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CANNABINOID RECEPTORS IN THE HUMAN BRAIN: A DETAILED ANATOMICAL AND QUANTITATIVE AUTORADIOGRAPHIC STUDY IN THE FETAL, NEONATAL AND ADULT HUMAN BRAIN

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Abstract—The anatomical distribution and density of cannabinoid receptors in the human brain was studied in one fetal (33 weeks gestation), two neonatal (aged three to six months) and eight adult (aged 21–81 years) human cases using quantitative receptor autoradiography following *in vitro* labelling of sections with the synthetic cannabinoid agonist [^{3}H]CP55,940.

Cannabinoid receptors were distributed in a heterogeneous fashion throughout the adult human brain and spinal cord. The allocortex contained very high concentrations of cannabinoid receptor binding sites in the dentate gyrus, Ammons's horn and subiculum of the hippocampal formation: high concentrations of receptors were also present in the entorhinal cortex and amygdaloid complex. Cannabinoid receptor binding sites were also present throughout all regions of the neocortex, where they showed a marked variation in density between the primary, secondary and associational cortical regions: the greatest densities of receptors were present in the associational cortical regions of the frontal and limbic lobes, with moderate densities in the secondary sensory and motor cortical regions, and with the lowest densities of receptors in the primary sensory and motor cortical regions. Relatively high concentrations of cannabinoid receptors were consistently seen in cortical regions of the left (dominant) hemisphere, known to be associated with verbal language functions. In all of the cortical regions, the pattern and density of receptor labelling followed the neocortical laminar organization, with the greatest density of receptors localized in two discrete bands—a clearly delineated narrow superficial band which coincided with lamina I and a deeper broader, conspicuous band of labelling which corresponded to laminae V and VI. Labelling in the intervening cortical laminae (II–IV) showed lower densities, with a well delineated narrow band of label in the middle of laminae IV in the associational cortical regions. The thalamus showed a distinctive heterogeneous distribution of cannabinoid receptors, with the highest concentration of receptors localized in the mediodorsal nucleus, anterior nuclear complex, and in the midline and intralaminar complex of nuclei, i.e. in thalamic nuclei which have connectional affiliations with the associational cortical areas. The basal ganglia showed a distinctive heterogeneous pattern of receptor binding, with the very highest concentrations in the globus pallidus internus, moderate concentrations in the globus pallidus externus and ventral pallidum, and moderately low levels of binding throughout the striatal complex. In the midbrain, some of the highest levels of cannabinoid receptor binding sites in the human brain were present in the substantia nigra pars reticulata, with very low levels of labelling in all other midbrain areas. The highest densities of cannabinoid receptor binding in the hindbrain were localized in the molecular layer of the cerebellar cortex and the dorsal motor nucleus of the vagus, with moderate densities of receptors in the nucleus of the solitary tract. The spinal cord showed very low levels of receptor binding. Studies on the distribution of cannabinoid receptors in the fetal and neonatal human brain showed similar patterns of receptor distribution to that observed in the adult human brain, except that the density of receptor binding was generally markedly higher, especially in the basal ganglia and substantia nigra. The pattern of cannabinoid receptor labelling in the striatum showed a striking patchy pattern of organization which was especially conspicuous in the fetal brain.

These results show that cannabinoid receptor binding sites in the human brain are localized mainly in: forebrain areas associated with higher cognitive functions; forebrain, midbrain and hindbrain areas associated with the control of movement; and in hindbrain areas associated with the control of motor and sensory functions of the autonomic nervous system. The possible role of these receptors is discussed with respect to the known behavioural and psychomotor effects of cannabinoids in humans. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: cannabinoid receptors, human brain, fetal, neonatal.

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Abbreviations: CP55,940, (*cis*)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl](*trans*)-4-(3-hydroxypropyl)cyclohexanal; Δ^9-THC, Δ^9-tetrahydrocannabinol.

Marijuana (*Cannabis sativa*) has long been recognized as a centrally acting cannabinoid with widespread effects on our higher cognitive functions. The behavioural effects of cannabinoids in humans are complex, consisting of both subjective and objective effects. The subjective events include enhancement of

the senses, errors in judgement of time and space, emotional changes, irresistible impulses, illusions and hallucinations (see Refs 12 and 33 for reviews). The more objective responses include decreased psychomotor performance,[50] an interference in attention span and a loss of efficiency in memory.[8,9,56] Thus, cannabinoids affect both cognitive and motor functions. The cognitive effects suggest that cannabinoids may affect forebrain structures which are known to play a major role in higher cognitive functions, while the motor activities may be mediated by basal ganglia, cerebellar and other motor regions of the human brain.

Δ^9-Tetrahydrocannabinol (Δ^9-THC) has been identified as the major psychoactive component of marijuana.[21] Synthetic cannabinoids, such as the cannabinoid receptor agonist (*cis*)-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl](*trans*)-4-(3-hydroxypropyl)cyclohexanal (CP55,940), have been shown to share many of the physicochemical properties and to produce similar behavioural and physiological effects to Δ^9-THC.[1,2,10,31,34–39,41] The synthetic cannabinoids inhibit adenylate cyclase via the guanine-nucleotide protein (G protein) Gi.[1,35,38] Binding assays with [^{3}H]CP55,940 show a saturable binding site[10,31] believed to be the recently cloned cannabinoid receptor,[51] and thus [^{3}H]CP55,940 has been used to study the distribution of cannabinoid receptors in a variety of quantitative autoradiography studies on the mammalian brain.[22,29–31,48,49]

Although there have been detailed studies on the distribution and localization of cannabinoid receptors in the rat brain using [^{3}H]CP55,940,[29–31] there has been no detailed study on the overall distribution of cannabinoid receptors in the human brain except for preliminary observations by ourselves[22] and others[31,48,49,61] showing high concentrations of cannabinoid receptors in the basal ganglia, cerebellum and hippocampus. In order to gain a better understanding of the widespread cognitive and motor effects of cannabinoids, we have undertaken a detailed quantitative study on the anatomical distribution of cannabinoid receptors throughout all major regions of the adult human brain using quantitative receptor autoradiography with the cannabinoid agonist [^{3}H]CP55,940. In addition, quantitative autoradiographic receptor studies were also undertaken on the distribution and density of cannabinoid receptors in the fetal and neonatal brain in order to investigate the ontogenic development of cannabinoid receptors in the human brain.

EXPERIMENTAL PROCEDURES

Tissue collection

The human brain tissue used in these studies was obtained from the New Zealand Neurological Foundation Human Brain Bank in the Department of Anatomy, University of Auckland. This study was performed unc ethical approval by the University of Auckland Hum Subjects Ethics Committee.

Table 1. Source of human tissue for cannabinoid recep autoradiography

Case	Sex	Age	*Post mortem* delay (h)	Cause of death
FH1	M	33 weeks	21	Bowel obstruction
N1	M	3 months	13	Asphyxia
N2	F	6 months	17	Congenital heart disease
H47	M	81 years	5	Subarachnoid haemorrha
H58	F	29 years	4.5	Asphyxia
H64	M	64 years	16	Myocardial infarction
H78	F	48 years	11.5	Myocardial infarction
H79	M	75 years	11	Myocardial infarction
H80	M	72 years	10	Myocardial infarction
H81	M	55 years	12	Myocardial infarction
H82	M	21 years	8.5	Asphyxia

All subjects had previously been in good health, with known history of neurological disease or drug treatme and all had died suddenly without the opportunity receiving any form of medical treatment. The brains w removed to the Department of Anatomy, University Auckland, immediately following autopsy. On arriv blocks were immediately selected from various regions the brain. The tissue blocks were frozen on dry ice, doub wrapped in tin foil and stored at −80°C prior to subseque autoradiographical processing as detailed below. The *p mortem* delay in each case is described as the interv between death and the freezing of the tissue blocks.

Post mortem human brains were obtained from one fet (33 weeks gestation), two neonates (aged three and months) and eight adult subjects (aged 21–81 years; avera age 55.6 years; see Table 1 for details). The interval betwe death and the freezing of the tissue blocks (i.e. the *p mortem* delay) ranged from 4.5 to 21 h (average 11.9 h).

Autoradiography

The autoradiograms were generated by incubating 16-μ cryostat sections with 2.5 nM [^{3}H]CP55,940 (Dupont/NE specific activity 125 Ci/mmol), as previously described detail.[22] In order to validate comparisons between brai and between different regions in the same brain, the recept autoradiographic labelling procedures were undertake using standardized procedures. Accordingly, sections fro blocks of the various regions studied in the eight adu human subjects were processed simultaneously using th same stock incubation solutions and under the same co ditions. Similarly, sections from the fetal and neonat brains were processed together. Briefly, sections were inc bated for 2 h with 2.5 nM [^{3}H]CP55,940 in a Tris–HC buffer (50 mM, pH 7.4) with 5% bovine serum albumin 37°C. Non-specific binding was determined with the ad dition of 10 μM cold CP55,940. Incubation was terminate and unbound ligand removed by washing twice in Tris–HC buffer (50 mM, pH 7.4) with 1% bovine serum albumin a 4°C for 2 h. The slides were placed in X-ray cassettes wit slide-mounted tritium micro-scale standards (RPA. 501 an RPA. 505; Amersham) and exposed to tritium-sensitiv hyperfilm for 10 weeks. The films were then developed u Kodak D19 for 4 min at 15°C, washed and fixed. Th autographic images were digitized by a solid state vide camera and Macintosh II computur-based system for quan titative densitometry using IMAGE software (Wayn Rasband, Research Services Branch, National Institute o Mental Health).

Myelin and cell staining

Following development of the autoradiograms, the slide-mounted cryostat sections used to generate the autoradiograms were fixed in 10% formalin for 1 h and counterstained for myelin with Luxol Fast Blue and for cells with Cresyl Violet in order to delineate the various nuclear subdivisions and cortical laminae using standard cytoarchitectonic and myeloarchitectonic criteria. Precise identification of the anatomical pattern of labelling was achieved by superimposing, at the same magnification, a projection of the counterstained section on to a photographic print of the corresponding autoradiogram.

