production of acquired adaptations might serve as an evolutionary precursor to a more innately produced analogue of this adaptation. The general logic of this evolutionary mechanism involves two phases: (1) the production of phenotypic plasticity (e.g., learned behaviors) making it possible for acquired adaptations to be conditionally produced that enable a lineage to persist despite a suboptimal match to the environment and (2) the appearance of new variants in that lineage that are selectively retained because they take over some fraction of the load of acquisition. This, presumably, described a Darwinian mechanism that would produce the evolutionary equivalent of Lamarckian inheritance of previously acquired traits. Proponents of innate UG invoked versions of this logic to argue that language-like behavior in our ancestors could have become progressively internalized as an innate faculty that is presently only minimally dependent on learning in the standard sense (e.g., Pinker, 1994). But the same logic could equally support the evolution of biases and aids to learning, without invoking a replacement of learned with innate knowledge of language (e.g., Deacon, 1992a, 1997). More recently, these arguments have been revisited in the context of the concept of niche construction (Odling-Smee *et al*., 2003; Deacon, 2003), in which persistent socially maintained language use can be understood as a human-constructed niche that exerts significant selection pressures on the organism to adapt to its functional requirements. This approach is compatible with the claim that language function is supported by many modest distributed evolutionary modifications of brain anatomy and chemistry. It also assumes that language-like communication was present in some form for an extensive period of human prehistory.

40.5.3 Degenerative Processes as Possible Contributors to Language Evolution

A co-evolutionary scenario for the evolution of language still does not account for the generation of the novel functional synergy between neural systems that language processing requires. The discontinuities between call control systems and speech and language control systems of the brain suggest that a co-evolutionary logic alone is insufficient to explain the shift in substrate. Recent investigation of a parallel shift in both complexity and neural substrate in birdsong may be able to shed some light on this.

In a comparative study of a long-domesticated bird, the Bengalese finch, and its feral cousin, the white-rump munia, it was discovered that the domesticated lineage was a far more facile song learner with a much more complex and flexible song than its wild cousin. This was despite the fact that the Bengalese Finch was bred in captivity for coloration, not singing (Okanoya, 2004). The domestic/feral difference of song complexity and song learning in these close finch breeds parallels what is found on comparisons between species that are song learners and nonlearners. This difference also correlates with a much more extensive neural control of song in birds that learn a complex and variable song. The fact that this behavioral and neural complexity can arise spontaneously without specific breeding for singing is a surprising finding since it is generally assumed that song complexity evolves under the influence of intense sexual selection. This was, however, blocked by domestication. One intriguing interpretation is that the relaxation of natural and sexual selection on singing paradoxically was responsible for its elaboration in this example. In brief, with the song becoming irrelevant to territorial defense, mate attraction, predator avoidance, and so on, degrading mutations and existing deleterious alleles affecting the specification of the stereotypic song would not have been weeded out. The result appears to have been the reduction of innate biases controlling song production. The domestic song could thus be described as both less constrained and more variable because it is subject to more kinds of perturbations. But with the specification of song structure no longer strictly controlled by the primary forebrain motor center (RA) (see Section 40.3.2), other linked brain systems can begin to play a biasing role. With the innate motor biases weakened, auditory experience, social context, learning biases, and attentional factors could all begin to influence singing. The result is that the domestic song became more variable, more complicated, and more influenced by social experience. The usual consequence of relaxed selection is genetic drift, increasing the genetic and phenotypic variety of a population by allowing random reassortment of alleles, but neurologically, drift in the genetic control of neural functions should cause constraints to become less specific, generating increased behavioral flexibility and greater conditional sensitivity to other neurological and contextual factors.

This is relevant to the human case, because a number of features of the human language adaptation also appear to involve a relaxation of innate constraints allowing multiple other influences besides fixed links to emotion and immediate context to affect vocalization. Probably the clearest evidence for this is infant babbling. This unprecedented tendency to freely play with vocal sound production occurs with minimal innate constraint on what sound can follow what (except for physical constraints on vocal sound generation). Babbling occurs also in contexts of comparatively low arousal state, whereas laughter, crying, or shrieking are each produced in comparatively specific high arousal states and with specific contextual associations. This reduction of innate arousal and contextual constraint on sound production opens the door for numerous other influences to begin to play a role. Like the domesticated bird, this allows many more brain systems to influence vocal behavior, including socially acquired auditory experience. In fact, this freedom from constraint is an essential precondition for being able to correlate learned vocal behaviors with the wide diversity of objects, events, properties, and relationships language is capable of referring to. It is also a plausible answer to the combinatorial synergy problem (discussed above) because it demonstrates an evolutionary mechanism that would spontaneously result in the emergence of multisystem coordination of neural control over vocal behavior.

But although an evolutionary dedifferentiation process may be a part of the story for human language adaptation, it is clearly not the whole story. This increased flexibility and conditionality likely exposed many previously irrelevant interrelationships between brain systems to selection for the new functional associations that have emerged. Most of these adaptations remain to be identified. However, if such a dedifferentiation effect has been involved in our evolution, then scenarios hypothesizing selection for increased innateness or extrapolation from innate referential calls to words become less plausible.

40.6 Conclusions

Despite decades of research to identify the distinctive neuroanatomical substrates that provide humans with an unprecedented faculty for language, no definitive core of uniquely human anatomical correlates has been demonstrated. Only a few distinctive anatomical differences can be directly associated with the human language adaptation. These are associated with the special motor adaptations for speech. There is an unprecedented direct projection from motor cortex to the laryngeal motor nucleus of the brainstem (nucleus ambiguus) allowing direct control of vocalization independent of arousal state or innate vocal motor pattern. There is also one known genetic correlate with language competence, the gene FOXP2. Although it is clearly not specifically a language gene, nor can we be sure that its few human sequence differences represent adaptive modifications with respect to language, it is clear that it plays a necessary supportive role in the development of brain systems involved in speech production. Damage to gene in humans results in both generalized vocal disarthria and disruption of the ability to automate certain highly regular syntactic operations, and is associated with reduction of anterior basal ganglia structures. Besides these specific effects, however, it also appears likely that the neural changes associated with language adaptations involve more generalized allometric deviations from the ape pattern. Correlated with the increase in brain size in hominid evolution, there appears to have been quantitative remodeling of relationships between brain structures that is likely to have produced quantitative connectivity changes as well. If, as now appears likely, human brain adaptations for language involve many systems' coordinated interactions, it is likely that some or all of the quantitative alterations of brain organization reflect language adaptations. Although there is still considerable controversy concerning the proper assessment of the allometry human brain structures, candidates include overall cortical expansion, disproportion between cerebral cortex and basal ganglia, disproportionate increase in eulaminate cortical areas with respect to specialized sensory and motor areas, prefrontal expansion, increases in proportions of corticocortical and corticocerebellar connections, among others. However, the relevance of any of these cannot be discerned until there is a better understanding of the contributions of these systems to language acquisition, comprehension, and production. But a definitive assessment of the significant allometric deviations of human brain structure from typical primate trends could likewise provide hints of major differences in cognitive processing relevant to language.

The highly robust and developmentally canalized nature of language acquisition suggests that this capacity does not depend on only a few subtle neurological changes from the ape pattern but instead likely reflects a prolonged process of selection involving many systems, and perhaps extending over a million years. The nature of this selection process appears to have involved early protolanguage use as

a kind of niche construction, providing selective pressure to better support the unusual demands imposed by language. If this is an accurate assessment, it means that the neurological adaptations supporting language can at least in part be understood as adaptations for language, rather than merely accidentally giving rise to language. Some aspects of this ability may also be the result of evolutionary degradation of other functional specializations, which has allowed more diverse and distributed neural systems to directly or indirectly influence vocalization.

Though human brains unquestionably include numerous species-unique innate adaptations supporting the acquisition and use of language, there is to date little evidence for a specific neuroanatomical substrate for an UG. So, progress in understanding the language-related evolutionary changes of human brain structure can mostly be marked by what we now know is not the case, and just a few clear correlates of language adaptation. But this imposes considerable constraint on the scenarios we can consistently entertain and focuses neural research on a few notable problem areas.

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41 The Evolution of Hemispheric Specializations of the Human Brain

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Glossary

allele One of several possible forms of a gene.

antisymmetry Form of bilateral asymmetry in which there are equal numbers of sinistral and dextral forms (e.g., equal numbers of left and right handers) in the population.

bilateral asymmetry Condition in which one side of the body is not the mirror image of the other.

bilateral symmetry Condition in which one side of the body is the mirror image of the other.

Bilateria The phylum of ancient origin that includes all organisms (including humans) whose body plan is for the most part bilaterally symmetrical.

bipedalism Standing or walking on two legs rather than four.

Broca's area Region in the left frontal convolution of the brain, usually on the left side, that plays a major role in the organization of speech.

cerebral asymmetry Systematic difference in structure or function between the two sides of the brain.

commissurotomy Section of the commissures connecting the two cerebral hemispheres of the brain, sometimes known as splitting the brain.

directional asymmetry Form of bilateral asymmetry in which the majority of the population exhibit the same direction of asymmetry (e.g., right handedness in humans).

functional magnetic resonance imaging (fMRI) Technique for measuring blood flow in the intact brain, indicating which parts of the brain are active.

handedness Systematic difference between the two hands, usually favoring the right hand in humans. Can be defined either in terms of preference for one hand over the other or in terms of greater skill or strength on one hand relative to the other.

hemispheric dominance Dominance of one side of the brain over the other.

hemispheric specialization Function performed exclusively or preferentially by one side of the brain rather than the other.

heterozygosity Condition in which an individual carries different alleles on a gene.

homozygosity	Condition in which an individual carries identical alleles at one or more loci in chromosome segments.
human revolution	Emergence of so called modern behavior in humans, including cave art, more sophisticated tools, and bodily ornamentation, and thought to date from around 40 000 years ago.
right shift	Theory that, in humans, the distribution of differences in function between the two hands is shifted to the right, explaining why most people are right handed. The right shift is thought to be due to a genetic effect.
temporal planum	Region in the posterior temporal lobe of the brain. This region on the left is part of Wernicke's area, and plays a major role in the comprehension of language.

41.1 Introduction

The most obvious mark of hemispheric specialization in the human brain is the near-universal preference for the right hand, implying a left-hemispheric dominance for manual action. This remarkable asymmetry has long been a source of fascination. It seems to apply to all human cultures and has served as a potent source of symbolism (Hertz, 1960). Because the right hand is generally the more skilled, the right is associated with positive values and the left with negative ones, as is evident in the contrast between terms such as 'dexterous', 'adroit', and even 'right' itself, with terms such as 'gauche' and 'sinister'. We speak of a 'right-hand man', but a 'left-handed compliment'. The Bible is said to contain over 100 favorable references to the right hand, and 25 unfavorable references to the left (Barsley, 1970). The negative values associated with the left are often manifest as discrimination against left-handers, even though left-handers often triumph in sports such as tennis or baseball, or even in intellectual ability. The quintessential Renaissance Man, Leonard da Vinci, was a left-hander.

The left cerebral dominance for speech and language, since it was first discovered in the nineteenth century, has been an equally potent source of myth, as is evident in the frequent references in popular culture to left-brain and right-brain values. In this case, though, the differences are not seen as quite so value laden, and if anything there may be more positive associations with the right hemisphere than with the left, even though it is the left hemisphere that controls the characteristically human functions of language and manual manipulation. The right is often portrayed as more creative, artistic, emotionally sensitive, and holistic than the more linear, rule-bound left (Corballis, 1980).

The potency of left and right may be due in part to the fact that both handedness and cerebral asymmetries are functional asymmetries that appear to emerge from symmetrical structures. Our hands look alike, yet function very differently. Similarly, the two sides of the brain are anatomically more or less left–right mirror images, yet one side can produce articulate speech and the other cannot. This discrepancy between function and structure seems to imply some nonmaterial, Cartesian influence, perhaps reinforcing our sense that we humans are unique and superior to other animals, closer to angels than to apes. We are, it has been argued, the lopsided ape (Corballis, 1991). Genetic theories of handedness and cerebral asymmetry are typically based on the premise that a genetic mutation at some point in the hominid lineage gave rise to both handedness and cerebral asymmetry in the majority of humans (e.g., Annett, 1995, 2002; Corballis, 1997; McManus, 1999). It has even been proposed that cerebral asymmetry is the result of a genetic mutation that not only resulted in cerebral asymmetry, but also gave birth to language, theory of mind, a vulnerability to psychosis, and the speciation of *Homo sapiens* (Crow, 2002).

In this article, I propose to challenge this view by summarizing the evidence on cerebral asymmetries in other species and showing that human cerebral asymmetry may in fact have ancient origins. It is almost certainly true that language itself is a uniquely human accomplishment, but its lateralized representation probably derives from asymmetries that go well back in evolution. A second aim of this article is to show that bilateral symmetry is as much a property of organisms, including humans, as is lateralization of function. The obsession with handedness and hemispheric specialization has tended to obscure the more obvious fact that our brains and bodies are built on a plan that duplicates the left and right sides in mirror fashion. The main theme of this article, then, is that there is a trade-off between bilateral symmetry and lateral specialization, and this trade-off informs differences both between and within species.