RESULTS

The principal aim of this study was to precisely define the anatomical localization and density of cannabinoid receptors in all major regions of the adult human brain and spinal cord. In addition, studies were also undertaken on the distribution and density of cannabinoid receptors in the fetal and neonatal brain in order to investigate the ontogenic development of cannabinoid receptors in the regions of the human brain which contain some of the highest concentrations of receptors in adults. In all cases, cannabinoid receptors were demonstrated using quantitative receptor autoradiography following *in vitro* labelling of cryostat cut sections with the synthetic cannabinoid agonist [^{3}H]CP55,940. In order to demonstrate the anatomical distribution of cannabinoid binding sites in the brain and spinal cord, after the development of the autoradiograms the cryostat sections which were used to generate the autoradiograms were stained for myelin and Nissl substance. The pattern of autoradiographic receptor labelling in the autoradiograms was then directly compared to the anatomical pattern of fibre and cell staining in the corresponding section. As shown in Figs 1–7, this enabled the precise anatomical localization of the pattern of labelling of cannabinoid receptor binding sites using standard cytoarchitectonic and myeloarchitectonic criteria. The density of the receptors in each of the identified anatomical regions was then determined using computerized densitometry methods (see Table 2).

Abbreviations used in the figures

AC	anterior commissure
Ag	amygdaloid complex
Am	nucleus ambiguus
AP	area postrema
AV	anteroventral nucleus of the thalamus
BSA	bovine serum albumin
CA1	CA1 field of Ammon's horn
CA2	CA2 field of Ammon's horn
CA3	CA3 field of Ammon's horn
CA4	CA4 field of Ammon's horn
CaS	calcarine sulcus
CC	crus cerebri
CCN	central cervical nucleus
CeM	central medial nucleus of the thalamus
CeS	central sulcus
CG	cingulate gyrus
CGr	central gray
Cl	claustrum
CL	central lateral nucleus of the thalamus
CM-Pf	central median and parafascicular nuclei of the thalamus
CN	caudate nucleus
CoS	collateral sulcus
Cu	cuneate nucleus
DCN	deep cerebellar nuclei
DMX	dorsal motor nucleus of the vagus nerve
EC	entorhinal cortex
G	granular layer of the cerebellar cortex
Ga	granular layer of the dentate gyrus
GP	globus pallidus
GPe	globus pallidus externus
GPi	globus pallidus internus
Gr	gracile nucleus
IC	internal capsule
ICP	inferior cerebellar peduncle
ICx	insular cortex
IO	inferior olivary nuclear complex
ITG	inferior temporal gyrus
LCu	lateral cuneate nucleus
LD	lateral dorsal nucleus of the thalamus
LR	lateral reticular nucleus
M	molecular layer of the cerebellar cortex
MB	mammillary body
MCx	primary motor cortex
MD	mediodorsal nucleus of the thalamus
MFG	middle frontal gyrus
MGB	medial geniculate body
ML	medial lemniscus
Mo	molecular layer of the dentate gyrus
MTG	middle temporal gyrus
MTT	mammillothalamic tract
MV	medioventral nucleus of the thalamus
NST	nucleus of the solitary tract
OTG	occipitotemporal gyrus
P	pontine nuclei
PaS	parasubiculum
PD	pyramidal decussation
PHG	parahippocampal gyrus
PRC	perirhinal cortex
PrS	presubiculum
PT	pyramidal tract
PTe	planum temporale
Pu	putamen
Ra	raphe nuclei of the pons
RF	reticular formation
RN	red nucleus
S	subiculum
SC	superior colliculus
SCx	primary somatosensory cortex
SG	substantia gelatinosa
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
TGH	transverse gyrus of Heschl
VA	ventral anterior nucleus of the thalamus
VH	ventral horn of the spinal cord
Vip	interpolar trigeminal nucleus
VL	ventral lateral nucleus of the thalamus
VLa	lateral vestibular nucleus
VMe	medial vestibular nucleus
Vmo	motor trigeminal nucleus
Vp	principal trigeminal nucleus
VP	ventral posterior nucleus of the thalamus
VPa	ventral pallidum
VS	ventral striatum
Vsp	spinal trigeminal nucleus
XII	hypoglossal nucleus

Cortical laminae are indicated by Roman numerals.
Brodmann's areas are indicated by Arabic numerals.

Table 2. The density of [^{3}H]CP55,940 binding in the adult human brain and spinal cord (fmol/mg)

Allocortex	
Hippocampus	
Dentate gyrus	
Molecular layer	150 ± 33
Granular layer	81 ± 8
Polymorphic layer	44 ± 10
Ammon's horn	
CA1	
Stratum moleculare	74 ± 36
Stratum lacunosum	115 ± 26
Stratum radiatum	125 ± 40
Stratum pyramidale	120 ± 53
Stratum oriens	102 ± 55
CA2	
Stratum pyramidale	131 ± 41
CA3	
Stratum pyramidale	130 ± 41
CA4	
Stratum pyramidale	93 ± 42
Subicular complex	
Prosubiculum	
Pyramidal cell layer	141 ± 43
Subiculum	
Pyramidal cell layer	131 ± 33
Presubiculum	
Layer II	127 ± 50
Parasubiculum	
Layer II	116 ± 47
Entorhinal cortex	
Layer I	127 ± 42
Layer II	112 ± 45
Layer III	126 ± 38
Layer IV	107 ± 38
Layers V and VI	125 ± 38
Amygdaloid complex	102 ± 19
Neocortex	
Middle frontal gyrus	
Layer I	113 ± 7
Layers II, III and IVA	98 ± 14
Layer IVB	114 ± 9
Layer IVC	94 ± 10
Layers V and VI	108 ± 10
Motor cortex	
Area 4	
Layer I	42 ± 11
Layers II–IV	29 ± 14
Layers V and VI	42 ± 15
Area 6	
Layer I	44 ± 5
Layers II, III and IVA	30 ± 5
Layer IVB	39 ± 7
Layer IVC	37 ± 13
Layers V and VI	47 ± 10
Somatosensory cortex	
Area 1	
Layer I	45 ± 8
Layers II, III and IVA	31 ± 10
Layer IVB	34 ± 11
Layer IVC	29 ± 8
Layers V and VI	35 ± 7
Area 2	
Layer I	40 ± 10
Layers II, III and IVA	25 ± 7
Layer IVB	31 ± 6
Layer IVC	20 ± 7
Layers V and VI	32 ± 6
Area 3	
Layer I	42 ± 13
Layers II–IV	29 ± 16
Layers V and VI	37 ± 14
Visual cortex	
Primary visual cortex (area 17)	
Layer I	64 ± 10
Layers II, III and IVB, C	24 ± 10
Layer IVA	27 ± 9
Layers V and VI	54 ± 13
Secondary visual cortex (area 18)	
Layer I	62 ± 11
Layers II–IV	36 ± 11
Layers V and VI	64 ± 8
Cingulate gyrus	
Layer I	102 ± 12
Layers II, III and IVA	98 ± 14
Layer IVB	111 ± 15
Layer IVC	98 ± 16
Layers V and VI	109 ± 9
Auditory cortex	
Primary cortex (area 41)	
Layer I	79 ± 9
Layers II–IV	41 ± 10
Layers V and VI	59 ± 9
Secondary cortex (area 22) left—Wernicke's area	
Layer I	93 ± 27
Layers II, III and IVA	76 ± 30
Layer IVB	93 ± 28
Layer IVC	72 ± 25
Layers V and VI	88 ± 14
Secondary cortex (area 22) right	
Layer I	78 ± 17
Layers II, III and IVA	66 ± 22
Layer IVB	84 ± 19
Layer IVC	65 ± 12
Layers V and VI	71 ± 11
Middle temporal gyrus	
Layer I	63 ± 16
Layers II, III and IVA	48 ± 17
Layer IVB	64 ± 11
Layer IVC	50 ± 13
Layers V and VI	70 ± 14
Occipitotemporal gyrus	
Layer I	75 ± 14
Layer II	74 ± 14
Layer III	75 ± 14
Layer IV	56 ± 20
Layer V and VI	79 ± 14
Thalamus	
Anteroventral nucleus	14 ± 5
Central medial nucleus	18 ± 5
Central lateral nucleus	14 ± 15
Central median and parafascicular nuclei	35 ± 27
Lateral dorsal nucleus	6 ± 4
Mediodorsal nucleus	41 ± 34
Medial geniculate body	23 ± 21
Mammillothalamic tract	7 ± 8
Medioventral nucleus	26 ± 5
Ventral anterior nucleus	10 ± 7
Ventral lateral nucleus	17 ± 14
Ventral posterior nucleus	12 ± 10
Hypothalamus	
Mamillary body	21 ± 13
Basal ganglia	
Caudate nucleus	40 ± 10
Putamen	44 ± 8
Ventral striatum	33 ± 9
Globus pallidus	
Internal	92 ± 16
External	72 ± 19
Ventral pallidum	48 ± 17
Claustrum	36 ± 8

Continued

Table 2. *Continued*

Midbrain		Medulla	
Substantia nigra, right	146 ± 55	Dorsal motor nucleus of the vagus	87 ± 29
Substantia nigra, left	129 ± 39	Hypoglossal nucleus	35 ± 9
Crus cerebri	4 ± 2	Inferior olive	31 ± 7
Red nucleus	8 ± 9	Cuneate nucleus	6 ± 5
Raphe nucleus	15 ± 12	Gracile nucleus	7 ± 5
Reticular formation	16 ± 11	Spinal trigeminal nucleus	7 ± 5
Central gray	21 ± 12	Pyramidal tract	7 ± 6
Superior colliculus	7 ± 5	Nucleus of the solitary tract	30 ± 18
		Raphe nucleus	16 ± 7
Pons			
Pontine nuclei	6 ± 2	Spinal cord	
Reticular formation	4 ± 2	Central cervical nucleus	26 ± 8
Motor trigeminal nucleus	15 ± 3	Substantia gelatinosa	39 ± 9
		Ventral horn	30 ± 11
Cerebellum			
Molecular	106 ± 15		
Granular	27 ± 8		
Deep cerebellar nucleus	24 ± 8		

The densities are expressed as means ± S.D. of femtomoles of [^{3}H]CP55,940 bound per mg of tissue. The values given for each brain region were determined from a minimum of nine sections (three sections for each of at least three brains). Which three or more brains were used was determined solely on the basis of tissue availability, and all brains labelled were included in the analysis.

The distribution and density of cannabinoid receptors in the adult human brain and spinal cord

The results show that [^{3}H]CP55,940 binding sites are distributed in a heterogeneous fashion throughout all major regions of the human brain and spinal cord. As detailed in Table 2 and illustrated in Figs 1–5, the highest concentrations of cannabinoid receptor binding sites in the human brain were found in the allocortex, in the substantia nigra, globus pallidus and cerebellum, and in regions of the association cortex. The detailed distribution of receptors in all of the major regions of the adult human brain and spinal cord is outlined below.

Forebrain.