41.2 Bilateral Symmetry

We belong to the phylum known as the Bilateria, an ancient lineage that includes over 1.5 million present-day animal species, including such diverse

creatures as soil nematodes, fruit flies, and mammals. The emergence of this lineage is said to mark the transition from stationary or drifting planktonic animals to active swimmers and burrowers. Bilaterian fossils have been dated to some 600 Mya, well before the Cambrian (Chen *et al.*, 2004). Bilateral symmetry may even precede the Bilateria, since it is also present in some species of the phylum Cnidaria, which is outside the Bilateria. In the sea anemone *Nematostella vectensis*, for example, bilateral symmetry is dependent on the expression of homologous *Hox* genes much as it is in the Bilateria, suggesting that bilateral symmetry arose even before the evolutionary split between the Cnidaria and Bilateria (Finnerty *et al.*, 2004).

Bilateral symmetry probably evolved in the first instance as an adaptation to directional movement, which effectively defines an anterior–posterior axis in addition to the dorsal–ventral one. These two axes are asymmetrical: the tops of organisms differ from their bottoms, and their fronts differ from their backs. The third axis, orthogonal to these two, is the left–right axis, and organs of movement are then arranged symmetrically to either side of this axis. It seems reasonable to suppose, then, that bilateral symmetry was selected to ensure linear movement, the most efficient way to travel between two points. With very few exceptions, legs, wings, swimming muscles (in fish), and flippers are bilaterally symmetrical. *Nematostella* is a directional swimmer, and the common ancestor we share with this interesting creature presumably lay close in time to the emergence of bilateral symmetry, perhaps as much as 900 Mya (Finnerty *et al.*, 2003).

Given linear movement, there is pressure to ensure bilateral symmetry of the sense organs. To a freely moving organism, it is as well to have the eyes, ears, and skin senses equally placed on either side, since any asymmetry could leave the organism exposed to attack or impervious to prey on the less receptive flank. It follows from the bilateral symmetry of both motor and sensory organs that the neural machinery for both action and perception is also likely to be symmetrical. For most animals, behavior is dominated by sensorimotor activity, involving reactions to an environment that is without overall left–right bias. Symmetry is much less apparent in parts of the body not involved in perception, locomotion, or sensorimotor behavior. The internal organs, including the heart, lungs, stomach, and liver, are arranged asymmetrically, presumably in the interests of more efficient packaging. Even the molecules of living tissue are asymmetrical, a property often taken to be a fundamental property of living matter (e.g., Monod, 1969). In a review of the evolution of bilateral asymmetry, Palmer (2004, p. 828) remarks that "bilateral symmetry is a default state once the anteroposterior and dorsoventral axes are defined." An extra step is therefore required to create bilateral asymmetry. Even the brain, with its origins in the control of sensorimotor activity, remains for the most part built on a bilaterally symmetrical plan.

Besides being largely symmetrical, the brain is also 'double'. In the treatment of intractable epilepsy in humans, surgeons have sometimes resorted to commissurotomy, in which the brain is split through the midsagittal plane, disconnecting the left and right halves. Roger W. Sperry, who pioneered the psychological investigation of the split brain in both humans and animals, wrote that split-brained patients behave as if they had "two separate conscious entities or minds running in parallel in the same cranium, each with its own sensations, perceptions, cognitive processes, learning experiences, memories and so on" (Sperry, 1966–1967, p. 318). Studies have also shown that people can function remarkably well following the removal of an entire cerebral cortex (e.g., Vargha-Khadem *et al.*, 1997; Hertz-Pannier *et al.*, 2002). Although differences between the two halves of the brain have been well documented, and are discussed further below, it is clear that there is considerable overlap.

41.2.1 The Trade-Off between Symmetry and Specialization

With respect to basic sensory and motor functions, then, there are clear benefits to bilateral symmetry, given that we live in a world that is for the most part without systematic left–right biases. We humans have nevertheless created left–right asymmetries in the manufactured world, such as the direction of script, traffic conventions, and so forth, and some of these work to the disadvantage of left handers: scissors, golf clubs, corkscrews, the placement of door handles, the sequence of pages in books and magazines. As a general rule, the advantages of symmetry apply more strongly to behaviors that are reactions to the environment than to behaviors that are operations on the environment. There may be advantages to having one hand or its controlling cerebral hemisphere specialized for intricate activities involving tools, as in writing, for example. One advantage of unilateral control is that it is not constrained by the relatively slow conduction time between hemispheres, so that computations can be carried out with greater speed (Ringo *et al.*, 1994), although an alternative solution, of course, would have been to evolve faster interhemispheric transfer!

Another advantage of hemispheric specialization is that it avoids duplication, and this may be especially important in complex functions, like language, that require large amounts of neural circuitry. Duplication may therefore be too wasteful of neural space, and also lead to interhemispheric conflict (Corballis, 1991).

Humans are cognitive specialists, with large brains even by primate standards (Passingham, 1982), and this may well have favored specialization to a greater extent than in other species. That is, the evolution of cognition and the relative independence from stimulus control may well have favored hemispheric specialization at the expense of bilateral symmetry. But it is becoming increasingly clear that cerebral and behavioral asymmetries are widespread in nature, and that at least some of the asymmetries observed in other species seem to operate according to principles similar to those documented in humans. Consequently, any sense that humans are special may derive not from cerebral asymmetry *per se*, but from the nature of the functions that are lateralized.

41.3 Hemispheric Specialization in Nonhuman Species

In this section, I review some of the evidence for directional asymmetries in animals, with an emphasis on those that that may provide insight into the nature and origins of our own lopsidedness. First, though, we need to distinguish two different kinds of asymmetry (see Palmer, 2004). One is antisymmetry, in which there are equal numbers of sinistral and dextral forms, as in the claws of male fiddler crabs, which are sometimes larger on the left and sometimes larger on the right. The other is directional asymmetry, in which most of the members of a species are asymmetrical in the same direction, as in the case of the vertebrate heart. In the case of antisymmetry, the direction of asymmetry is almost never inherited, while in the case of directional asymmetry, it typically is. This article is concerned primarily with directional asymmetry, which might arise directly, through some genetic mutation, from symmetry, or it might arise through genetic assimilation from antisymmetry.

41.3.1 Handedness

In humans, at least, the most obvious manifestation of cerebral asymmetry is handedness, since the majority of us are right-handed. It seems likely that handedness is one example of a directional asymmetry that arose through genetic assimilation of one form of asymmetry from a character that was previously antisymmetric. Mice are equally divided into left- and right-handers, and selection for left-handedness fails to influence the relative proportions in succeeding generations (Collins, 1969). There does appear to be a genetic component underlying the direction of the handedness component in chimpanzees, however, where the evidence suggests a 65:35 split in favor of right-handedness (Hopkins *et al.*, 2001). In humans, the split is about 90:10 in favor of right-handedness, and there is strong evidence for a genetic component to human handedness (McManus, 1999; Annett, 2002). This progression suggests canalization and genetic assimilation, and may apply more generally to cerebral asymmetry.

Aside from the evidence for weak right-handedness in chimpanzees, and perhaps in other great apes (Hopkins *et al.*, 2001), there is relatively little evidence for comparable asymmetries in other species. One reason for this is that the limbs are generally involved in locomotion, which, as noted earlier, creates a pressure toward bilateral symmetry. The hominids are characterized by bipedalism, which freed the hands from locomotion, allowing for manual specialization to emerge, or at least to become apparent in everyday activities. Nevertheless, there are other isolated examples of consistent handedness in nonhuman species.

Oddly enough, the clearest case of limb asymmetry comes not from primates, but from parrots. Most species of parrot show a strong preference for the left foot in picking up objects, and the proportion of left footers is close to 90%, comparable to the proportion of right-handed humans (Rogers, 1980). Second place may go to the walrus, since there is evidence that 77% of walruses display a preference for the right flipper when feeding, and there is evidence that several bones (scapula, humerus, and ulna) are longer in the right than in the left flipper (Levermann *et al.*, 2003). There have been claims that monkeys show a slight population-level preference for the left hand (MacNeilage *et al.*, 1987). Subsequent evidence has been mixed (see commentaries to the article by MacNeilage *et al.*, 1987), but if true the asymmetry may reflect a right-hemispheric bias for spatial perception. At least one study has shown a slight right-hand advantage for rhesus monkeys, but no bias in capuchins (Westergaard and Suomi, 1996).

McGrew and Marchant (1997) sound a cautionary note. In a comprehensive review, they conclude that among nonhuman primates, "only chimpanzees show signs of a population bias ... to the right, but only in captivity and only incompletely"

(McGrew and Marchant, 1997, p. 201); see also McGrew and Marchant (2001). The evidence for a population-level right-hand preference in chimpanzees comes from Hopkins and his colleagues, but appears to be restricted to certain activities, such as extracting peanut butter from a glass tube (Hopkins, 1996), gestural communication (Hopkins and Leavens, 1998), and throwing (Hopkins *et al.*, 2005), and the ratio of right-to-left-handers is only about 2:1, whereas in humans the ratio is about 8:1. McGrew and Marchant (2001) suggest that the bias in captive chimpanzees is a consequence of contact with right-handed humans, although Hopkins *et al.* (2004) have disputed this, claiming that right-handedness occurs in three distinct populations of captive chimpanzees and is unrelated to the proportion of animals raised by humans, and more recently Lonsdorf and Hopkins (2005) have documented population-level right-handedness for tool use in wild chimpanzees.

41.3.2 Vocalization

The left-hemispheric specialization for speech and language in humans may well derive from left-hemispheric control of vocalization. This has been demonstrated even in the frog, suggesting an ancestry that may go back to the very origins of the vocal cords some 170 Mya (Bauer, 1993). In passerine birds, too, vocalization seems to be controlled by the left hemisphere (Nottebohm, 1977), although it has been argued that the mechanisms underlying this asymmetry are not comparable to those in humans (Goller and Suthers, 1995).

Asymmetries for vocalization apply to perception as well as to production. A left-hemispheric advantage for the perception of species-specific vocalizations has been demonstrated in mice (Ehert, 1987), rhesus monkeys (Hauser and Anderson, 1994), and Japanese macaques (Heffner and Heffner, 1984). In chimpanzees, the left temporal planum is larger on the left than on the right (Gannon *et al.*, 1998; Hopkins *et al.*, 1998), an asymmetry that seems not to be present in rhesus monkeys or baboons (Wada *et al.*, 1975) but is well documented in humans (Geschwind and Levitsky, 1968; Jäncke and Steinmetz, 1993; Foundas *et al.*, 1996). This too may reflect an asymmetry in the perception, and perhaps comprehension, of species-specific vocal communication.

41.3.3 Facial Asymmetries

The asymmetry for species-specific vocalization may also be manifested in facial movements, but may be reversed for vocalizations and facial movements that are more emotionally based. Hook-Costigan and Rogers (1998) found that marmosets opened the right side of the mouth wider when making social contact calls, implying left cerebral dominance, but the right side of the mouth wider when expressing fear, implying right cerebral dominance for emotion. Curiously, however, Hauser and Akre (2001) found only a bias toward the left side of the mouth in rhesus monkeys, regardless of the nature of the calls, implying uniform right cerebral dominance. Hook-Costigan and Rogers' finding mimics that found in humans, with the right side of the mouth dominant for speech (e.g., Graves and Potter, 1988) and the left for emotional expression. These asymmetries are also evident in 5- to 12-month-old human babies, who open the right side of the mouth wider when babbling, and the left side when smiling (Holowka and Petitto, 2002). In adults, the asymmetry of the mouth when speaking also influences the McGurk effect, in which the perception of spoken syllables depends on movements of the mouth as much as on the actual sounds emitted (McGurk and MacDonald, 1976). This effect depends on movements of the right side of the mouth, not the left (Nicholls *et al.*, 2004).

More generally, there are facial asymmetries associated with emotional expression. For example, human observers see the left side of chimpanzee faces as more emotional than the right side (Fernández-Carriba *et al.*, 2004), implying right-hemispheric dominance. In humans, though, there is controversy as to whether there is a general bias to the left (and thus the right hemisphere) for emotional expression, or whether there is a leftward bias for negative emotions and a rightward bias for positive ones (see Davidson, 1995, for a review of human evidence). The bulk of evidence now seems to support this second view (e.g., Brockmeier and Ulrich, 1993; Jansari *et al.*, 2000).

41.3.4 Visual Asymmetries

Lateral asymmetries in the visual system have been widely documented in birds. One striking example comes from the New Caledonian crow, which appears to favor the right eye, and therefore the left hemisphere, when constructing digging tools from Pandanus leaves (Hunt *et al.*, 2001). Not only does this finding parallel the human preference for the right hand (and therefore the left hemisphere) in tool-making and tool use, but it also suggests that manufacture itself, as well as cultural transmission of tool-making techniques, may not be unique to humans (Hunt and Gray, 2003).

Other birds also show visual asymmetries. For example, chicks show a right-eye advantage in fine visual discrimination, suggesting a left-hemispheric advantage. This advantage arises because the right eye is exposed to light in the egg, prior to hatching, whereas the left eye is not (Rogers, 1990). Nevertheless it appears to have adaptive significance. Chicks raised without this prehatching asymmetry do not show the discrimination bias and are at a disadvantage relative to lateralized birds in a situation where they monitor a hovering predator while at the same time discriminating grain from nonedible grit (Rogers, 2002a). This suggests that the lateralized brain is better able to carry out two tasks at once, with the right eye (left hemisphere) picking out the grain and the left eye (right hemisphere) monitoring the hawk. Other evidence shows that the avian right hemisphere is better able to make use of the large-scale geometry of the environment to deal with problems in spatial reorientation (Vallortigara *et al.*, 2004). A left-hemispheric advantage for fine-grained visual analysis and a right-hemisphere advantage for more global vision have also been well documented in humans (Ivry and Robertson, 1998).

The advantage of asymmetry over symmetry is further illustrated in a study with pigeons (Güntürkun *et al.*, 2000). Like chickens, pigeons show a right-eye advantage in discriminating grain from grit. There was a positive correlation between the degree of asymmetry under monocular conditions and the discrimination performance under binocular conditions, suggesting that visual foraging is accomplished more effectively if mediated by a single hemisphere, perhaps because there is less risk of interhemispheric conflict (cf. Corballis, 1991).

41.3.5 Behavioral Asymmetries

Many species also show biases in overt behavior, such as turning to escape predators or to attack prey. Faced with a barrier through which a learned predator was visible, some species of fish showed population-level biases to turn left or right, while others did not (Bisazza *et al.*, 2000). This bias was related to the gregariousness of the species, suggesting a social influence: presumably, species that swim together must turn together to avoid collisions. Tadpoles have been shown to have a bias to turn left when escaping a predator, but a bias to turn right when turning to take in air at the surface (Rogers, 2002b), suggesting hemispheric differences. A right-hemisphere bias has also been documented for social responses in a number of species of fish (Sovrano *et al.*, 2001), chicks (Vallortigara and Andrew, 1994), sheep (Peirce *et al.*, 2000), and monkeys (Vermeire, *et al.*, 1998), and may relate to the right-hemispheric involvement in social understanding in humans (e.g., Sperry *et al.*, 1979). There may be a dark side to this, as there is also evidence that the right hemisphere is the more specialized for aggressive behavior in a number of species, including toads (e.g., Rogers, 2002b), lizards (Deckel, 1995), chicks (Howard, *et al.*, 1980), baboons (Casperd and Dunbar, 1996), and humans (Devinsky *et al.*, 1994). Right-handed boxers typically hold a stance in which their opponents are in their left visual fields, perhaps to ratchet up the aggression in their right hemispheres, but also, of course, to give greater momentum to the stronger right hand.

Complementary to the right-hemispheric dominance for attack, there is a left-hemisphere dominance for feeding. Chicks (Deng and Rogers, 1997), pigeons (Güntürkun, 1985), zebra finches (Alonso, 1998), and toads (Vallortigara *et al.*, 1998) respond to prey or to feeding matter preferentially with the right eye. Andrew *et al.* (2000) have suggested that this asymmetry may be related to left-hemispheric control of the mouth structures, an asymmetry that may be widespread in vertebrates and may relate to the left-hemispheric control of vocalization.

41.3.6 Summary

The above review is by no means an exhaustive coverage of the now voluminous literature on behavioral and cerebral asymmetries in nonhuman species. It should serve, however, to illustrate that humans are not unique in displaying such asymmetries. Moreover, some of the principles underlying these asymmetries seem to apply to both human and nonhuman species. There appears to be right-hemispheric specialization for emotion (or perhaps for negative emotions), aggression, social behavior, and for the more holistic aspects of perception. The left hemisphere seems to be the more specialized for detailed visual analysis, feeding behavior, and species-specific communication. It is likely that these asymmetries vary between species, perhaps depending on ecological or social factors.

Rarely, if ever, are the asymmetries absolute: each hemisphere, for example, appears to have some capacity to undertake the speciality of the other. Further, not all members of the species show the same directional asymmetry, despite the overall population bias. The percentage of individuals who reverse the asymmetry shown by the majority ranges from 10% to 35% (Ghirlanda and

Vallortigara, 2004). This again suggests that there is a trade-off between symmetry and asymmetry, and one or the other may dominate depending on survival contingencies.

41.4 Cerebral and Manual Asymmetries in Humans

As the foregoing review illustrates, the idea that cerebral asymmetry is unique to humans is wrong and may reflect an age-old desire to place humans on a pedestal above other species, closer to angels than to apes. Nevertheless, part of the reason our own lopsidedness seems so salient is that it applies to activities that are themselves characteristically human, if not uniquely so. Handedness is most obvious in tool use, as in writing, hammering, throwing, or, in the present age, texting. Such activities are indeed essentially and in most cases uniquely human, although whether they place us close to angels may be debated. Moreover, it is generally agreed that true language is uniquely human (e.g., Chomsky, 1966; Pinker, 1994). Hence any sense of human uniqueness applies to the activities that are lateralized, rather than to the lateralization itself.

41.4.1 Cerebral Asymmetry

The left-cerebral dominance for language nevertheless remains a distinctive feature of the human brain, and may well be more pronounced than asymmetries associated with communication in other species, although this has yet to be established. Patients with damage to the language-mediating areas of the left hemisphere effectively lose the power of speech or of comprehension if the damage occurs in adolescence or adulthood. Since the pioneering discoveries of Broca (1861), speech production has been typically identified with an area in the third frontal convolution of the left hemisphere, known as Broca's area, but more exacting analysis now suggests that the left precentral area of the insula, a cortical structure underling the frontal and temporal lobes, may be more critical (Dronkers, 1996). The important characteristic of language that distinguishes it from other forms of communication is grammar, and although grammar has also been associated with Broca's area, it probably also involves widespread and diffuse regions of the left hemisphere (Dick *et al.*, 2001).

Cerebral asymmetry for language was corroborated by Sperry's work on the split brain, which again revealed that only the left side of the brain was capable of producing articulate speech (Sperry, 1966–1967, 1974, 1982). This work also rather surprisingly showed that the right hemispheres of at least some of these patients were capable of comprehension, albeit at a less sophisticated level than that displayed by the left hemisphere (Zaidel, 1976). Gazzaniga (1983) has maintained, however, that right-hemisphere comprehension is the exception rather than the rule among commissurotomized patients, but Zaidel (1983) has in turn disputed this. This issue remains unresolved.

Brain imaging has further confirmed the dominant role of the left hemisphere in language. Broca's area is typically larger on the left than on the right in most people (Foundas *et al.*, 1995, 1996), as is the temporal planum, as we have seen. We have also seen that the asymmetry of the temporal planum appears to be present in chimpanzees, suggesting that the asymmetry may not be related to language *per se*, but may originate in a left-hemisphere advantage in the processing of species-specific vocalizations. Nevertheless any such asymmetry was no doubt carried over into the processing of language in humans. Functional imaging also shows that that areas of the left hemisphere are activated during both the production (e.g., Huang *et al.*, 2002; Heim and Friederici, 2003) and comprehension (e.g., Springer *et al.*, 1999) of spoken language. Left-hemisphere dominance for speech perception is evident from functional magnetic resonance imaging (fMRI) recordings even in 3-month-old infants (Dehaene-Lambertz *et al.*, 2002).

Although the right hemisphere has some involvement in language processing (e.g., Gernsbacher and Kaschak, 2003), it is clear that in most people language is largely a left-hemispheric enterprise, and indeed occupies widespread circuits in that hemisphere. It is probably the sheer complexity of language, therefore, that makes the asymmetry stand out. It may also explain a striking right-hemisphere dominance for spatial attention. Patients with lesions to the right hemisphere often show a neglect of the left side of space, whereas those with comparable lesions of the left side do not show the reverse asymmetry, or show it only transiently. This follows especially from lesions in the posterior half of the brain, and although the parietal lobe is usually implicated, it has been claimed that the critical area involves the temporal lobe rather than the parietal lobe. According to Karnath *et al.* (2001), this area is homologous to Wernicke's area in the left hemisphere, suggesting that the asymmetry may have been a secondary consequence of language representation in the left hemisphere (cf. Corballis, 1991). There is no evidence for any asymmetry in spatial attention in animals comparable to that

demonstrated by left hemineglect in humans (Driver and Vuilleumier, 2001), although it could be argued that it is related to the right-hemispheric advantage for more global aspects of perception, which has also been documented in birds, as outlined earlier.

41.4.2 Handedness

Right-handedness in humans may also be a consequence of cerebral asymmetry for speech. Along with others, I have argued that language itself may derive from manual gestures, rather than from primate calls (Hewes, 1973; Armstrong *et al.*, 1995; Givón, 1995; Rizzolatti and Arbib, 1998; Corballis, 2001a). If this is so, the shift from gestural to vocal language was presumably gradual, so that language for much of our recent evolutionary history was a combination of the two. As we saw earlier, left-hemispheric control of vocalization may go back far in evolution, perhaps to the origins of the vocalization, so the gradual assimilation of vocalization into the language system may have lateralized the neural circuits involved (Corballis, 2003). Indeed, people habitually gesture with their hands while they speak, and in right-handers the right hand predominates (Kimura, 1973). This may have had a spin-off. As vocalization gradually took over, so the hands were released for other activities, such as toolmaking, resulting in the manual specialization that we see in present-day human activities. The release of the hands, and along with it the release also of right-handedness, may also explain what has come to be termed the human revolution that took place some 40 000 years ago in Europe and probably earlier in Africa (Corballis, 2004).

This account explains why the extreme right-handedness observed in the human population is not evident in nonhuman primates. It is likely, though, that right-handedness itself was a species-wide characteristic well before 40 000 years ago. Cornford (1986) analyzed the asymmetries of flakes recovered from La Cotte de St. Brelade in Jersey, dating from 150 000 to 200 000 years ago, and estimated that the incidence of right-handedness among the toolmakers was between 80% and 90%, which is close to present-day estimates. Toth (1985) provides even earlier estimates from asymmetrical flakes found in Lower Pleistocene sites at Koobi Fora in Kenya, dated at 1.4–1.9 Mya. Flakes favoring right-handed action outnumbered those favoring left-handed action in a ratio of about 57:43. Although this bias may seem relatively slight, Toth found that the same ratio was obtained by present-day right-handers given a similar task, which suggested to McManus (1999, 2002) that right-handedness was universal among the early flakers, and that left-handedness was the result of a later mutation. Yet the ratio observed by Toth is not dissimilar to the ratio of right- to left-handers among present-day captive chimpanzees, as reported by Hopkins (1996), and I have suggested that a later mutation may have raised the ratio from 2:1 to about 8:1 (Corballis, 1997). A more conservative conclusion, more aligned with my current thinking, is that right-handedness emerged gradually as vocalization increasingly accompanied manual gesture in the evolution of language. However, the data do not yet permit a clear distinction between big bang and continuity theories of the emergence of handedness.

41.5 Genetic Models

Regardless of which of these theories is correct, there seems good reason to suppose that handedness is at least partially under genetic control. Despite the strong human bias toward right-handedness, a minority of the population remains stubbornly left-handed, and a few ambidextrous individuals display no overall preference. Similarly, the right hemisphere controls language in a minority of people, and in some language appears to be represented bilaterally. The asymmetry in language representation, moreover, is loosely correlated with handedness (Knecht *et al.*, 2000). There is at least a weak parental influence on handedness, as revealed in data summarized by McManus and Bryden (1992) and shown in Table 1. Although it is clear that handedness does not 'breed true', single-gene models can accommodate the data reasonably well.

Over 30 years ago, Annett (1972) proposed that the true distinction was not between left- and right-handers, but between those carrying a right shift (RS) factor and those not carrying this factor; in more recent terminology, there is a right-shift allele, RS+, and an allele without directional specification, RS–. To put it simply, right-handedness is inherited but left-handedness is not (Annett, 2002). It should

Table 1 Percentage of left-handed offspring by parental combination, and prediction from McManus' model

	Parental handedness		
	R R	*R L*	*L L*
% Left-handed offspring	9.5	19.5	26.1
Predicted by McManus' model with *p*(D) 0.76	9.45	20.24	28.87

be emphasized, though, that in Annett's model most of the variation in handedness is random, and the RS+ allele shifts a normal distribution of intermanual differences to the right. For individuals homozygous for the RS+ allele, designated RS++, the shift is about two standard deviations to the right of neutrality; for heterozygotes, designated RS+–, the shift is about one standard deviation to the right; and for those homozygous for the RS– allele, designated RS– –, the distribution is centered on the point of neutrality, that is, the direction of handedness is essentially assigned at random. Annett also makes it clear that the RS gene influences cerebral dominance rather than handedness *per se*. (Since the left hemisphere controls the right hand, it should really be termed a left-shift gene.)