Allocortex. The allocortex contained some of the highest concentrations of cannabinoid receptor binding sites in the human brain (Fig. 2B). The highest densities of receptors in the allocortex were present within the hippocampal formation, where they showed a distinctive heterogeneous distribution. As shown in Fig. 2B, very high concentrations of receptors were present in the molecular layer of the dentate gyrus, in the strata pyramidale of fields CA1, CA2 and CA3 of Ammon's horn and in the subicular complex (Table 2).

High concentrations of receptors were also present in the entorhinal cortex (Fig. 2B) and amygdaloid complex (Fig. 2C), although the binding was less than that in the hippocampal formation. In the perirhinal cortex lying in the floor of the collateral sulcus, which is the transitional cortical region between the allocortex and neocortex (see PRC, Fig. 2B), the density of cannabinoid receptor binding gradually decreased (Fig. 2B) to approach the lower, moderately high levels of receptors in the immediately adjacent neocortex which comprises the occipitotemporal gyrus of the temporal association cortex (Fig. 2B, Table 1).

Neocortex. Cannabinoid receptor binding sites were present throughout all regions of the neocortex. However, the density of receptor labelling varied markedly between the primary, secondary and associational cortical regions of the neocortex. In general terms, as shown in the autoradiograms of Figs 1 and 2 and in Table 2, the density of receptor labelling was lowest in the primary sensory (somatosensory, visual and auditory; Figs 1B, E, F, 2A) and primary motor (Fig. 1B) regions of the cerebral cortex. Higher densities of receptors were consistently present in the secondary sensory (somatosensory, visual and auditory; Figs 1B, E, F, 2A) and secondary motor (Fig. 1B) regions. The greatest densities of receptors in the neocortex were present in the associational cortical regions of the frontal lobe (Fig. 1A) and limbic lobe (Fig. 1C). In addition, relatively high concentrations of cannabinoid binding sites were also seen in the cortical regions of the left (dominant) hemisphere, known to be associated with verbal language functions. For example, the density of receptors in the planum temporale of the secondary auditory cortex in the left hemisphere, known as Wernicke's area (i.e. Brodmann's area 22; Fig. 1E, Table 2), was 10–19% greater than that in the corresponding regions of the non-dominant right hemisphere (Fig. 1F, Table 2), and was comparable to that seen in the frontal and limbic associational cortical regions detailed above.

In particular, in all of the cortical regions, the pattern and density of labelling precisely followed the neocortical laminar organization as demonstrated by

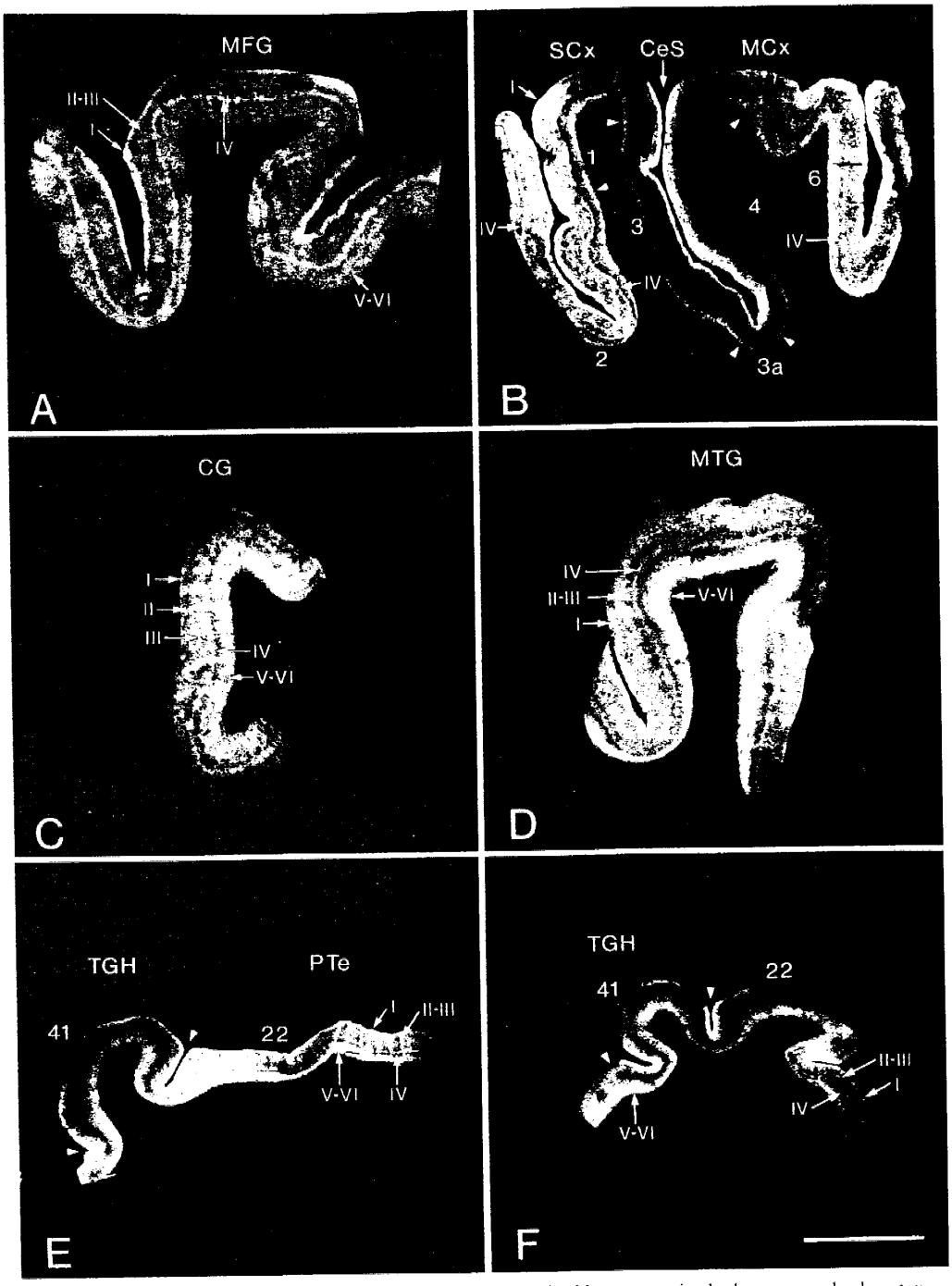

Fig. 1. Autoradiograms showing the distribution of cannabinoid receptors in the human cerebral cortex. (A) Middle frontal gyrus. (B) Primary somatosensory cortex (postcentral gyrus, Brodmann's areas 3, 1, 2) and primary motor cortex (precentral gyrus, Brodmann's areas 4, 6) on either side of the central sulcus. (C) Cingulate gyrus. (D) Middle temporal gyrus. (E) Left primary auditory cortex (transverse gyrus of Heschl, Brodmann's area 41) and secondary auditory cortex (planum temporale, Brodmann's area 22). (F) Right primary auditory cortex (transverse gyrus of Heschl, Brodmann's area 41) and secondary auditory cortex (Brodmann's area 22). Scale bar=1 cm.

the cytoarchitecture and myeloarchitecture of the cortex. The laminar organization of the pattern of cannabinoid receptor labelling in representative regions of the neocortex is shown in Figs 1A–F and 2A–C and is detailed below.

In general, all regions of the neocortex showed clearly delineated, moderately dense band of labell in the most superficial region of the cortex, wh precisely coincided with lamina I (Figs 1A–F, 2A. As detailed in Table 2, the density of labelling

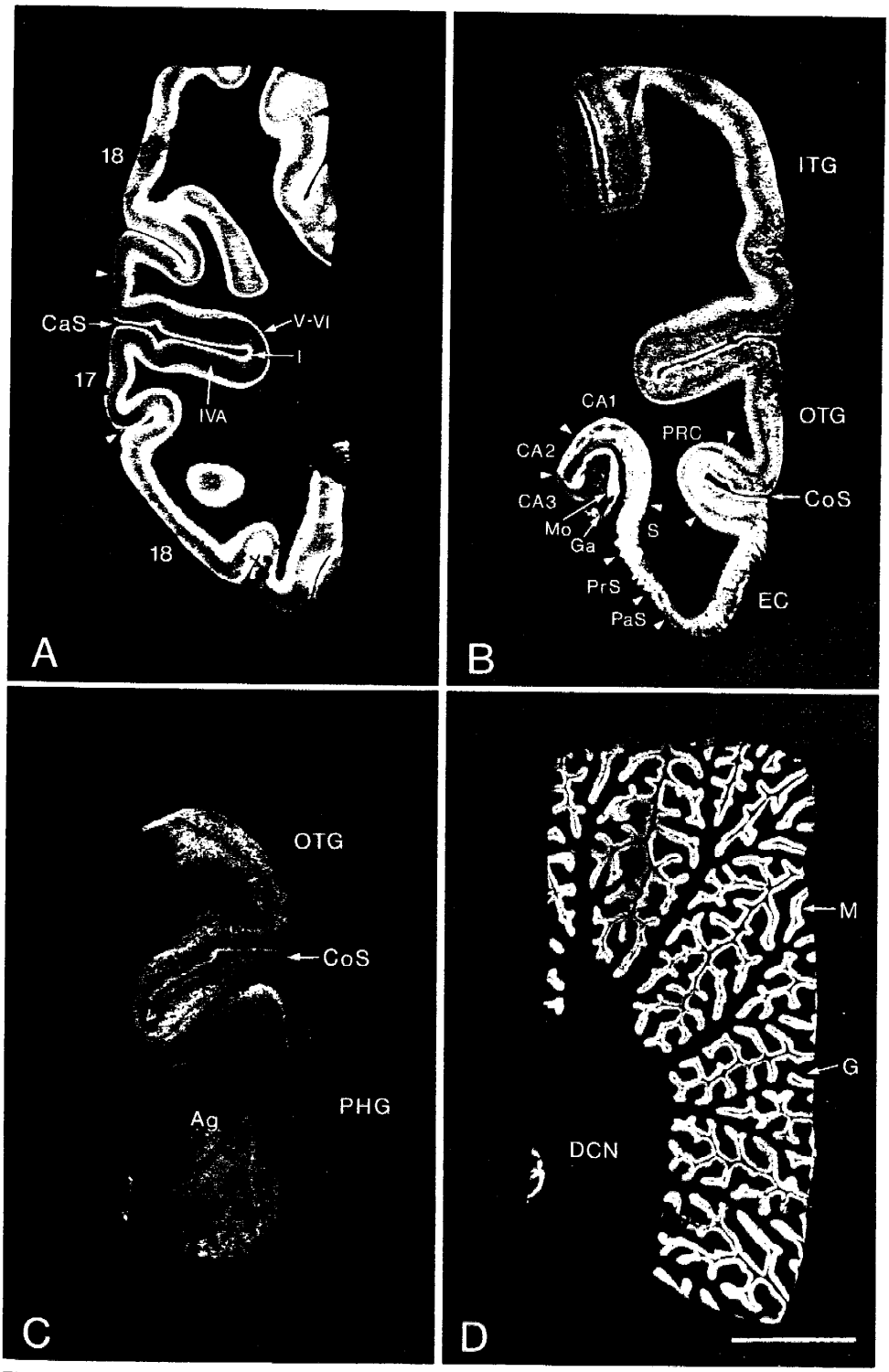

Fig. 2. Autoradiograms showing the distribution of cannabinoid receptors in the human occipital cortex, temporal lobe and cerebellum. (A) Primary (Brodmann's area 17) and secondary (Brodmann's area 18) visual cortex. (B) The temporal lobe, comprising of the hippocampal formation (consisting of the dentate gyrus, Ammon's horn, subicular complex and entorhinal cortex), perirhinal cortex, occipitotemporal gyrus and inferior temporal gyrus. (C) The amygdaloid complex and adjacent parahippocampal gyrus and occipitotemporal gyrus. (D) The cerebellum, comprising of the cerebellar cortex and deep cerebellar nuclei. Scale bar=1 cm.

lamina I was generally higher in the regions of the association cortex of the frontal lobe (Fig. 1A, middle frontal gyrus), limbic lobe (Fig. 1C, cingulate gyrus) and temporal lobe (Fig. 2B, inferior temporal gyrus and occipitotemporal gyrus) with generally lower densities of binding sites in the primary motor cortex (Fig. 1B) and in the primary sensory cortical regions (for example, see Fig. 1B, primary somatosensory cortex, areas 3, 1, 2; Fig. 1E, F, primary auditory cortex, area 41; Fig. 2A, primary visual cortex, area 17).

A second, broader and more diffuse conspicuous band of moderately dense labelling was also present in the deeper region of all neocortical areas—this band of receptor labelling corresponded to laminae V and VI. This band of cannabinoid receptor labelling was particularly conspicuous in the visual cortex (Fig. 2A), especially in the secondary visual cortex (Brodmann's area 18). In general, as detailed in Table 2, in the sensory cortical regions the density of receptor labelling in laminae V and VI was generally greater in the secondary cortical regions compared with the primary cortical regions; for example, compare the density of autoradiographic receptor labelling between the secondary (area 22) and primary (area 41) auditory cortices in Fig. 1E, and between the secondary (area 18) and primary (area 17) visual cortices in Fig. 2A. Quantitative analysis (Table 2) showed that, as for the superficial band of cannabinoid receptor labelling, the density of labelling in the deep band of receptors was generally greatest in regions of the association cortex (see, for example, Fig. 1A, middle frontal gyrus; Fig. 1C, cingulate gyrus; and Fig. 2B, inferior and occipital temporal gyri): comparable binding densities were also seen in laminae V/VI of the secondary auditory cortex (area 22) of the left (dominant) hemisphere (Wernicke's area; Fig. 1E, Table 2).

Labelling in the intervening middle laminae of the cerebral cortex (laminae II–IV) varied markedly in the pattern and density of receptors in different neocortical regions. This was especially evident in the pattern of labelling in lamina IV. For example, careful comparison of the pattern of labelling in lamina IV in the autoradiograms shows that all regions of the association cortex are distinguished by a very well delineated narrow band of label which corresponds precisely to the middle of lamina IV, i.e. to lamina IVB. This clearly demarcated band of dense cannabinoid receptor binding in middle lamina IV was especially evident in the prefrontal (Fig. 1A), cingulate (Fig. 1C) and temporal (Figs 1D, 2B) associational cortical regions. By contrast, this narrow band was generally not present in the primary sensory (Figs 1B, E, F, 2A) and the primary motor (Fig. 1B) neocortical regions, although a very faint narrow band of label was just visible in laminae IVA in the original autoradiogram of the primary visual cortex (see region labelled IVA in Fig. 2A). A very narrow band of cannabinoid receptor labelling in lamina IV was also clearly delineated in the secondary somatosensory (Fig. 1B), secondary auditory (Fig. 1E, F) and secondary motor (Fig. 1B) cortices. The autoradiograms and quantitative analyses (Table 2) showed that the density of cannabinoid receptor labelling in the middle laminae (II–IV) was generally higher in the secondary motor, secondary sensory and associational cortical regions in comparison to the labelling in the primary sensory and motor regions. In particular, in agreement with the general pattern of receptor binding in the superficial (lamina I) and deep (laminae V/VI) laminae of the neocortex detailed above, the density of binding in the middle laminae (laminae II–IV) was markedly greater in the associational cortical regions compared with the secondary cortical regions. The notable exception was the consistently high level of binding in the secondary auditory cortex in the dominant hemisphere (Wernicke's area), where the density of binding in all laminae was comparable to that in the associational cortical regions.

Thalamus. The density of receptor labelling in the thalamus was considerably less than that in the cerebral cortex. However, as with other regions of the human forebrain, the thalamus showed a distinctive heterogeneous distribution of cannabinoid binding sites. Following the general pattern of labelling in the neocortex, the highest concentrations of receptors in the human thalamus were generally localized within the thalamic nuclei, which have known connectional affiliations with the associational cortical regions. In particular, the highest densities of receptors in the thalamus were present within the mediodorsal nucleus (Fig. 3B, Table 2); as shown in Fig. 3B, the pattern of labelling in the mediodorsal nucleus showed a distinctive patchy distribution which appeared to correspond to regions of higher acetylcholinesterase staining seen in adjacent sections. Moderate densities of receptors were also present within the anterior nuclear complex (anteroventral nucleus, Fig. 3A), and in the midline (medioventral nucleus, Fig. 3A) and intralaminar (central medial nucleus, Fig. 3A; central lateral, centre median and parafascicular nuclei, Fig. 3B) complex of nuclei. By contrast, very low densities of receptors were present within: (i) the thalamic sensory "relay" nuclei, which are known to have close connectional affiliations with the primary somatosensory cortex (ventroposterior nucleus, Fig. 3B), primary auditory cortex (medial geniculate body, Fig. 4D) and primary visual cortex (lateral geniculate body); and (ii) within the "motor" thalamic nuclei of the thalamus (ventral anterior and ventral lateral nuclei, Fig. 3A, B), which are known to receive afferents from the cerebellum and basal ganglia and which connect with the primary and secondary motor cortical regions.

Basal ganglia. The basal ganglia of the forebrain showed a very distinctive heterogeneous pattern of

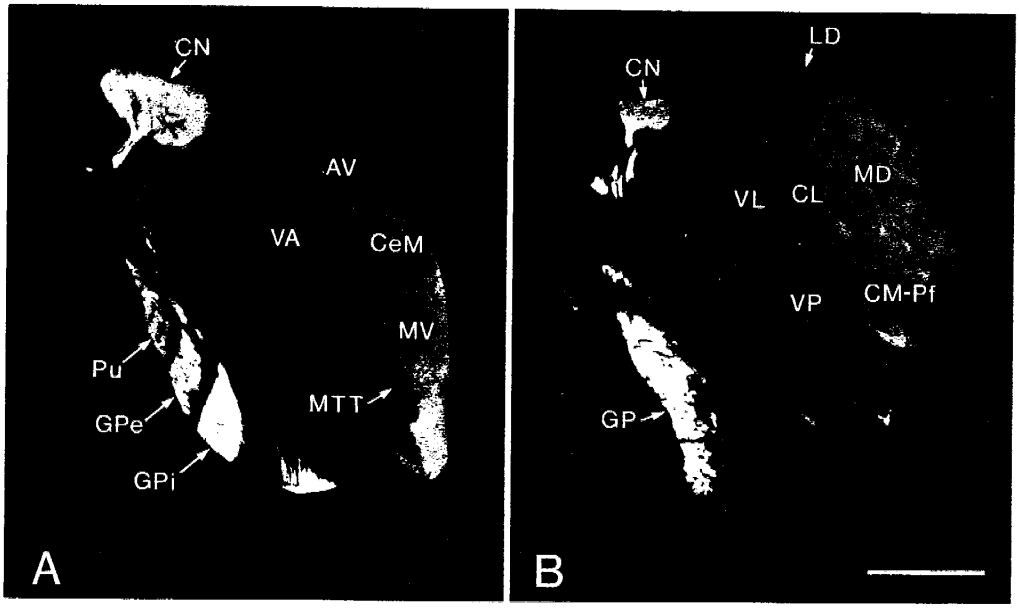

Fig. 3. Autoradiograms showing the distribution of cannabinoid receptors in the human thalamus. (A) The anterior region of the thalamus at the level of the mammillothalamic tract. (B) The mid-thalamic level. Scale bar=1 cm.

cannabinoid receptor binding which corresponded to the major anatomical nuclear components of the basal ganglia. The striatum, which is the largest nuclear complex of the basal ganglia and comprises of the caudate nucleus, the putamen and the ventral striatum, showed a moderately low level of cannabinoid receptor binding (Fig. 4A–C). Careful examination of the pattern of receptor labelling in the striatum suggests a patchy distribution of receptors, especially in the caudal putamen at the level of the lenticular nucleus (Fig. 4B, C). By contrast, the globus pallidus, which is the other major nuclear component of the basal ganglia, showed moderate to very high densities of cannabinoid receptor binding sites. The highest densities of receptors were present in the internal segment of the globus pallidus (Fig. 4C). However, moderate densities of receptors were present throughout the rostrocaudal extent of the external segment of the globus pallidus (Fig. 4B, C) and in the ventral pallidum, which is a ventral subcommissural extension of the globus pallidus externus lying immediately ventral to the transverse limb of the anterior commissure (Fig. 4B). Closer examination of the pattern of autoradiographic receptor labelling in the globus pallidus externus (Fig. 4B, C) revealed some regional variations in the density and pattern of receptor binding: higher density patches appeared to be present in some regions, with the highest density of labelling being present in the rostrolateral region of the complex and with lower densities of binding in the ventral pallidum (Fig. 4B).

In addition, clearly delineated longitudinal bands of receptor binding sites immediately medial to the internal segment of the globus pallidus (see arrowheads in Fig. 4C) were consistently seen in all cases. The pattern of labelling corresponded precisely to heavily myelinated fibre bundles (identified by myelin staining) coursing from the level of the lenticular nucleus in the forebrain towards the rostral pole of the substantia nigra in the midbrain.

Midbrain.