The idea that genes can influence the presence versus the absence of an asymmetry, rather than the direction of the asymmetry, may be a general principle in the genetics of asymmetry (Morgan and Corballis, 1978) and applies for example to the asymmetry of the heart and other visceral organs (Layton, 1976). The same principle is embodied in McManus' (1999) genetic model of handedness. Like Annett, McManus proposes a two-allele gene, with a dextral (D) allele specifying right-handedness and a chance (C) allele, which does not specify the direction of handedness, but leaves it to chance. Unlike Annett, though, McManus argues that handedness is fundamentally dichotomous, so that all DD individuals are right-handed, 75% of CD individuals are right-handed, and CC individuals are equally divided between left- and right-handers. This model makes predictions about the inheritance of handedness that are essentially indistinguishable from those of Annett's model. Table 1 shows how McManus' version fits the data, with the proportion p(D) of D alleles in the population estimated at 0.76.

This estimate might seem high, but perhaps reflects a society in which dextrality, or left cerebral dominance, has greater adaptive fitness than the lack of consistent handedness or cerebral dominance. Variations in this parameter might explain cultural differences in handedness, although the heterozygotic advantage ensures that both alleles are maintained in the population. Figure 1 shows how the asymptotic value of p(D) varies depending on the relative fitness of CC and DD genotypes.

The models can also account for the relations between handedness and cerebral dominance for language. Studies based on the Wada test (Rasmussen and Milner, 1977), electroconvulsive therapy (ECT) (Warrington and Pratt, 1973), and brain imaging (Pujol *et al.*, 1999; Knecht *et al.*, 2000) are reasonably consistent in showing that over 90% of right-handers are left-cerebrally dominant for language, as are some 70% of left-handers. McManus' model, on the assumption that p(D) = 0.76 and that handedness and cerebral asymmetry are assigned independently in CD and CC genotypes, predicts that 90.6% of right-handers and 69% of left-handers will be left-cerebrally dominant for language, figures reasonably close to empirically determined values. Annett's model makes similar predictions.

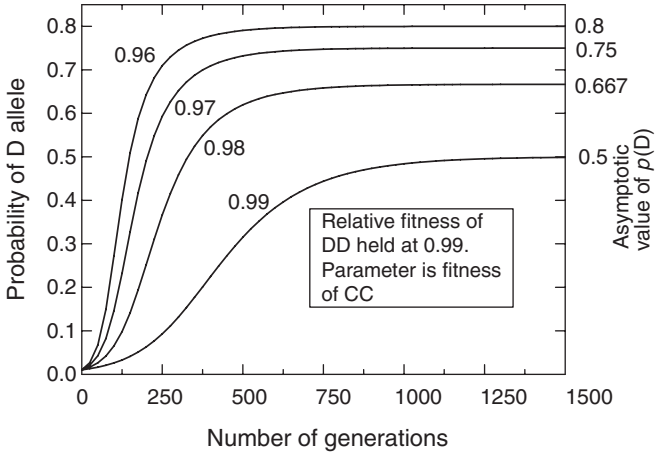

Figure 1 Suppose a mutation occurs creating a dextral allele D in 1% of a population. The figure shows the increase in the probability of the D allele, p(D), over successive generations, given a fitness advantage to the CD genotype. In this example, the relative fitness of the CD genotype is held at 1.0 and that of the DD genotype at 0.99. The rise of the D allele is shown for four different fitnesses: 0.99, 0.98, 0.97, and 0.96, yielding different asymptotic values of p(D). The asymptote would be less than 0.5 if the fitness of the CC genotype were to exceed that of the DD genotype. At asymptote, the ratio of p(D):p(C) is given by (1 f_{CC}):(1 f_{DD}), where f_{DD} and f_{CC} are the fitnesses of DD and CC genotypes relative to that of the CD genotype. Reprinted from Corballis, M. C. 1997. The genetics and evolution of handedness. *Psychol. Rev.* 104, 714 727, with permission from APA.

Both Annett and McManus assume that the mutation that gave rise to the RS+ or D allele occurred in hominid evolution. McManus (1999) has speculated that it may have occurred in a copy of one of the genes creating the leftward asymmetry of the heart, perhaps allowing the asymmetry to affect neural tissue as well as the heart rudiment. It seems entirely possible, though, that similar genetic influences may explain the cerebral asymmetries evident in other species. We have seen, in fact, that the proportion of individuals that reverse the population-level bias ranges from approximately 10% to approximately 35% (Ghirlanda and Vallortigara, 2004), and in fact both extremes are evident in humans. Previc (1991) has summarized evidence on what he terms natural forms of auditory and motor asymmetries in humans, and these favor one side over the other in a ratio of 2:1. They include the right-ear advantage in dichotic listening, right-eye dominance, a host of postural asymmetries, and a tendency, especially among newborns, to turn the head to the right. These asymmetries may relate to the fact that about two-thirds of human fetuses are confined to an asymmetrical fetal position, with the right side facing toward the mother's front, during the final trimester. Consequently, approximately 33% of people reverse this asymmetry. The variations between these different asymmetry ratios may reflect the relative fitnesses of homozygotes, under the assumption of an overall heterozygotic advantage, as illustrated in Figure 1.

The gene or genes that create these biases remain hypothetical, although there have been some leads. For example, Crow (2002) has suggested that the laterality gene is located in the *Xq21.3/Yp11.2* region of homology on the X and Y chromosomes, and suggested protocadherin XY as a likely candidate. I have myself argued against this on the grounds that polymorphisms are unstable on the Y chromosome, but suggested, following McKeever (2000), that the gene may be on the X chromosome (Corballis, 2001b). However, a genome-wide search for the handedness gene has since offered little support for X-linkage, and suggests that the region *2p11.2-12* on chromosome 2 may be a better bet (Francks *et al.*, 2002). Although the authors report that this failed to replicate in an independent sample, a further analysis has revealed significant paternal linkage within this site (Francks *et al.*, 2003), suggesting that imprinting may play a role. It is perhaps unlikely that the single-gene models proposed by Annett and McManus will provide the whole answer, but they provide a useful starting point.

41.5.1 The Trade-Off between Symmetry and Specialization: A Genetic Perspective

The models proposed by Annett and McManus capture something of the trade-off between symmetry and asymmetry, at least insofar as the D allele stands for asymmetry and the C allele for symmetry. (For simplicity, I use McManus' terminology rather than Annett's, but I do not mean to imply that one model is to be preferred to the other.) Thus DD genotypes are strongly lateralized, with right-handedness and left-cerebral dominance for language, whereas CC genotypes are subject only to random lateralizing influences. The two genes are held in balance by the superior fitness of the CD genotype.

Part of the trade-off may have to do with the relative advantages of language and spatial ability. There is some evidence that left-cerebral dominance for language is achieved through the pruning of the right hemisphere, with some loss of spatial function (Annett, 2002). Thus DD individuals may benefit from having language mechanisms contained within a hemisphere, but while displaying superior skills of oratory may tend to get lost on the way to the Forum. CC individuals may be at risk of divided hemispheric control over speech, with increased risk of stuttering (Foundas *et al.*, 2003) and reading disability (Annett, 2002), but may benefit from greater spatial awareness and more balanced motor skill. Heterozygotic DC individuals may be less prone to either deficiency, and so have the better of both worlds.

A study of 12 770 11-year-olds in the United Kingdom suggests, however, that the academic deficits shown by CC individuals may extend beyond language abilities. Handedness in these children was assessed in a test of skill (checking squares) and ranged from extreme left- to extreme right-handedness. Their scores on tests of verbal ability, nonverbal ability, reading comprehension, and mathematical ability showed a pronounced dip at the point of equality between the hands (Crow *et al.*, 1998). That is, both left- and right-handers scored above those who were ambidextrous. Since CC individuals were more likely to be represented at the point of equality than either DC or DD individuals, this result suggests that they are at greater risk of poor academic performance. This result was not replicated in a German study by Mayringer and Wimmer (2002), who tested a smaller (but still large) sample of 530 boys. Crow *et al.* (1998) refer to ambidexterity as "the point of hemispheric indecision"; the symmetrical brain, so to speak, is unable to make up its mind. The risk of impediment in CC individuals is still relatively small, since many with

this genotype will display asymmetry in one or the other direction by chance.

What, then, are compensatory advantages of a bilaterally symmetrical brain? Many prominent athletes and sportspeople have mixed dominance, and may derive their benefit from an extra degree of perceptual and motor balance. Annett (2002) also claims that surgeons include a disproportionately large number of non-right-handers. It is possible that the benefits of altered handedness derive precisely from being in a minority. Suppose, for example, that members of a group tend to stick together to avoid predation and run off to the left when a predator threatens. By being one of many, each individual is less likely to be singled out by the predator. The predator may nevertheless choose to attack the mob rather than the strays, since the chances of catching at least one victim are maximized. Some individuals may therefore benefit from joining a minority that veers off to the right, a strategy that works only if this group remains a minority. This may have resulted in a subtle selection dynamic that held left- and right-turning in balance (Ghirlanda and Vallortigara, 2004), but with left-turning implying a right-hemisphere dominance for this behavior, maintained for the majority. One might argue similarly that left-handers hold an advantage in fighting, but only so long as they are in the minority (Raymond *et al.*, 1996).

There is also some reason to suppose that the lack of consistent cerebral asymmetry may lead to different styles of thought. In a study of magical ideation and handedness, Barnett and Corballis (2002) reported a relationship that was exactly the reverse of that reported for intellectual achievements by Crow *et al.* (1998). People with mixed-handedness were the most prone to magical ideation, characterized by mild paranoia and superstition, and scores on magical ideation decreased systematically as handedness became more extreme in either direction.

There is also evidence that mixed-handedness – or perhaps hemispheric indecision (Crow *et al.*, 1998) – is associated with a greater sensitivity to sensory illusions (Niebauer *et al.*, 2002) – which was not replicated, however, in a study by Barnett-Cowan and Peters (2004) – and a higher risk of schizophrenia (Claridge *et al.*, 1998; Upadhyay *et al.*, 2004) and strong belief in the paranormal seems to be associated with symmetrical brain activity (Pizzagalli *et al.*, 2000). Another study has shown that people who score relatively low on magical ideation show a left visual field advantage on a lexical-decision task, whereas those who score relatively high show no difference between visual fields, implying a lack of cerebral dominance (Pizzagalli *et al.*, 2001).

Jaynes (1976) speculated that cerebral asymmetry emerged in the second millennium BCE, in response to assorted catastrophes, such as floods, invasions, and the like. Prior to this, people were governed by hallucinations, invoking the gods, but cerebral asymmetry allowed the left hemisphere to create a sense of self, so that people took responsibility for their own actions. Jaynes' theory makes little evolutionary sense, since handedness and cerebral asymmetry almost certainly go back at least 2 My, and perhaps even earlier, in hominid evolution (Corballis, 1997). Nevertheless, there may well be some truth to the idea that cerebral asymmetry underlies rational thought, and that a lack of asymmetry may well lead to more delusional and perhaps hallucinatory thought processes.

At least some of the characteristics associated with the lack of cerebral asymmetry, including paranormal experience, hallucinations, and so on, may be linked to religion. Although religious activities may seem irrational and sometimes counterproductive, it has been argued that religious behaviors has been favored by selection because they promote intergroup alliances (Hayden, 1987). Others have argued that religion is a system used by elites to maintain social control (e.g., Cronk, 1994). While this implies a social rather than an evolutionary origin, there may well have been selection of those predisposed to accept arbitrary leadership. Obedience is a common religious virtue. Religion is undoubtedly a complex phenomenon, but still a universal one, and is in many respects at odds with scientific rationalism. This raises the possibility that the trade-off between symmetry and asymmetry, or the C and D alleles, may have a bearing on the age-old struggle between religion and science, as exemplified in religious opposition to such scientific luminaries as Charles Darwin and Galileo.

Nevertheless, magical thinking may also be related to creativity, with positive implications for science and mathematics. Leonhard and Brugger (1988) note a link between paranormal thought, delusional thought, and creativity, and suggest that these characteristics relate to heightened right-hemispheric activation and relatively coarse semantic activation in that hemisphere. This in turn results in a loosening of associations and enhanced creativity. Although Leonhard and Brugger's account focuses on the right hemisphere, it is possible that the profile has to do with lack of cerebral dominance rather that any specialization of the right hemisphere itself. Despite the evidence of Crow *et al.* (1998) that mixed-handers are deficient in

arithmetic ability, Singh and O'Boyle (2004) report that mathematically gifted adolescents show no hemispheric asymmetry on tasks involving global–local judgments and matching letters, whereas average-ability adolescents and college students show a left-hemispheric advantage, suggesting that the mathematically gifted may lack consistent cerebral asymmetry. Although Singh and O'Boyle selected right-handers for this study, they also characterize the mathematically gifted as "typically male, left-handed, and myopic" (Singh and O'Boyle, 2004, p. 371).