Substantia nigra. The substantia nigra consistently showed very high levels of cannabinoid receptor binding sites in all cases (Fig. 4D). In confirmation of our previous findings,[22] comparison of the autoradiograms with Nissl- and fibre-stained sections showed that the region of dense receptor binding corresponded with the pars reticulata of the substantia nigra. By comparison, the pars compacta region of the substantia nigra showed negligible levels of receptor binding (Fig. 4D).

Other regions. In comparison to the very dense receptor binding in the substantia nigra, the remaining regions of the midbrain showed very low levels of labelling. Only the central gray, raphe nucleus and reticular formation showed significant binding above background levels.

Hindbrain.

Pons. The raphe nucleus and central gray showed low levels of receptor binding. Very low levels of binding were present in other nuclear regions.

Cerebellum. Within the cerebellum, cannabinoid receptor binding sites were mainly present within the cerebellar cortex, with only very low densities of

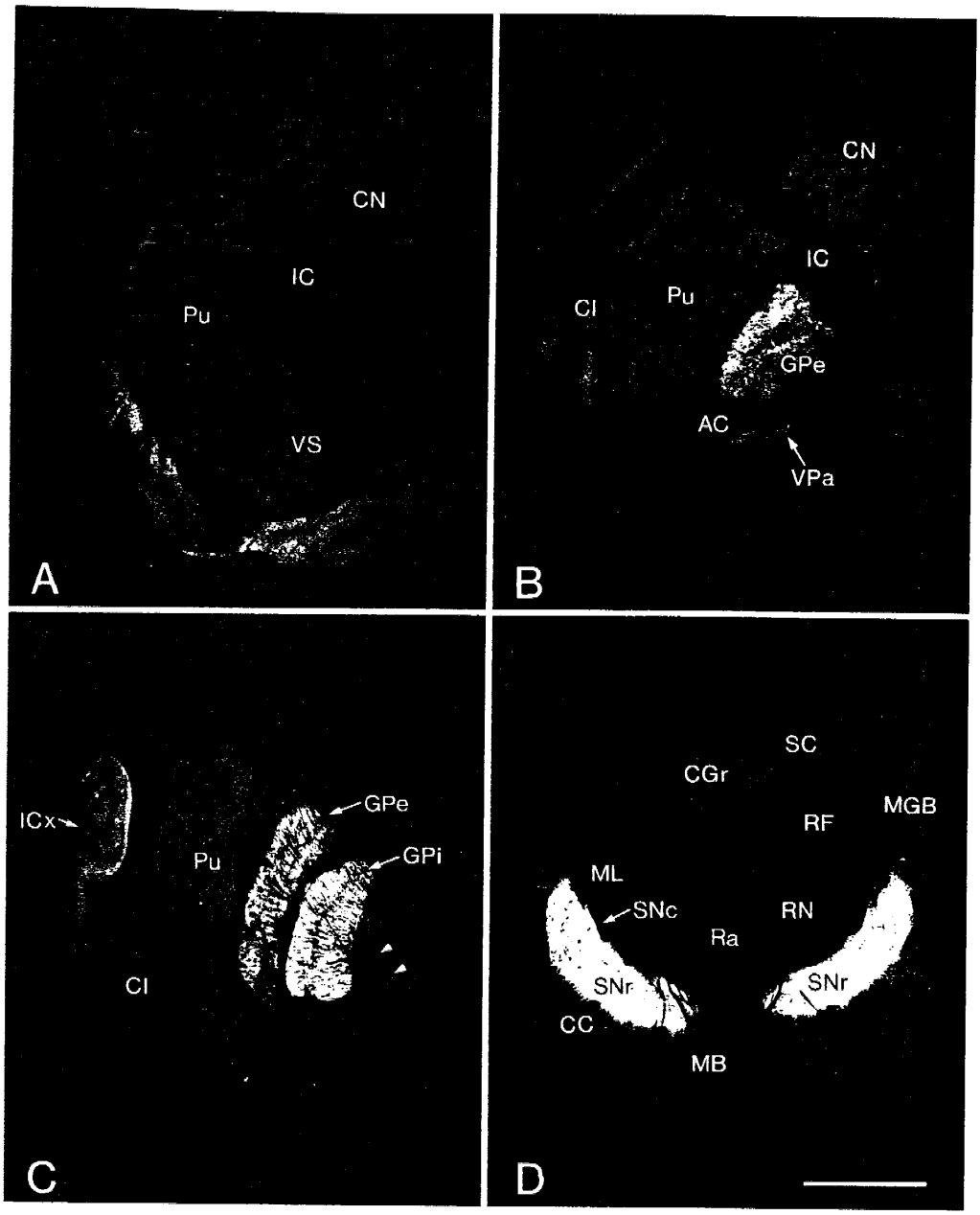

Fig. 4. Autoradiograms showing the distribution of cannabinoid receptors in the human basal ganglia in the forebrain (A–C) and midbrain (D). (A) Striatal complex at the level of the ventral striatum. (B) Caudate–putamen–pallidal complex at the level of the ventral pallidum. (C) Putamen–pallidal complex at the mid-level of the lenticular nucleus. (D) Substantia nigra in the midbrain. The arrowheads in C indicate cannabinoid receptor binding sites in fibre bundles coursing from the level of the lenticular nucleus towards the substantia nigra in the midbrain. Scale bar=1 cm.

receptors in the deep cerebellar nuclei (Fig. 2D). The cerebellar cortex was distinguished in the autoradiograms (Fig. 2D) by the presence of a conspicuous solid band of high densities of receptors throughout the full extent of the molecular layer. By contrast, very low levels of receptor labelling were present in the Purkinje cell layer and in the granular layer of the cerebellar cortex.

Medulla. Cannabinoid receptor distribution in the medulla was characterized by very high levels of binding localized in the dorsal motor nucleus of the vagus (Fig. 5B–E). Although receptor labelling was present throughout the rostrocaudal extent of the dorsal motor nucleus (Fig. 5B–E), there was a distinctive regional variation in the density of binding. At the rostral level of the nucleus (Fig. 5B), two

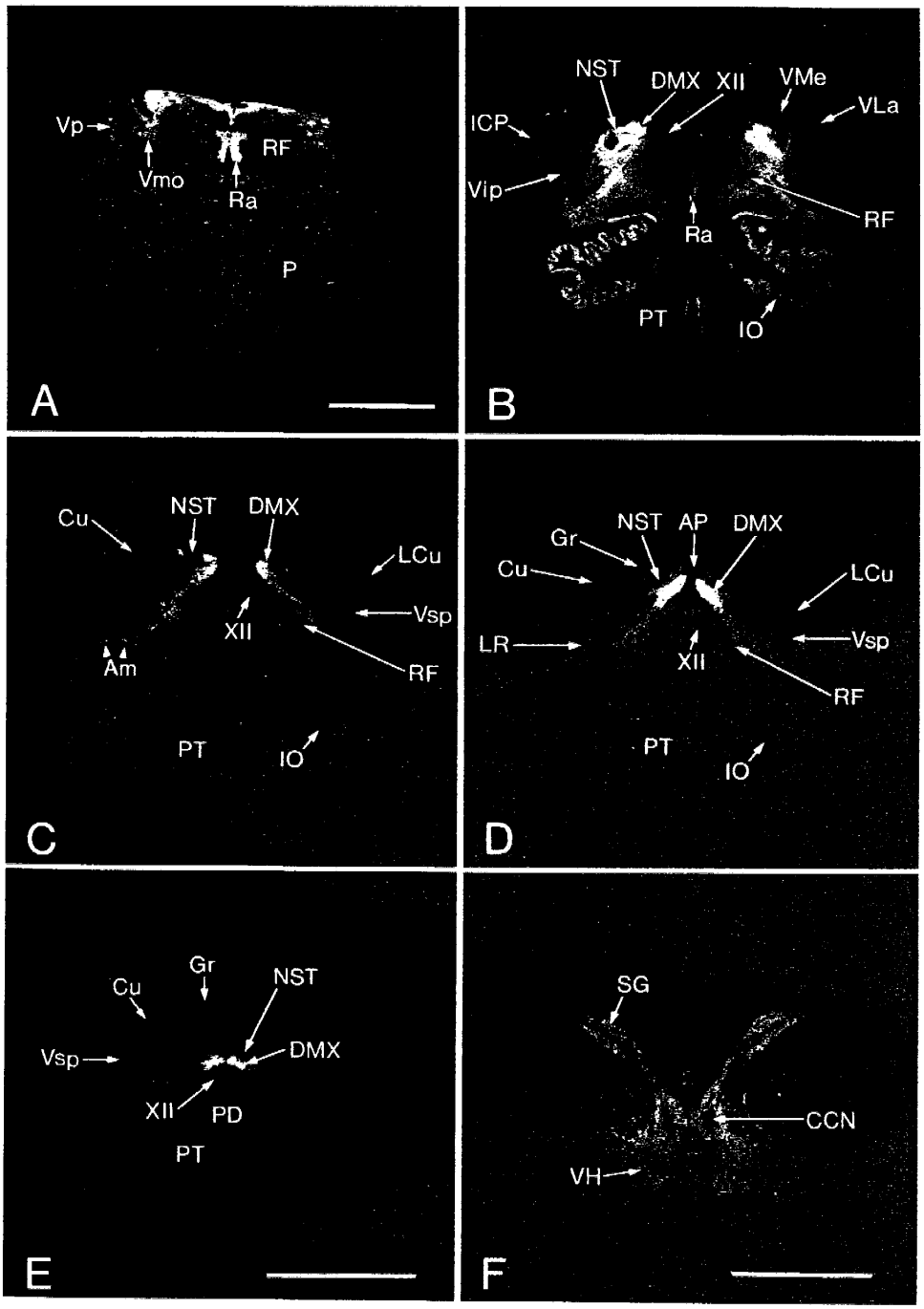

Fig. 5. Autoradiograms showing the distribution of cannabinoid receptors in the human hindbrain (A–E) and spinal cord (F). (A) Pons. (B) Upper medulla. (C) Middle medulla. (D) Lower medulla. (E) Spinomedullary junction. (F) The first cervical level of the spinal cord. Scale bar=1 cm.

discrete high-density patches of binding were consistently evident, and the caudal third of the nucleus showed a homogeneous high density of receptor labelling (Fig. 5D, E). The intermediate region of the dorsal motor nucleus of the vagus showed a lower and more heterogeneous pattern of receptor labelling—less well defined moderate densities of cannabinoid receptor binding were localized in two patches of the medial region of the nucleus (Fig. 5C).

Moderate densities of receptor binding were present throughout the full extent of the nucleus of the solitary tract, with some regional variation in the density of binding evident in the autoradiograms (Fig. 5B–E).

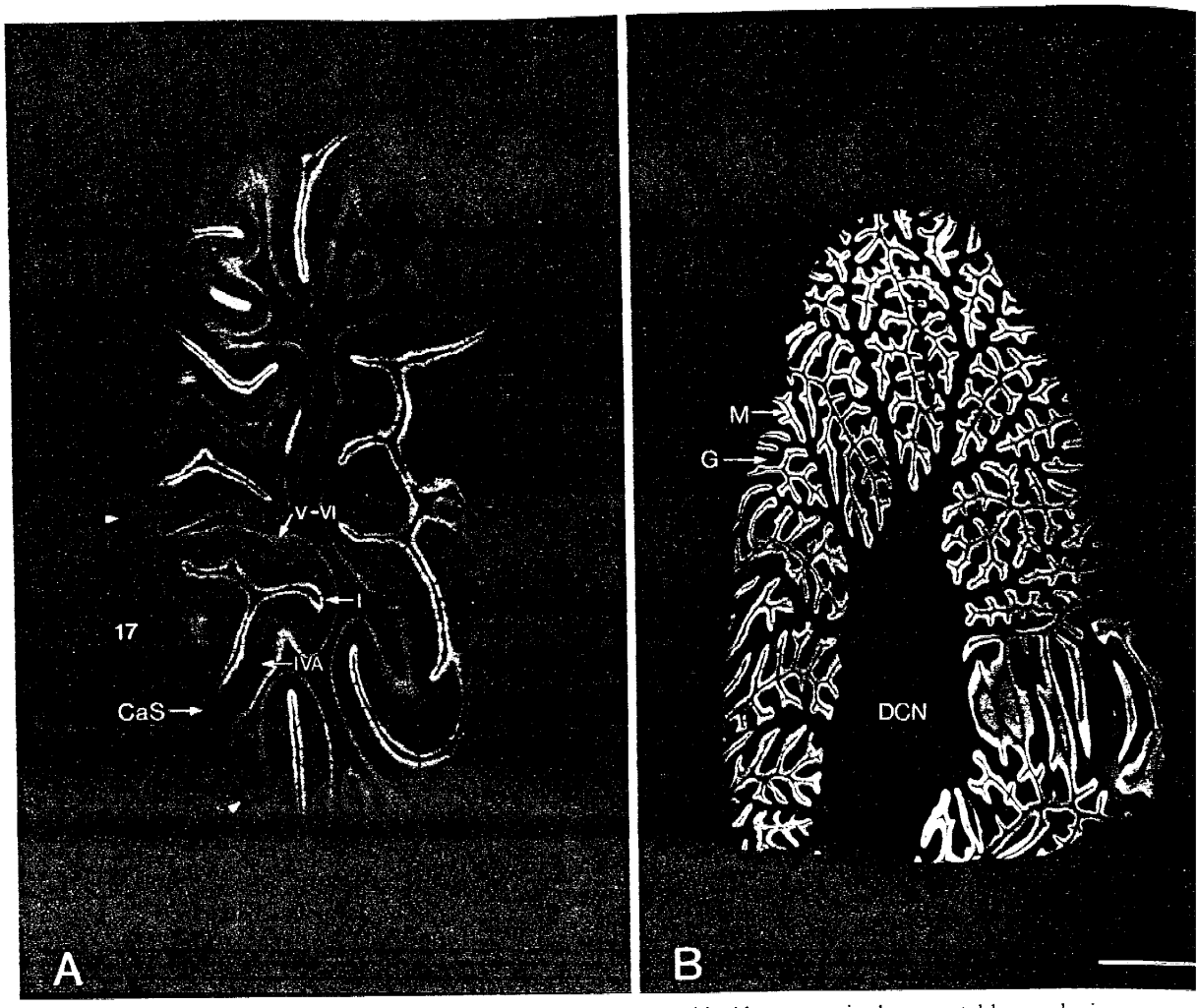

Fig. 6. Autoradiograms showing the distribution of cannabinoid receptors in the neonatal human brain (case N2; six months of age). (A) The occipital cortex. (B) The cerebellum. Scale bar=1 cm.

Other nuclear groups in the medulla (hypoglossal nucleus, nucleus ambiguus, inferior olive, lateral reticular nucleus, reticular formation) showed moderately low densities of receptor binding. The nuclei in the medulla associated with the somatosensory pathways (gracile nucleus, cuneate nucleus, sensory trigeminal nuclear complex) showed the lowest levels of receptor binding in the autoradiograms.

Spinal cord. The spinal cord was distinguished by very low levels of receptor binding. Low densities of receptors were present in the substantia gelatinosa, the intermediate gray horn (excluding the central cervical nucleus) and in the ventral horn. Other areas showed no significant specific binding.

The distribution and density of receptors in the fetal and neonatal human brain

In this study, in order to investigate the ontogenic development of cannabinoid receptors in the human brain, quantitative autoradiographic receptor studies were also undertaken on regions of the fetal and neonatal brain which contained some of the highest concentrations of receptors in the adult brain. Because of the difficulty in obtaining fetal and neonatal human tissue, only three cases were available—one fetal brain (33 weeks gestation) and two neonatal brains (aged three and six months; see Table 1 for details)—and only a very small number of tissue blocks were available for analysis. The results are shown in Figs 6 and 7 and in Table 3.

Forebrain. The occipital cortex (Fig. 6A) and basal ganglia (Fig. 7A–C) were the only regions of the forebrain available for study.

Visual cortex. There was a laminar pattern of distribution and density of cannabinoid receptors in the neonatal visual cortex (Fig. 6A, Table 3). As in the adult brain, the neonatal brain showed a clearly delineated, moderately dense narrow band of labelling in the most superficial region of the cortex, which corresponded to lamina I (Fig. 6A), and a second

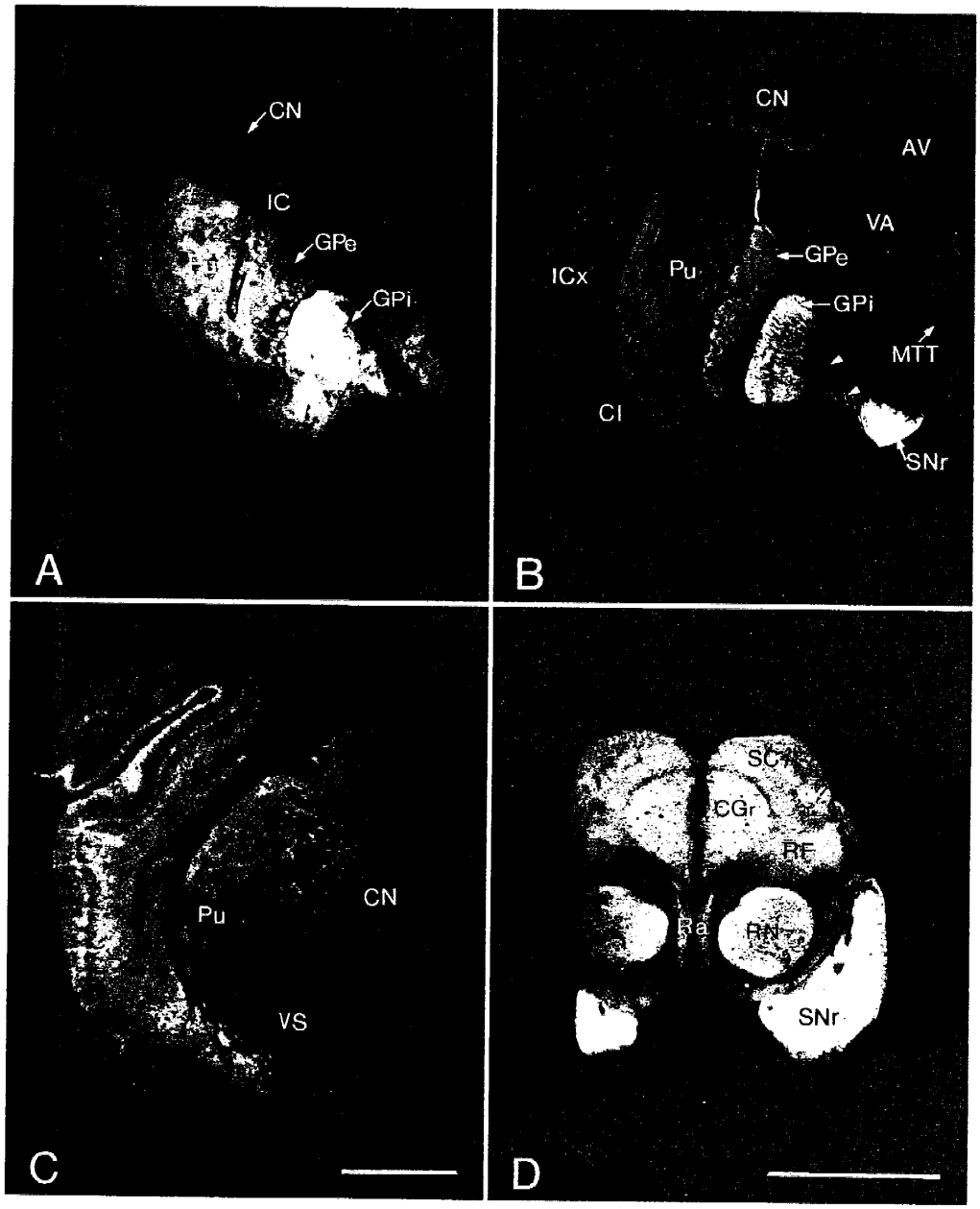

Fig. 7. Autoradiograms showing the distribution of cannabinoid receptors in the lenticular nucleus (A) of the fetal human brain (case FH1; 33 weeks of gestation), and in the lenticular nucleus (B), striatal complex (C) and midbrain (D) of the neonatal human brain (case N2; six months of age). The arrowheads in B indicate cannabinoid receptor binding sites in fibre bundles coursing from the level of the lenticular nucleus towards the substantia nigra in the midbrain. Scale bar=1 cm.

broader and more diffuse conspicuous band of dense receptor labelling which was localized in the deepest laminae of the cerebral cortex, laminae V and VI (Fig. 6A). The intervening middle laminae (laminae II–IV) showed much lower densities of receptors and, as in the adult human brain, a very faint narrow line of receptor labelling was only just visible in laminae IVA of the autoradiograms from the primary visual cortex (Fig. 6A).