Two individuals who may serve as CC icons are Leonardo da Vinci and Albert Einstein. Leonardo – the prototypical Renaissance Man, artist, scientist, and inventor – is generally regarded as being several centuries before his time. He was left-handed, and habitually wrote backwards, in mirror writing, but was also capable of writing normally. Einstein seems to have been right-handed, but was said to be slow to develop speech and a slow learner, and postmortem analysis of his brain revealed "an unusual symmetry between the hemispheres" (Witelson *et al.*, 1999, p. 2151), especially in the occipital and parietal lobes. He is also reported to have declared "I want to know how God created this world."

41.6 Conclusions

The brain and nervous system are built according to a plan that is bilaterally symmetrical. This probably goes back to the first organisms that moved linearly, perhaps even earlier than Bilateria, the lineage that includes nearly all present-day insects and animals, from humans to fruit flies to nematodes. Yet bilateral symmetry is readily broken if there are advantages in lateralization of function. Human handedness and cerebral asymmetry are examples, but there are countless other examples in the animal world, some of which are precursors to our own characteristic lopsidedness. Yet these asymmetries are not absolute. There is considerable overlap of function even in the lateralized human brain, and there is nearly always a minority of individuals within each lateralized species who show the opposite direction of asymmetry, although this may be born of chance fluctuation rather than systematic reversal. This minority appears to range from approximately 10% to some 35%, a range that may also apply to asymmetries that occur within our own species. Again, this suggests phylogenetic continuity rather than human uniqueness.

Genetic theories can explain a number of features of individual differences, at least in humans. The simplest theories are those in which there is a single laterality gene, with two alleles, one specifying a directional asymmetry and the other leaving the direction of asymmetry to chance. Such models remain speculative, since there is no sure evidence as to where the gene might be located in the genome, or even whether such a gene exists. If there is a genetic component to cerebral asymmetry, it may well provide an important basis for individual differences. Despite the widespread belief that right-handedness and left-cerebral dominance for language arose from a genetic mutation that occurred in hominid evolution, the more parsimonious view is that the same mechanisms that underlie these asymmetries also underlie the asymmetries observed in other species. A balance between directional and chance influences is then maintained by a heterozygotic advantage, and the relative costs of the two homozygotic genotypes then determines the relative proportions of lateralized and nonlateralized individuals. This, then, could be the universal mechanism underlying the trade-off between symmetry and specialization.

In any event, the widespread notion that cerebral asymmetry is uniquely human is wrong. Nevertheless, some of the functions that are lateralized in the human brain may well be unique to our species. This applies especially to language, which requires widespread neural circuitry, and if lateralized occupies a good proportion of the hemisphere in which it is housed. This in turn may have created a complementary asymmetry in the other side of the brain for spatial attention. Hand skill is another characteristic that is exceptionally highly developed in humans, and is an asymmetry that is strikingly obvious in everyday human behavior. Yet not all humans show this high degree of asymmetry, and there may be some advantages to an unlateralized brain that offset the advantages of lateralization. Some of these advantages may come about precisely because those who possess them may be in a minority. If nearly all tennis players were left-handed, the advantage of surprise and unorthodoxy would be transferred to the right-handed. If a lack of cerebral dominance were also a characteristic of creative visionaries, the impact of such people would be lessened if we were all like that. The world needs accountants.

Is there a common source for the evolution of lateralization? Perhaps the best candidate is the mouth. MacNeilage (1998) has proposed that speech is based on masticatory movements of the mouth, and Andrew *et al.* (2000) note that left cerebral control of the mouth and its internal structures is widespread in vertebrates. The left-hemispheric control of speech may therefore have

ancient origins. I have proposed that handedness may then have come about because of the association of speech and manual gesture (Corballis, 2003): if language went from hand to mouth, so lateralization went from mouth to hand. But there are perhaps earlier associations between hand and mouth that could have favored the right-hand preference. In some animals, the forelimbs are involved in bringing food to the mouth. We have seen that walruses show a general preference for the right flipper in feeding, and there is controversial evidence that the great apes also show a right-hand preference for a number of activities, including feeding (Hopkins, 1996). Any such preference, however, might be countered by the advantages of bilaterality, allowing an animal to reach with equal facility to either side. The mouth is also a more general manipulative organ, and manipulation also involves the hands, so that right-handedness may have emerged in the context of manipulation, again driven by lateralized control of the mouth. These hand–mouth associations are likely to have been especially decisive in the most manipulative of creatures, *H. sapiens*.

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42 Neurological Specializations for Manual Gesture and Tool Use in Humans

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Glossary

apraxia	A deficit affecting manual skills that can not be attributed to elementary sensory or motor disturbances. Generally pro nounced when pantomiming or imitating transitive actions.
prehension	Manual reaching, grasping, and object manipulation.
transitive actions	Behaviors that involve the use of objects other than one's own body (e.g., using tools or utensils).

42.1 Neural Bases of Manual Prehension in Primates

Establishing homologies between brain structures of species whose most recent common ancestor lived 30 Mya is a nontrivial challenge (Kaas and Reiner, 1999). This is made even more difficult when comparisons involve contrasting the response properties of single neurons in macaques with indirect measures of the activity of several million neurons in the human brain recorded with functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) (Orban *et al.*, 2004). With growing evidence for differences between human and macaque cortical architecture (Preuss and Coleman, 2002) including areas of the dorsal visual processing stream that are implicated in manual prehension (Vanduffel *et al.*, 2002; Orban *et al.*, 2004), caution is warranted when evaluating claims of homologies between species. Nevertheless, human research in this area is guided largely by results in macaques, and, as reviewed below, there appear to be marked similarities between species in the gross organization of systems involved in manual actions.

42.1.1 Ventral and Dorsal Pathways

The extrastriate visual areas of nonhuman primates are grossly organized into two primary pathways with significant reciprocal interconnections (Morel and Bullier, 1990): (1) an occipital temporal (i.e., ventral) stream; and (2) an occipital parietal (i.e., dorsal) stream (Ungerleider and Mishkin, 1982; Mishkin *et al.*, 1983; Felleman and Van Essen, 1991). Areas within these streams are also reciprocally interconnected with regions of prefrontal cortex involved in working memory and planning, and their sensory responses may be modulated via feedback from these higher cognitive centers (Wilson *et al.*, 1993; Goldman-Rakic, 1996). This organization is found in both Old and New World monkeys (Preuss and Goldman-Rakic, 1991), and it is believed to be relatively preserved in human beings (Ungerleider and Haxby, 1994; Ungerleider *et al.*, 1998; Braddick *et al.*, 2000). It is generally agreed that areas within the dorsal stream constitute the major source of input to premotor regions of frontal cortex involved in the organization and control of action (Jeannerod *et al.*, 1995; Johnson *et al.*, 1996; Andersen *et al.*, 1997; Luppino and

Rizzolatti, 2000; Marconi *et al.*, 2001; Rizzolatti and Luppino, 2001).

42.1.2 Subdivisions Within the Dorsal Pathway

As illustrated in Figure 1, on the basis of anatomical connectivity with frontal cortex, the macaque's dorsal visual pathway can be subdivided further into dorsal–dorsal (d–d) and ventral–dorsal (v–d) streams (Rizzolatti and Matelli, 2003). Inputs to dorsal (PMd) or ventral premotor cortex (PMv) arise from segregated regions of parietal cortex (Tanne-Gariepy *et al.*, 2002), and these frontal areas are not highly interconnected. In macaques, the primary visual input to the d–d stream is from area V6 along with V6A, parietooccipital (PO), and medial intraparietal (MIP) area in the superior parietal lobule (SPL).

The primary visual input to the v–d pathway is from areas MT/MST (middle temporal/medial superior temporal) along with visual areas of the inferior parietal lobule (IPL: anterior intraparietal area (AIP), PG, PFG, and PF). The v–d pathway projects to PMv cortex and can be further subdivided: neural tracing studies demonstrate that parietal area AIP projects to F5, while the adjacent ventral intraparietal area (VIP) projects selectively to F4 (Luppino *et al.*, 1999). As discussed below, these subdivisions may be involved in constructing representations for grasping objects versus delineating peripersonal space, respectively.

Consensus on the functional significance of the v–d and d–d subdivisions of the dorsal stream is currently

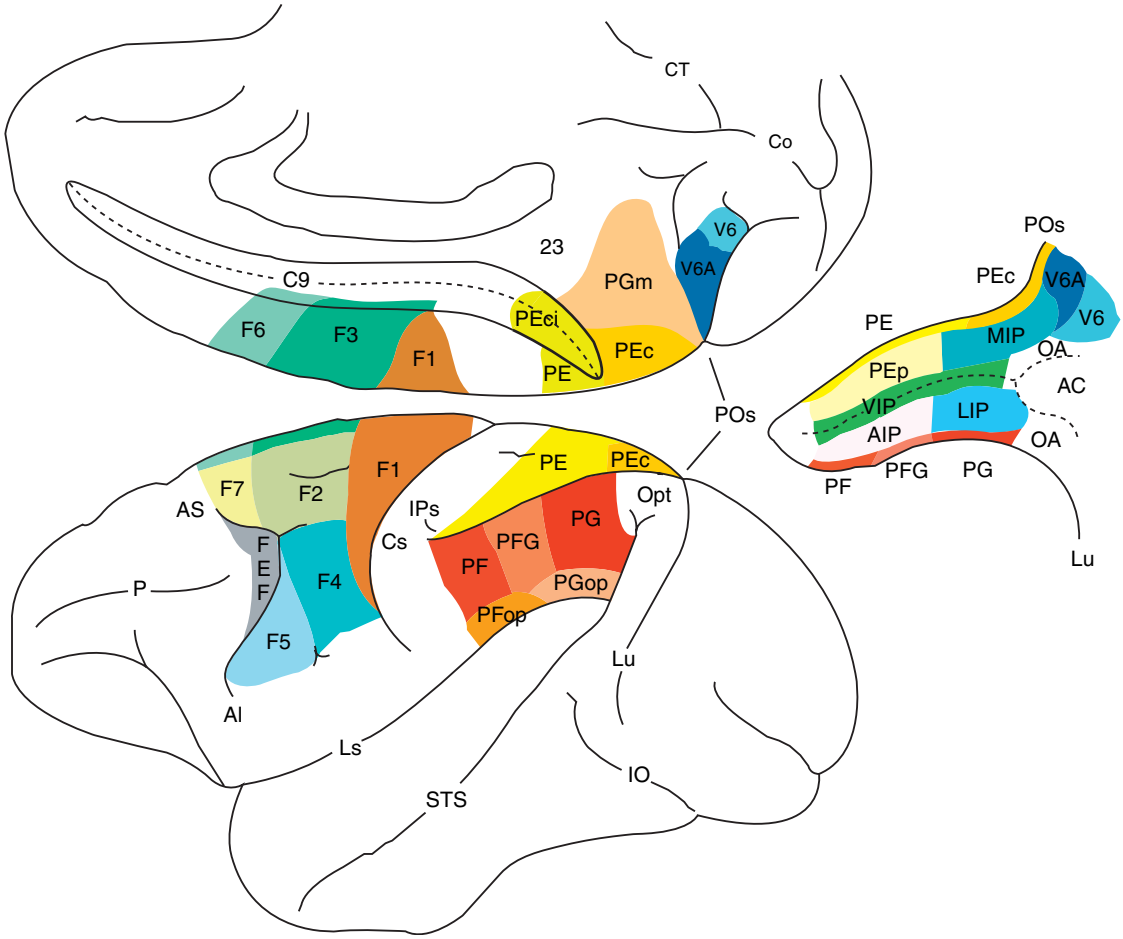

Figure 1 A schematic representation of the parietal and frontal areas of the macaque brain involved in manual prehension. Frontal areas are labeled according to the systems of Matelli and colleagues (Matelli *et al.*, 1985, 1991), and parietal areas use the system of Pandya and Seltzer (Pandya and Seltzer, 1982). Lower: View of the lateral surface of the left cerebral hemisphere. Upper: The medial surface of the left cerebral hemisphere. Inset: The intraparietal sulcus unfolded to reveal areas located within. These include medial (MIP), ventral (VIP), and anterior (AIP) intraparietal areas (from Rizzolatti and Matelli, 2003). AG, annectant gyrus; AI, inferior arcuate sulcus; AS, superior arcuate sulcus; Ca, calcarine sulcus; Cg, cingulated sulcus; Cs, central sulcus; Ls, lateral sulcus; Lu, lunate sulcus; OT, occipitotemporal sulcus; P, principal sulcus; POs, parietooccipital sulcus; STS, superior temporal sulcus. Reproduced from *Exp. Brain Res.*, vol. 153, 2003, pp. 146 157, Two different streams form the dorsal visual system; Anatomy and function, Rizzolatti, G. and Matelli, M. With kind permission of Springer Science and Business Media.

lacking. For the past decade the predominant view has been that reaching and grasping are controlled by parallel dorsal versus ventral visuomotor channels, respectively (Jeannerod *et al.*, 1995). Yet, as articulated by Tanne-Gariepy *et al.* (2002), accruing data suggest that the truth is likely more complex. PMd and PMv both contain representations of distal (F5) and proximal (F4) musculature of the upper limbs involved in grasping versus reaching, respectively. Likewise, both areas contain cells that code movement direction (Kakei *et al.*, 2001). As an alternative, Rizzolatti and Matelli (2003) hypothesize that the d–d pathway is exclusively involved in online motor control while the v–d stream participates in both the execution of prehensile actions as well as premovement organization (Glover, 2004). Another view is that premotor representations are entirely goal-specific and effector-independent (Rijntjes *et al.*, 1999). Consistent with this hypothesis are findings showing that microstimulation of motor and premotor cortex can evoke complex, multijoint movements that appear to be organized on the basis of the final position or action goal (e.g., bringing the grasping hand to the opening mouth regardless of the direction of movement) (Graziano *et al.*, 2002). Regardless of which organizational scheme is ultimately shown best to capture the properties of these systems, the majority of research in this area has been organized around the use of specific functional tasks. In the following sections I will consider evidence regarding those areas contributing to three requisites for dexterous manual prehension in macaques and humans: reaching, grasping, and the representation of peripersonal space.

42.1.3 The Dorsal–Dorsal Subdivision

Cells within the medial intraparietal sulcus of the SPL (area MIP), including the so-called parietal reach region (PRR), are involved in the representation of reaching actions (Wise *et al.*, 1997; Snyder *et al.*, 2000; Batista and Andersen, 2001; Andersen and Buneo, 2002). Area PMd also receives visual information via a direct connection with area PO (Caminiti *et al.*, 1996) and proprioceptive input via a circuit interconnecting PEc/PEip-F2 (Lacquaniti *et al.*, 1995; Matelli *et al.*, 1998). Neurons in PMd use this input to compute representations of both the location of visual targets and the direction of forelimb movements needed to acquire them (Johnson and Ferraina, 1996; Johnson *et al.*, 1993, 1996). A subpopulation of PMd neurons responds to specific combinations of sensory cues specifying target location and which limb to use when performing a manual pointing task, suggesting that single PMd units represent plans for specific reaching actions (Hoshi and Tanji, 2000).

Early PET studies identified activation within PMd, intraparietal sulcus (IPS), and SPL during reaching, pointing, and finger-tracking movements in humans (Colebatch *et al.*, 1991; Deiber *et al.*, 1991; Grafton *et al.*, 1992; Kertzman *et al.*, 1997). More recent findings using fMRI are consistent with these earlier results in suggesting the existence of a parietofrontal reach circuit in humans that can be activated by either overt movements (Connolly *et al.*, 2003) or motor imagery (Johnson *et al.*, 2002).

42.1.4 The Ventral–Dorsal Subdivision: Grasping

Single-unit electrophysiological recordings indicate that a parietofrontal circuit interconnecting areas AIP and F5 is involved in the transformation of sensory information into motor commands for grasping (Jeannerod *et al.*, 1995; Luppino and Rizzolatti, 2000; Rizzolatti and Luppino, 2001). Reversible inactivation studies with muscimol injections to either AIP (Gallese *et al.*, 1994) or F5 (Fogassi *et al.*, 2001) cause a selective deficit in visually guided grasping without affecting reaching. Electrophysiological recordings in macaques identified cells in the lateral bank of the IPS that are involved in the visual guidance of object-oriented hand movements (Taira *et al.*, 1990). A subpopulation of these cells are highly shape-selective in their responses (Sakata *et al.*, 1995). These object-selective cells can be divided into three categories on the basis of their receptive field (RF) properties. Motor dominant neurons require no visual input and therefore discharge in either the light or dark during manipulation. These cells do not respond to object fixation, and may therefore be coding hand movements necessary to engage objects. Visuomotor neurons respond strongest when objects are manipulated in the light, and less when either the hand or target object is invisible. A subpopulation of the visuomotor neurons also responds when the preferred object is fixated in the absence of manipulation, suggesting that these cells are coding hand movements relative to objects' visual properties. Finally, visual neurons only respond when an object is manipulated in the light, or when it is fixated. These cells are likely coding visual properties of objects that are useful for manipulation (Sakata *et al.*, 1995, 1997).

The object-selective cells within these three classes are distributed in a gradient along the lateral bank of the IPS. Visual neurons are found in higher concentrations in the lateral intraparietal (LIP) areas, known to also be involved in saccades (Colby *et al.*, 1995, 1996; Andersen *et al.*, 1998).

Movement-related motor and visuomotor units are also found in LIP, but are more concentrated immediately posterior to the primary somatosensory area (SI) hand representation in area AIP (Sakata *et al.*, 1995). Injections of a gamma-aminobutyric acid (GABA) agonist (muscimol) into area AIP cause a reversible deficit in preshaping the hand when grasping visual objects while leaving reaching intact (Gallese *et al.*, 1994).

Cells within area F5 of the macaque code the goals of specific prehensile actions rather than the movements of which they are composed. These units can be categorized on the basis of their RF properties into those that represent specific actions such as holding, grasping, or tearing objects. If the same hand movements are made as part of a different action, e.g., grooming instead of feeding, responses are weak or absent (Rizzolatti *et al.*, 1988). This observation has led to the hypothesis that area F5 contains a vocabulary of hand actions (Rizzolatti *et al.*, 1988), in which the goals of hand–object interactions are represented explicitly.

As discussed extensively in Michael Arbib's article, a subclass of neurons in area F5 (Gallese *et al.*, 1996; Rizzolatti *et al.*, 1996) and rostral inferior parietal cortex (PF; Rizzolatti and Craighero, 2004), known as mirror neurons, discharge not only when the monkey produces a specific action but also when it observes the experimenter undertake a comparable behavior (Rizzolatti and Luppino, 2001). This mirror system may be relevant to understanding the acquisition of complex skills through observation (Buccino *et al.*, 2004), as they provide a mechanism for mapping perceived actions on to one's own motor representations. Of potential relevance to the upcoming discussion of tool use, a recent paper reports tool-responding mirror neurons that respond selectively when macaques observe food items being captured with a simple tool and not the hand (Ferrari *et al.*, 2005).

On the basis of cytoarchitectonic similarities, it has been suggested that macaque area F5 is homologous with pars opercularis of the human inferior frontal gyrus (Petrides and Pandya, 1984; Preuss *et al.*, 1996), while F4 may be homologous with human inferior precentral gyrus (Rizzolatti *et al.*, 2002). Several studies report activation of pars opercularis as well as inferior precentral gyrus during visually guided grasping (Ehrsson *et al.*, 2000, 2001), haptic object manipulation (Binkofski *et al.*, 1999), and action observation (Iacoboni *et al.*, 1999; Johnson-Frey *et al.*, 2003). Because the RF characteristics of neurons in AIP and VIP are not strictly segregated, but rather distributed along a gradient (Sakata *et al.*, 1995), it may not be possible to differentiate these regions and adjacent areas of cortex (PF) using current neuroimaging techniques. I will therefore refer to the anterior portion of the human IPS and adjacent cortex as aIPS.

Recent fMRI studies identify activity within aIPS and surrounding cortex in tasks similar to those that evoke responses in cells of macaque AIP and/or VIP. Manipulation of complex versus simple shapes without vision is associated with mean activation in human aIPS (Binkofski *et al.*, 1999). This location is also activated during haptic recognition of shapes (Jancke *et al.*, 2001). Activation within this vicinity is also observed during object discrimination tasks involving both visual-to-tactile and tactile-to-visual transfer (Grefkes *et al.*, 2002). Activity associated with grasping visually presented three-dimensional objects (Binkofski *et al.*, 1998; Johnson-Frey *et al.*, 2005a), or grasping at two-dimensional projected objects (Culham *et al.*, 2003), is centered within a slightly more lateral and anterior site, as is visual discrimination of objects' surface orientations (Shikata *et al.*, 2001). Finally, as reviewed in Johnson-Frey (2004), a number of studies report activation in aIPS and surrounding areas of the IPL when viewing manipulable tools (Chao and Martin, 2000; Kellenbach *et al.*, 2003). Recent work using transcranial magnetic stimulation demonstrates that disruption of aIPS compromises the ability to update plans for visually guided grasping on the basis of rapidly changing visual feedback (Tunik *et al.*, 2005).

42.1.5 The Ventral–Dorsal Subdivision: Peripersonal Space

Electrophysiological investigations suggest that visuotactile representations of peripersonal space are constructed in a circuit connecting IPL area VIP with area F4 in PMv (Fogassi *et al.*, 1992, 1996). Area F4 contains a representation of the face, neck, trunk, and limbs and lies caudal to grasp-related area F5 (Figure 1). The majority of units in F4 are bimodal, having tactile RFs that are in register with three-dimensional visual RFs of space immediately adjacent to the animal and are not affected by variations in gaze direction. Similar RF properties can be found in area VIP (Colby *et al.*, 1993; Duhamel *et al.*, 1998), which provide direct afferent input to F4 (Luppino *et al.*, 1999). These observations have prompted the hypothesis that the VIP–F4 circuit constructs representations of peripersonal space in a frame of reference centered on the body part involved in a given visually guided action such as object manipulation (Graziano *et al.*, 1994; Fogassi *et al.*, 1996).

The paucity of neuroimaging studies attempting to identify areas of the human brain involved in the representation of peripersonal space may reflect the technical challenges associated with stimulus delivery and response recording. One successful PET investigation found differential activation in ventral premotor cortex and the IPS in association with bisecting lines located in near versus far space (Weiss *et al.*, 2000). While more work is necessary, these results are consistent with those predicted by the VIP–F4 circuit in macaques.

42.2 Primate Tool Use and the Ventral–Dorsal Stream

An interesting possibility to consider is whether tool use in primates might be accounted for by extensions of the sensorimotor mechanisms involved in manual prehension. Similar to using a limb, tool use involves implementing a sensorimotor transformation. However, this transformation must now include parameters that capture the physical and mechanical properties of the tool. Macaques can be trained to use simple tools in the laboratory, and this work suggests that these behaviors may involve acute plasticity within existing neural circuits involved in manual prehension. As noted above, cells in macaque area VIP have visuotactile RF properties similar to those observed in area F4 (Iriki *et al.*, 2001; Obayashi *et al.*, 2000). Interestingly, as illustrated in Figure 2, the RFs of these units appear to increase over time as Japanese macaques learn to use rakes to extend their reach (Iriki *et al.*, 1996). Specifically, visual RFs that are normally in register with tactile RFs of the hand expand to encompass peripersonal space now occupied by the tool. This expansion is only observed when tools are actively used to accomplish a goal-directed action (food retrieval), and not when tools are merely held or when physically similar yet ineffective tools are manipulated. Learning to use tools is associated with increased expression of brain-derived neurotrophic factor within the anterior bank of the IPS, that may reflect neuronal remodeling via axonal sprouting (Ishibashi *et al.*, 2002). Some neurons in PF and AIP also exhibit plasticity with tool use, but the highest density of such cells is

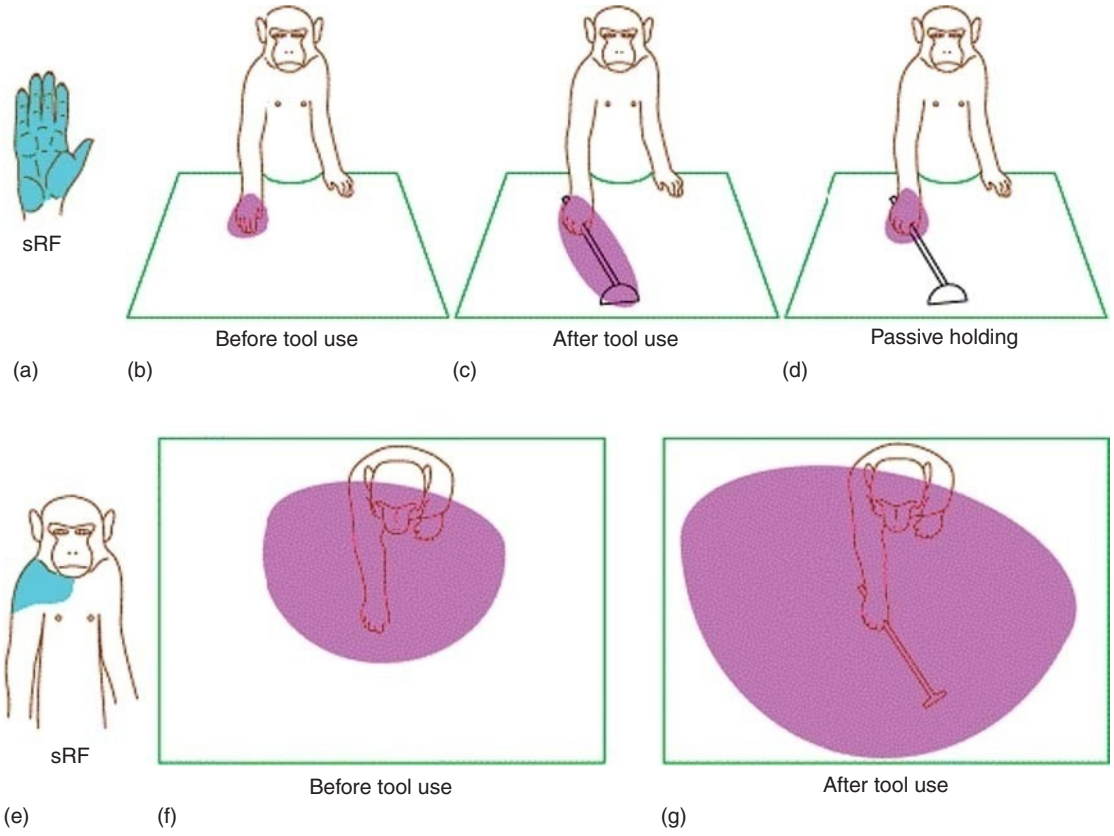

Figure 2 Experience-dependent changes in area VIP neurons with tool use. Distal-type neurons (top): Changes associated with extended tool use in the receptive field of a visuotactile neuron in area VIP that represents the distal forelimb. Proximal-type neurons (bottom): Changes associated with extended tool use in the receptive field of a visuotactile neuron in area VIP that represents the proximal forelimb. Reproduced from Maravita, A. and Iriki, A. 2004. Tools for the body (schema). *Trends Cogn. Sci.* 8, 79 86, Elsevier.

found on the other side of the IPS, i.e., roughly lateral (or anterior) to MIP including VIP and the fundus of the sulcus (Iriki, personal communication). A PET study reveals that performance of these simple tool use behaviors is accompanied by increased activity within a widely distributed network in the macaque brain including regions within v–d pathway (IPS and PMv), as well as basal ganglia, pre-supplementary motor area (pre-SMA), and cerebellum (Obayashi *et al.*, 2001).