Basal ganglia. As in the adult human brain, the neonatal brain (Fig. 7B, C) showed the same pattern of high concentrations of cannabinoid receptors in the various component nuclei of the basal ganglia. However, in the neonatal brain the density of cannabinoid receptors in the basal ganglia was on average more than 40% higher in the globus pallidus and 80% higher in the striatum than the concentration of receptors in the basal ganglia of the adult

Table 3. The density of [^{3}H]CP55,940 binding in the neonatal human brain (fmol/mg)

Neocortex	
Primary visual cortex (area 17)	
Layer I	66 ± 7
Layers II, III and IVA	19 ± 7
Layer IVB	25 ± 9
Layer IVC	19 ± 9
Layers V and VI	61 ± 11
Secondary visual cortex (area 18)	
Layer I	62 ± 14
Layers II–IV	29 ± 15
Layers V and VI	56 ± 14
Basal ganglia	
Caudate nucleus	72 ± 11
Putamen	86 ± 18
Ventral striatum	73 ± 16
Globus pallidus	
Internal	135 ± 20
External	100 ± 15
Midbrain	
Substantia nigra	181 ± 26
Red nucleus	151 ± 15
Central gray	157 ± 11
Superior colliculus	137 ± 13
Cerebellum	
Molecular	103 ± 13
Granular	29 ± 9
Deep cerebellar nucleus	16 ± 7

The densities are expressed as means ± S.D. of femtomoles of [^{3}H]CP55,940 bound per mg of tissue. Because of the difficulty in obtaining neonatal human brain tissue, the values given are the average of two sections taken from either case N1 or case N2.

brain. The highest concentration of receptors was present in the internal segment of the globus pallidus (135 fmol/mg), with somewhat lower levels in the external segment (100 fmol/mg; Fig. 7B). The striatum, comprising of the caudate nucleus, putamen and ventral striatum (Fig. 7B, C), showed moderately high levels of receptors (Table 3) and, as in the adult, a patchy pattern of receptor labelling was evident in the striatum (for example, see Fig. 7C). Just medial to the globus pallidus internus, clearly delineated longitudinal bands of receptor binding sites were identified which corresponded to longitudinal coursing bands of myelinated fibres passing from the level of the lenticular nucleus to the substantia nigra pars reticulata (see arrowheads in Fig. 7B).

As shown in Fig. 7A, the fetal brain showed markedly higher densities of cannabinoid receptors in the basal ganglia. In particular, the globus pallidus internus showed a density of receptors (333 fmol/mg) which was more than twice that in the neonatal brain (135 fmol/mg; compare Fig. 7A and B). Also, the density of receptors in the globus pallidus externus in the fetal brain (146 fmol/mg) was 46% higher than that in the neonatal brain. In addition, the density of receptors in the putamen of the fetal brain (169 fmol/mg) was double that in the neonatal brain, and showed a very conspicuous patchy distribution of cannabinoid receptors. The patchy pattern of labelling in the putamen of the fetal brain (Fig. 7A) was considerably more striking than that in the neonate (Fig. 7B) and adult (Fig. 4C) brains.

Midbrain. In comparison with the adult human brain, all regions of the midbrain in the neonate showed substantially higher levels of cannabinoid receptors (compare Figs 7D and 4D, and Tables 3 and 2). In particular, the density of cannabinoid receptors in the substantia nigra pars reticulata in the neonate was especially high (181 fmol/mg), showing a 24% increase in density compared with the adult substantia nigra pars reticulata. Also, the concentration of receptors in the other major regions of the midbrain (red nucleus, central gray, superior colliculus) in the neonate averaged 148 fmol/mg, compared with an average of 12 fmol/mg for the same areas in the adult human brain.

Hindbrain.

Cerebellum. The cerebellum in the neonate showed the same distinctive laminar pattern of cannabinoid receptor labelling (Fig. 6B) that was evident in the adult human brain (Fig. 2D). A very conspicuous band of dense receptor labelling was present in the molecular layer, where the density of labelling (103 fmol/mg) was comparable to that in the adult cerebellum (106 fmol/mg). Labelling in the other regions of the neonatal cerebellum (granular layer, Purkinje cell layer, deep cerebellar nuclei) was comparable in density to the adult cerebellum.

DISCUSSION

This study represents the first detailed investigation on the overall distribution of cannabinoid receptors throughout the human CNS. The study has clearly demonstrated a heterogeneous distribution of cannabinoid receptors throughout the adult human brain and spinal cord. The results show that cannabinoid receptor binding sites in the human brain are localized mainly in: forebrain areas associated with higher cognitive functions; forebrain, midbrain and hindbrain areas associated with the control of movement; and in hindbrain areas associated with the control of motor and sensory functions of the autonomic nervous system. As discussed below, of particular interest is the correlation between the anatomical pattern of cannabinoid receptor localization in the allocortex, neocortex, thalamus, basal ganglia, cerebellum and medulla with the known effects of cannabinoids on higher cognitive and motor functions.

The results of this study confirm and considerably extend recent studies by ourselves and others on the distribution of cannabinoid receptors in the human basal ganglia, hippocampus and cerebellum,[22,31,48,49,61,72] and on the overall pattern of cannabinoid receptors reported in primate and

subprimate brains.[29,30,31,59] In particular, our studies confirm and extend the detailed quantitative autoradiographic receptor studies of Herkenham *et al.*[29-31] on the distribution of cannabinoid receptors in the rodent brain, showing that the highest concentrations of receptors in the mammalian brain are localized in the basal ganglia, cerebellar molecular layer and hippocampal formation. Also the general pattern of distribution of cannabinoid receptors demonstrated here in the human brain is essentially similar to that seen by Herkenham *et al.*[30] in the subprimate rodent mammalian brain; however, because of the increased complexity and relative massive enlargement of the human forebrain with the resultant complex elaboration of the associational cortical regions, the distribution of cannabinoid receptors in the human forebrain shows a greater complexity and a more detailed morphological pattern to that seen in the rodent brain. For example, in general agreement with our findings on the human cerebral cortex, Herkenham's detailed plots of receptor density across the cortical surface in the rodent brain[30] showed that cannabinoid receptors were localized in higher concentrations in the superficial and deep laminae of the cerebral cortex. However, in contrast to our findings detailed here in the human brain, no distinct differences were identified in the rodent[30] between the densities of receptors in the primary and secondary "associational" cortical regions; presumably this is due to the fact that the rodent cerebral cortex is relatively less differentiated and shows little development of "true" associational cortex compared with the highly elaborate human cerebral cortex. Paralleling the development of the human cerebral cortex, the human thalamus is also more elaborate than the thalamus in the rodent brain, and this is dramatically reflected in the anatomical pattern and complexity in the distribution of cannabinoid receptors demonstrated here in the human thalamus. Thus, in comparison to the findings in the rodent thalamus,[30] our studies on the human brain have shown that cannabinoid receptors are present in highest concentrations in the mediodorsal nucleus and anterior nuclear complex of the human thalamus. Furthermore, as detailed below, these particular thalamic nuclei have close connectional affiliations with the associational cortical regions, which are also enriched with cannabinoid receptors in the human brain. Thus, it appears that in comparison to the findings in the rodent brain,[30] the increased complexity in the distribution of cannabinoid receptors demonstrated here parallels the phylogenetic development of the mammalian forebrain.

Allocortex

Very high levels of cannabinoid binding were found in the human allocortex, with especially high levels in Ammon's horn, the subicular complex and the molecular layer of the dentate gyrus; high concentrations of receptors were also present in the entorhinal cortex and amygdaloid complex. The role of the hippocampus and related allocortical structures in the coding of sensory information[16] and the storage of memories[66] is well established. Thus, it may be that cannabinoids produce deficits in memory function by interacting with cannabinoid receptors in the hippocampus. Memory impairment has been suggested to be the most frequently reported psychological deficit produced by the cannabinoids.[56] Memory tasks requiring a delay in recall are affected by cannabis use.[14] Furthermore, marijuana intoxication slows the speed of processing of visual information from image-based memory to a more permanent form, a process thought to be mediated by the hippocampus.[3] These effects in humans are paralleled by observations of decreased performance in signal detection and discrimination tasks in rats and primates.[6,32,65] These deficits may be due to effects of cannabinoids depressing neural activity, since studies by Campbell *et al.*[5] indicate that the transmission of sensory information between the entorhinal cortex and the dentate gyrus via the perforant path is decreased during the action of Δ^9-THC, and high doses of cannabinoids both *in vivo* and *in vitro* depress evoked responses.[44,71] Furthermore, Heyser *et al.*[32] demonstrated the effects of Δ^9-THC on delayed match to sample performance in rodents to be similar to the effects produced by damage to the hippocampus and related structures, and correlated to decreased hippocampal cell discharge during the task performance. Thus, the very high levels of cannabinoid receptors in the hippocampus and subiculum may provide the anatomical basis of the amnesic effects of cannabis.

Neocortex

Cannabinoid binding sites were also present throughout all regions of the neocortex, where they showed a marked variation in density between the primary, secondary and associational cortical regions. In general, the greatest densities of receptors in the neocortex were in the associational cortical regions of the frontal lobe (Fig. 1A), limbic lobe (Fig. 1C) and temporal lobe (Figs 1D, 2B), with moderate densities of receptors in the secondary sensory and motor cortical regions, and the lowest densities of receptors in the primary sensory and motor cortical regions. This study therefore shows that cannabinoid receptors are localized in greatest density in the major regions of the human associative neocortex, which are involved with higher order cognitive functions. The complexity of the effects mediated by cannabinoid receptors on cortical function in these regions is dramatically illustrated by the pattern of receptor labelling in the autoradiograms, which show a corresponding pattern in the anatomical laminar distribution of the receptors in each of these cortical regions in the human brain. Although cannabinoid

receptors are present in all cortical laminae of the associational cortices, there are quite distinct laminar differences in the density of receptors which coincide with the anatomical boundaries of the laminae. Thus, laminae I, IVB and V/VI are characterized by clearly delineated bands of very dense receptor labelling, while laminae II/III are distinguished by lower densities of receptors and sublaminae IVA and IVC are characterized by very low levels of receptors. Since the supragranular layers (i.e. laminae I–III) of the cerebral cortex are thought to play a major role in associative cortical functions via their pattern of afferent and efferent connections with other cortical regions in the homolateral and contralateral hemispheres,[42] cannabinoid receptors localized in these superficial laminae may be involved in the modulation of associative cortical activity crucial for higher cognitive functions. By contrast, neurons in the infragranular laminae (laminae V/VI) provide the efferent (or motor) projections from the cerebral cortex to subcortical regions of the brain and spinal cord; therefore, the high-density bands of cannabinoid receptors localized discretely in laminae V/VI are strategically located to enable cannabinoids to modulate and monitor cortical efferent or "motor" activity. Finally, since lamina IV (the granular lamina) is mainly concerned with receiving afferent or "sensory" information from subcortical thalamic nuclei, the discrete dense band of cannabinoid receptors shown in this study to be localized in the middle of this layer (laminae IVB) in the frontal, limbic and temporal association cortical regions (Figs 1A, C, D, 2B) suggest a possible role for cannabinoid receptors in the modulation of subcortical "sensory" input to the association cortex. The results of this study therefore show that the localization of cannabinoid receptors in the neocortex of the forebrain correlate with the known observed complex effects of marijuana on cognitive and motor behavioural functions in animals and humans (see Introduction). Indeed, the complex psychoactive effects of marijuana on higher order cognitive processing associated with thought disturbances, difficulties in concentration and the euphoria or cannabinoid "high" are also likely to be mediated by the actions of cannabinoids on receptors localized especially in neocortical associational regions. Furthermore, an "amotivational syndrome" associated with cannabis use has been suggested (for example, see Refs 43 and 52) and, while being difficult to establish in humans,[20,54,55] it has been supported in animal models[60] and could well be mediated by prefrontal cannabinoid receptors.