Recent studies of humans with parietal lesions reveal behavioral effects that nicely complement the observations of expanded visuotactile hand representations with tool use in macaques (Farne and Ladavas, 2000; Maravita *et al.*, 2001; for a comprehensive review, see Maravita and Iriki, 2004). For instance, some patients suffering right parietal injuries demonstrate left hemineglect when performing a line bisection task positioned within reach. That is, they neglect portions of the line located to the left of fixation, biasing their estimates of center toward the right. However, when bisecting distant lines with a laser pointer they may perform normally. Importantly, when bisecting lines at a distance with a hand-held stick, one such patient again demonstrated neglect (Berti *et al.*, 2001). Similar to changes in the RF properties of IPS neurons in monkeys, use of a stick appears to cause distant space to be remapped as within reach, leading to neglect-related bias in performance. Likewise, there is kinematic evidence in healthy adults suggesting that the same motor representation may be involved in grasping with the fingers and grasping with a tool (Gentilucci *et al.*, 2004).

Results of an fMRI experiment with humans show that employing a set of tongs to extend one's grasp is associated with increased activity centered within the IPS (Inoue *et al.*, 2001). Curiously, these activations are ipsilateral rather than contralateral to the involved limb, as would be expected on the basis of macaque electrophysiology. Reasons for this pattern are uncertain and more work is necessary to determine the source. However, because the control condition involved performing the same grasping actions with the hand without a tool, it is possible that activations within the contralateral sensorimotor regions may have been eliminated during the subtractive comparison.

42.3 Human Specializations for Tool Use and Manual Gesture

Whether the explosion of tool use behaviors in hominids reflects the emergence of specialized brain mechanisms is an important and unresolved question. This seems probable for at least two reasons. First, human tool use differs in a variety of ways from that known to occur in nonhumans. Only hominids are known to fashion compound tools by joining together multiple parts and/or materials. Likewise, nonhominids do not appear to make one tool in order to create another (e.g., shaping a rock cutter to manufacture a wooden spear). This latter behavior has been taking place for a relatively long time; the available fossil record indicates that ancestral hominids were using rocks to manufacture stone-cutting implements for at least the past 2.5 million years (Ambrose, 2001). In contrast to other species in which tool use is typically found in specific subpopulations, tool use is a universal characteristic of all human cultures, and highly refined procedures for skillful use have co-evolved with complex tools, and are actively transmitted to successive generations.

Second, it is not possible to understand many complex forms of human tool use exclusively in terms of sensorimotor transformations (Johnson-Frey, 2003a; Johnson-Frey and Grafton, 2003). As is illustrated in Figure 3a, a variety of different postures can be used to achieve a stable grip that will enable an actor to grasp familiar tools stably for

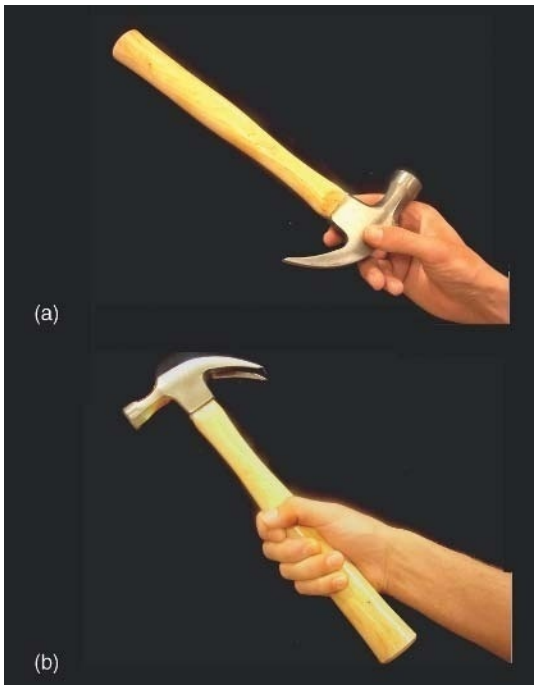

Figure 3 Different ways of grasping a familiar tool. a, The most stable way of gripping a hammer is at its perceived center of mass. b, However, effective use of a hammer requires adopting a less stable grip that is better suited to generating force when pounding. This latter grip depends on both perceived visual properties of the object and access to semantic knowledge.

purposes such as moving them from one location to another, handing them to another individual, or manipulating them. These solutions are clearly not suitable for using familiar tools to perform their ordinary functions, however. As shown in Figure 3b, grips appropriate for tool use frequently differ in nontrivial ways from those chosen solely on the basis of perceived structural information. In addition to mechanisms for online sensorimotor transformations, these actions are influenced by semantic knowledge of the objects' functional properties, the goals it can be used to accomplish, and the user's specific intentions on that occasion. The critical question for understanding these behaviors is how semantic knowledge interacts with and influences sensorimotor representations during the planning and performance of these skills (Johnson-Frey, 2003b). As detailed below, available evidence suggests that the human left cerebral hemisphere may be specialized for constructing representations of these meaningful actions (Johnson-Frey, 2004; Johnson-Frey *et al.*, 2005c; Lewis, 2006; Rumiati *et al.*, 2004).

The majority of what is known about the neural bases of skilled tool use in humans derives primarily from over a century of studies of apraxic patients who have difficulties with praxis skills that cannot be explained in terms of more elementary sensory or motor deficits. A review of this vast literature is well beyond the scope of this article. I will focus instead on findings from recent human neuroimaging studies that have attempted to delineate the neural substrates involved in planning and/or producing familiar tool use actions in the healthy brain.

42.3.1 Functional Neuroimaging Investigations of Human Tool Use

As summarized in a review (Johnson-Frey, 2004), the available data from functional neuroimaging studies have two points of convergence with the apraxia literature: First, while systems involved in sensorimotor control are organized in a largely contralateral fashion, the vast majority of evidence shows that mechanisms involved in representing familiar tool use actions are lateralized to the left cerebral hemisphere. Maximal lesion overlap in these patients is found in the left cerebral hemisphere in parietal regions (within and adjacent to the IPs, including angular and supramarginal gyri and ventral SPL) and/or the middle frontal gyrus (GFm) (Haaland *et al.*, 2000).

Second, within this left-lateralized system, mechanisms involved in representing conceptual knowledge about tools and their functions appear to be relatively independent yet interactive with those areas supporting the manual skills involved in their usage.

42.3.2 Conceptual-Level Representations

Functional neuroimaging studies show that naming tools selectively activates posterior-left middle temporal gyrus (MTG) (Martin *et al.*, 1996), an area that is also engaged when subjects generate action words (Martin *et al.*, 1995) or answer questions about tools (Chao *et al.*, 1999). Likewise, Damasio and colleagues report activation in this region when subjects identify actions or spatial relations performed with or without a tool (Damasio *et al.*, 2001). On the basis of its proximity to motion-processing centers (putative MT/MST) and its selectivity for manipulable versus nonmanipuable artifacts, it has been suggested that activations in left MTG may be involved in representing nonbiological motions associated with tool use (Heilman *et al.*, 1997; Chao *et al.*, 1999; Beauchamp *et al.*, 2002).

In addition to left posterior temporal cortex, functional neuroimaging studies consistently demonstrate that identification of tools and conceptualization of associated actions activate left frontal and anterior parietal areas. As introduced above, PMv is involved in visuomotor transformations for grasping and manipulating objects in both macaques (Rizzolatti *et al.*, 2002) and humans (Binkofski *et al.*, 1999). In the left hemisphere, this region is also activated when naming (Martin *et al.*, 1996; Chao and Martin, 2000) and viewing (Chao and Martin, 2000) tools, while a larger region including left GFm is activated when identifying the actions with which tools are associated (Grabowski *et al.*, 1998). Activations of left posterior parietal cortex (aIPS/supramarginal gyrus) are also frequently observed in association with these tasks (Martin *et al.*, 1996; Chao and Martin, 2000; Damasio *et al.*, 2001; Kellenbach *et al.*, 2003).

42.3.3 Production-Level Representations

In contrast to the sizable literature on conceptual-level action representations, relatively few studies have employed functional neuroimaging techniques to investigate mechanisms involved in organizing and/or producing tool use skills. When activations associated with complex yet meaningless finger and limb movements are removed, pantomiming tool use gestures with either hand activates left posterior parietal cortex in and around the IPS and dorsal lateral premotor cortex (Moll *et al.*, 2000). A similar pattern is also present when tool use pantomimes involving either hand are contrasted with repetitive

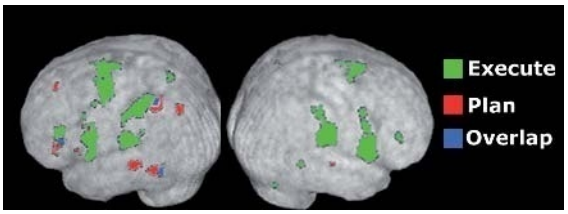

Figure 4 Human cortical regions associated with planning and executing tool use actions. When preparing to pantomime how familiar tools are used, areas of the left frontal, temporal, and parietal cortices are activated, regardless of the hand involved (red). During the execution of tool use pantomimes with either hand, additional regions of frontal and parietal cortex are engaged in both cerebral hemispheres (green). There is modest overlap between regions that are active during both planning and execution (blue). Reproduced from Johnson-Frey, S. H., Newman-Norlund, R., and Grafton, S. T. 2005a. A distributed left hemisphere network active during planning of everyday tool use skills. *Cereb. Cortex* 15, 681 695, by permission of Oxford University Press.

finger movements (Choi *et al.*, 2001), and when gestures are made as though the limb itself is the object (Ohgami *et al.*, 2004). A recent PET study of tool use pantomime found activations of left dorsolateral prefrontal cortex (DLFPC), inferior frontal cortex, and supramarginal gyrus when controlling for effects related to lexical and semantic processing (Rumiati *et al.*, 2004). Interestingly, none of these experiments observed activations in left GFm, as would be expected given the lesion analysis data introduced above. This is also true for results of two recent fMRI studies that used event-related designs to distinguish regions involved in planning (i.e., identifying, retrieving, and preparing actions associated with a familiar tool's usages) versus executing tool use gestures with the dominant right and nondominant left hands (Johnson-Frey *et al.*, 2005b). As illustrated in Figure 4, planning tool use actions for either limb activates a distributed network in the left cerebral hemisphere consisting of areas within the IPL (supramarginal and angular gyri) and frontal cortex (inferior frontal and ventral premotor cortices as well as DLFPC). These studies also detected activation during action planning within the ventral visual pathway (posterior superior temporal sulcus along with proximal regions of the MTG and superior temporal gyri). Involvement of these latter areas likely reflects activation of conceptual-level representations.

An important and unresolved question concerns whether these distributed cortical networks are specific to behaviors involving tools (i.e., transitive skills) or whether they are more generally involved in all manner of familiar skills, including those that do not involve objects (i.e., intransitive skills). In apraxia transitive gestures are often more substantially affected than intransitive gestures (Roy *et al.*, 1991; Rapcsak *et al.*, 1993) and both left and right brain-injured patients may show difficulties pantomiming and/or imitating intransitive actions (Heath *et al.*, 2001). There have been few studies investigating neural bases of familiar intransitive gestures to date, and unfortunately none that has directly contrasted familiar transitive and intransitive skills.

Although the focus has largely been on cortical contributions to skilled actions, both the apraxia (Pramstaller and Marsden, 1996; Hanna-Pladdy *et al.*, 2001) and neuroimaging literatures suggest involvement of subcortical structures. Imamizu and colleagues have demonstrated involvement of the posterior superior cerebellar fissure in learning to control computer mice with novel input–output mappings (Imamizu *et al.*, 2000, 2003).

42.4 Conclusions

In summary, results from studies of nonhuman primates indicate the existence of parallel parietofrontal networks involved in the organization and/or execution of prehensile behaviors. Functional neuroimaging studies suggest that aspects of this organization are preserved in the human brain. Findings in macaques demonstrate that use of a tool to extend reach induces an expansion of RFs within parietal visuotactile neurons. Specifically, representations of peripersonal space are remapped to include areas accessible with the tool. Along with complementary results in the human literature, these findings suggest that some forms of tool use may arise from relatively rapid, experience-dependent changes in existing sensorimotor circuits. More complex forms of tool use, that constitute a large potion of the human repertoire, demand input from semantic as well as sensorimotor representations, however. Data from apraxic patients and results of recent functional neuroimaging investigations of healthy adults converge on two points. First, the human left cerebral hemisphere is dominant for the representation of complex tool use actions. Second, although both are necessary for complex tool use behaviors, there appears to be a separation between conceptual and production-level representations.

The story of how complex manual actions are acquired and represented in the primate brain is only now beginning to unfold. Future efforts should be directed at identifying both the similarities in functional organization across primate species, as well as differences that may account for unique, species-specific behaviors.

Acknowledgment

Preparation of this manuscript was supported by a grant from NIMH (#MH002022-02).

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43 Frontal Cortex Evolution in Primates

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43.1 Primate-Specific Adaptations

The frontal cortex can be defined as the neocortex anterior to the motor somatosensory–cortex border. This is a large region in primates, containing areas involved directly or indirectly in the control of almost every behavior. It has long been thought that the frontal cortex played an important role in primate evolution. Modern evidence supports this view. Indeed, given the diversity of functions in the region and the variety of unique behaviors exhibited by primates, it would be surprising if the frontal cortex had not evolved unique adaptations.

The common ancestor of eutherian mammals probably had a small body and a small brain. Comparative work in modern mammals suggests it had a basic complement of cortical areas including primary visual, auditory, and somatosensory areas. It also probably had a primary motor area (M1). This means that we could define a frontal cortex in that mammal and that the region of the frontal cortex in modern eutherians (taken as a whole) can be thought of as being homologous.

It is likely however that this broad homology obscures substantial differences in frontal cortex structure between primates and nonprimates. One piece of evidence to this effect is that the frontal cortex scales differently in primates and nonprimates. In primates, the frontal cortex hyperscales with the brain size. This can be seen in the reconstructions in Figure 1 which show the brain of a small primate, the galago, and a larger primate, the macaque. A primate with a larger brain tends to have a disproportionately large frontal cortex. In contrast, in a nonprimate order, carnivores, the frontal cortex size does not vary systematically with brain size. This suggests that the structure and development of the frontal cortex differs substantially in the two orders.

When we focus on specific cortical areas and regions, we again find that the primate frontal cortex differs in important ways from that found in other orders. A variety of evidence suggests that the two main branches of primates, strepsirrhines and anthropoids, share up to ten motor areas in the frontal cortex. Of these, only one or two have clear homologs outside primates. Most eutherians have an agranular M1, with large layer 5 pyramidal cells, somatotopy, and relatively low thresholds of stimulation. Rostral and medial to M1 is a supplementary motor area (SMA, also called M2) in primates. It is also somatotopically organized, and has slightly smaller layer 5 pyramidals than M1. This area may have homologs in other mammals such as rats.

The remaining motor areas which are shared among primates appear not to have one-to-one homologs in other orders. Among these areas are several cingulate motor areas and a number of premotor areas involved in higher-level coordination of movement. Several of these are particularly interesting. The frontal eye field (FEF) is connected with both the sensory visual areas and the prefrontal cortex. Microstimulation here produces saccadic and smooth pursuit eye movements. The FEF may be involved in the voluntary control of eye movements, an attribute of great importance for highly visual primates. Strepsirrhines and haplorhines also share a ventral premotor area (PMV), which appears to have representations of the upper body. One possibility is that PMV is involved in the visually guided grasping of objects, something that would be especially important to a small visual predator. Early primates are thought to have been small arboreal visual predators, and it is tempting to view the evolution of primate motor areas in this light. These areas could be seen as motor adaptations of the earliest primates for a very demanding arboreal niche.

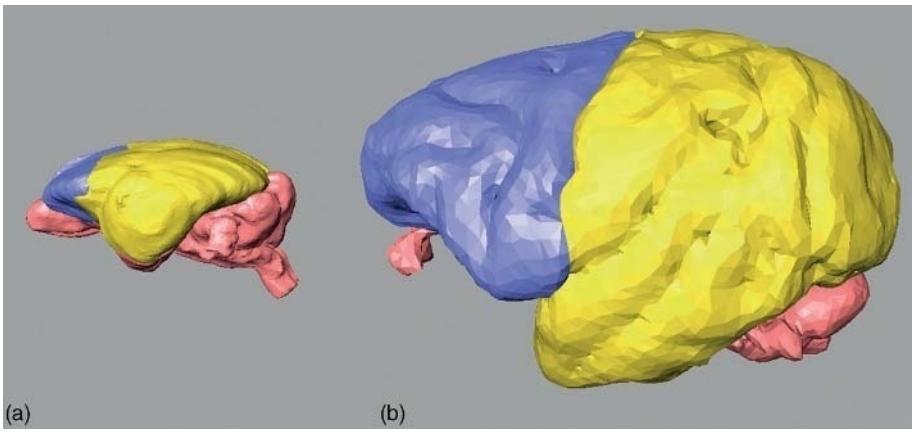

Figure 1 Reconstructions of the brains: (a) *Galago senegalensis*; (b) *Maccaca mulatta*. Frontal cortex is indicated in blue. The position of the motor-somatosensory cortex border was used to delineate frontal cortex. The two brains are shown here in their proper proportions relative to one another.

A frontal region of particular interest in primate evolution is the prefrontal cortex. This region has a wide variety of connections with other brain regions, and patients with prefrontal damage show deficits in the ability to plan and organize behavior. This suggests that it is involved in aspects of behavior which have been important in primate evolution.

The question of whether the prefrontal cortex is unique to primates has been controversial. Brodmann originally argued that it was, identifying prefrontal cortex with the well-developed granular layer 4 which is seen in primates. This was later disputed based on connectivity data. Today, there is general agreement that neither of these two types of data alone provides an adequate answer. However, there is less consensus on whether the prefrontal cortex is unique to primates. What is clear, however, is that even if a broadly homologous prefrontal cortex exists in other mammals, it is substantially different from that found in primates. There is no evidence that rats possess anything like the diversity of prefrontal areas found in primates, and many of the details of the primate prefrontal cortex are likely to be unique to the order.

43.1 Specializations within Primates

43.1.1 Anthropoids versus Strepsirrhines

There is also important variation in frontal cortical structure between the two large divisions of primates, anthropoids and strepsirrhines (represented by the macaque and galago, respectively). Among the premotor areas we have discussed, area PMV appears to have developed interesting adaptations in anthropoids. In monkeys, it seems to have an increasing representation of distal forelimb movements. At the same time, it has strong connections to parts of somatosensory cortex which represent information from the cutaneous receptors of the hands. Such information is particularly useful for reaching and grasping motions. These differences have led to the suggestion that anthropoid PMV evolved mechanisms for the improved guidance of reaching and grasping.

In terms of the broader arrangement of areas, however, the premotor cortices in the two groups have many similarities. As already mentioned, electrophysiological data suggest that the motor and premotor regions of anthropoids and strepsirrhines share up to ten areas. Architectonic evidence supports this view. This broad similarity in the arrangement of areas can be found in several other frontal regions. Comparisons in cytoarchitecture and myeloarchitecture suggest that anthropoids and strepsirrhines have a similar arrangement of areas in the orbital and medial regions of the frontal cortex.

In contrast, it seems that there are important differences between the two groups in the prefrontal cortex. Macaques appear to have a much larger number of areas in this region than galagos do. This is suggested by patterns of connectivity between the prefrontal cortex and other regions. Labeling experiments show that the prefrontal cortex in both species has numerous connections with other parts of the cortex. But the pattern of connectivity suggests that galago has fewer prefrontal areas. For example, tracer injections in regions of the parietal cortex produce fewer discrete zones of labeling in the galago prefrontal cortex than in the macaque. A similar result has been found in comparisons of architectonic parcelations in the two species.

Current architectonic and connectional data suggest that most prefrontal areas in galagos can be identified with a homologous area in the macaque. For example, the posterior-most prefrontal areas in galago appear to have homologs in the macaque arcuate cortex region. These conclusions reflect similarities in cell density and size, fiber-staining patterns, and connections to the parietal, superior temporal, and inferotemporal cortices. Similar conclusions have been reached about other areas in the galago prefrontal cortex (e.g., areas in the superior, polar, and ventral parts of the prefrontal cortex).

However, there are a number of areas in the macaque which have no clear homolog in galagos. These areas are concentrated especially around the principal sulcus in macaques, and they may represent anthropoid-specific specializations. This region of the cortex, often referred to as the dorsolateral prefrontal cortex, is thought to be involved in working memory. Classic lesion studies showed that monkeys with lesions in this region have deficits on delayed-response tasks. Such tasks may involve spatial working memory, for example, requiring the monkey to remember a physical position where food was last given. They can also involve nonspatial problems which require the monkey to remember an object's identity over a delay. Working memory of this type is thought to play an important role in cognition, and in light of this, it is especially interesting that the dorsolateral prefrontal cortex appears to have anthropoid-specific specializations. Also, it is interesting to note that area PMV has developed connections to the dorsolateral prefrontal cortex which are especially prominent in Old World monkeys.

43.1.2 Great Apes and Humans

The fronto-polar cortex (Brodmann's area 10) expands in apes and especially in humans both absolutely and relative to total cortical size. Functional imaging studies indicate that area 10 is activated in the retrieval of episodic memory, in the receipt of monetary rewards, in weighing cost versus benefit, in the formulation of auction bids, in deciding how much to spend to punish cheaters, and in moral decision making. The retrieval of specific past episodes contributes to the complex deliberative socioeconomic decision making that this structure participates in. The slow deliberative nature of this form of cognition stands in contrast to rapid intuition, which another hominoid specialization, the von Economo neurons (VENs) may participate in.

The VENs are large bipolar cells located in anterior cingulate (aCC) and fronto-insular (FI) cortex. They are distinguished from pyramidal cells because they have only a single, large, basal dendrite, whereas pyramidal cells have an array of smaller basal dendrites extending from the cell body. The VENs are present only in humans and great apes and are far more abundant in humans than in apes.

The apical dendrites of VENs are very similar to those of the apical dendrites of neighboring pyramidal cells. The radial orientation and narrow width of the dendritic arborization indicate that the VENs sample a sharply circumscribed cylinder of cortex, possibly corresponding to a minicolumn. They may thus constitute a fast-fire output from minicolumns that provides a rapid relay to other parts of the brain. VEN functions are revealed by immunocytochemical staining with antibodies to neurotransmitter receptors. The VENs are strongly labeled with antibodies to the dopamine D3 receptor, which may signal the expectation of reward under uncertainty. The activation of the FI and aCC increases with the degree of uncertainty. FI and aCC activity is coupled to situations in which the subject sustains a gambling loss (punishment) and then switches to a different behavioral strategy, implying that in normal subjects these areas are involved in adaptive decision making and cognitive flexibility.

The VENs may participate in intuition, a form of cognition in which many variables are rapidly evaluated to yield a fast decision. Typically we are unaware of the logical steps or assumptions underlying the process although intuition is based on experience-based probabilistic models. We experience the intuitive process at a visceral level. Intuitive decision making enables us to react quickly in situations that involve a high degree of uncertainty, which commonly involve social interactions. Frequently we do not have the luxury of sufficient time to perform a deliberative cost-benefit analysis to determine the most appropriate course of action but, instead, must rely on rapid intuitive judgments. The aCC and FI are active when subjects make decisions under a high degree of uncertainty. These areas are also active when subjects experience guilt, experience embarrassment, and engage in deception. The aCC and FI are also active in humor, trust, empathy, and the discrimination of the mental states of others. All these social emotions are influenced by the degree of uncertainty involved. Their large size suggests that the VENs may relay a fast intuitive assessment of complex social situations to allow the rapid adjustment of behavior in quickly changing social situations. For example, humor, which activates the FI and aCC in proportion to subjective ratings of funniness, may serve as a way

to recalibrate intuitive judgments in changing social situations, thus resolving uncertainty and relieving tension. The VENs can thus be seen as an adaptation supporting the increased complexity of hominoid and especially human social networks.

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