An unexpected but very interesting result of our studies on the cortical localization of cannabinoid receptors was the finding of higher levels of receptor binding in cortical regions of the left (dominant) hemisphere, known to be associated with verbal language functions, than in the corresponding region of the non-dominant right hemisphere. These findings suggest that cannabinoid receptors show lateralization in the human forebrain and that they may play a role in verbal language control mechanisms. Indeed, subtle difficulties with speech have been reported in users of marijuana;[70] however, as suggested[70] this may be due more to interference with retrieval of information from immediate memory storage rather than specific effects on language control regions. Nevertheless, the action of cannabinoids at these language processing centres may additionally contribute to this effect.

Thalamus

The pattern of cannabinoid receptor distribution in the thalamus corresponds closely to the pattern of receptor distribution in the neocortex. In particular, the highest concentrations of receptors in the thalamus are localized within thalamic nuclei which are known to have very close connectional affiliations with the regions of the cortex which show the highest concentraitons of cannabinoid receptors, i.e. the associational cortical regions. For example, relatively high concentrations of cannabinoid receptors in the thalamus are localized within the mediodorsal nucleus and the anterior nuclear complex, which are closely associated with the prefrontal associational cortex and the limbic cortex (including the cingulate, subiculum and retrosplenial cortex), respectively.[42] Furthermore, moderate densities of cannabinoid receptors were present within the midline and intralaminar complex of the thalamic nuclei, which are known to provide diffuse projections to widespread regions of the neocortex.[28,42] Interestingly, the projections from the intralaminar nuclei are known to terminate within laminae V and VI of the entire cerebral cortex,[28] which this study has shown to be selectively enriched with cannabinoid receptors throughout all cortical regions. By contrast, and corresponding with the low level of receptor binding in the primary sensory and motor cortical regions, very low concentrations of receptors were found in: (i) the thalamic primary sensory "relay" nuclei, which have close connectional affiliations with the primary somatosensory cortex (ventral posterior nucleus), primary auditory cortex (medial geniculate body) and primary visual cortex (lateral geniculate body); and (ii) the ventral anterior–ventral lateral motor thalamic nuclei, which are connected with the primary and secondary motor cortical regions.[42]

In summary, there is a corresponding pattern of cannabinoid receptor distribution in the neocortex and thalamus, with the highest concentrations of receptors being localized in those forebrain regions which are concerned with "associative" or cognitive functions. This anatomical distribution of receptors corresponds with the known widespread complex cognitive effects of cannabinoids (for a review see Ref. 12).

Basal ganglia and cerebellum

The effects of cannabinoids on motor activity have been well characterized (see Ref. 12 for a review). In the majority of species studied, cannabinoids lead to a depression of motor activity, characterized by static ataxia in dogs[13,69] and monkeys.[15,27] In these animals, as well as in mice, this depression is accompanied by a state of hyper-reflexia. High doses lead to catalepsy in rats[27] and mice.[46] Furthermore, Δ^9-THC injected directly into the caudate nucleus, but not into the globus pallidus, causes catalepsy in rats.[23] Thus, it is not surprising to find high densities of receptors within the regions of the brain involved in the control of movement, namely the basal ganglia and cerebellum. In these regions, as in others, the general distribution of cannabinoid binding sites was very closely related to that observed in the rat brain.[30] However, in the rat brain the region of the caudate nucleus which receives afferent inputs from the primary sensory and primary motor cortex in the rat[71] (i.e. the dorsolateral sector of the head of the caudate–putamen) is greatly enriched with receptors, compared to lower levels in the ventromedial sector of the striatum. No gradient in receptor levels was identified in this study, confirming the finding of Herkenham *et al.*[31] in suggesting that this lateral to medial gradient may be a unique feature of the rodent brain.

As in the rat,[30] and confirming recent studies in the human,[22,61,72] the outflow nuclei of the human basal ganglia exhibit extremely high levels of cannabinoid receptor binding, with the highest levels being observed in the internal segment of the globus pallidus and the substantia nigra pars reticulata. In our studies, the substantia nigra pars reticulata showed some of the highest densities of binding in the human brain. Previous studies have suggested that the cannabinoid receptors in the globus pallidus and substantia nigra are localized on the terminals of striatal projection neurons by rat lesion studies[29] and in studies on the pattern of receptor losses in Huntington's disease.[22,61] This has also been further confirmed by receptor mRNA studies showing cannabinoid receptor gene expression in the striatum but not in the globus pallidus or substantia nigra.[48,72] In particular, further confirmation that the high-density of cannabinoid receptors in the basal ganglia are localized to the striatopallidal and striatonigral projection neurons is provided by two further lines of evidence. First, by the mRNA studies of Westlake *et al.*[72] in the normal and aged human brain showing that, in the striatum, cells expressing cannabinoid receptor mRNA belong to the population of medium-sized striatal neurons which are well established as the neuronal population which provides the GABAergic striatal efferent projections to the globus pallidus and substantia nigra. Second, the presence of moderate densities of cannabinoid receptors along the trajectory of the GABAergic striatonigral projection pathway in the human[31] (Figs 4C, 7B) and rat.[29,30] This labelling presumably represents receptors in transit in striatonigral fibres *en route* to terminals in the substantia nigra pars reticulata and suggests that there may be a high turnover of cannabinoid receptors on striatal efferent terminals in the substantia nigra. These various findings suggest that endogenous cannabinoids may have a role in modulating the release of GABA from striatal efferent terminals in the globus pallidus and substantia nigra, and therefore suggest a role for cannabinoids in the control of movement and possibly in the therapeutic treatment of hyperkinetic neurodegenerative basal ganglia diseases[58] such as Huntington's disease.[57]

Medulla

The highest concentrations of cannabinoid receptors in the medulla were localized in the dorsal motor nucleus of the vagus (Fig. 5B–E), which provides the preganglionic parasympathetic motor innervation of the foregut and midgut, and in the nucleus of the solitary tract, which receives sensory afferent fibres from the autonomic nervous system. The distribution of receptors in the dorsal motor nucleus of the vagus showed a distinctive heterogeneous pattern, which appears to correspond to specific cytoarchitectural and chemoarchitectural subnuclei of the nucleus recently described by Huang *et al.*[40] in a detailed study of the nuclear subdivisions of the dorsal motor nucleus of the vagus in the human brain. For example, the two distinct high-density patches of receptor binding in the rostral region of the nucleus (Fig. 5B) appear to correspond to the dorsorostral and ventrorostral subdivisions, while the high density of receptors in the caudal third (Fig. 5D, E) appears to correspond to the caudal subnucleus. Although little is known about the functional importance of these nuclear subdivisions in the human dorsal motor nucleus of the vagus, studies in the rabbit and pigeon have shown that the subnuclei in the rostral regions of the nuclear complex innervate the abdominal organs and lungs, and the caudal region innervates the oesophagus (for a review see Ref. 40). Although there has been little study to date on the effects of cannabinoids on the autonomic nervous system, the high density of cannabinoid receptors identified in the autonomic medullary nuclei in this study would clearly suggest a role for cannabiniods in the control and modulation of motor and sensory fuctions of the human autonomic nervous system. Some of the physiological actions of cannabinoids which may be attributed to these hindbrain structures include tachycardia, hypotension and dry mouth.[12] In addition, cannabinoids have a well established anti-emetic effect following cancer chemotherapy (see Ref. 67), although the mechanism of this effect is poorly understood. Studies in the cat suggest that nabilone, a cannabinoid analogue, produces its anti-emetic effects within the cerebral cortex by inhibiting the

vomiting control mechanism located in the medulla oblongata via its descending corticomedullary pathways.[47] Furthermore, the anti-emetic effect and appetitite-stimulating properties of cannabis suggest that cannabinoids may be useful in the treatment of AIDS patients (for example, see Refs 4 and 45). Wasting is the leading cause of death for AIDS patients, making the potential of cannabinoids to stimulate appetite in these patients of particular therapeutic importance.

Ontogeny of cannabinoid receptors

Due to the small numbers of cases available for this study, it is not possible to draw any definitive conclusions on the precise levels of cannabinoid receptor binding within the developing human brain. Also, since the fetal/neonatal and adult human tissue was not processed together, considerable care must be taken in comparing the results of the fetal/neonatal studies with the results in the adult human brain. However, it is clear from this study that cannabinoid receptors are present in the prenatal and neonatal brain at levels which are substantially higher than those seen in the adult human brain. In particular, the basal ganglia in the fetal and neonatal brains showed a very conspicuous patchy pattern of receptors in the putamen, and extremely high levels of binding were present in the globus pallidus internus (Fig. 7A) and within the substantia nigra pars reticulata. The striking patchy pattern of CP55,940 binding sites in the fetal human striatum (Fig. 7A) suggests that cannabinoid receptors may be distributed in the same heterogeneous striosome/matrix compartmental fashion that has been shown to be a characteristic feature of the distribution of a wide variety of neurotransmiiters, receptors and other neurochemical markers in the neostriatal complex in the adult human[17–19,68] and other mammalian brains.[25] Indeed, the finding that this patchy pattern of receptor distribution in the striatum is especially conspicuous in the fetal brain (Fig. 7A) and is less pronounced in the neonatal (Fig. 7B, C) and adult (Fig. 4A–C) human brains is consistent with other studies showing that the intensity and pattern of other neurochemical markers in the striatum changes during development[24,26] and may be related to the organization of the modular development of the dopamine-containing innervation of the striatum.[24]

Also of interest was the finding of very high levels of receptors in the neonatal red nucleus and periaquaductal gray regions, areas which demonstrate minimal binding in the adult. This study therefore differs from a previous study, which showed cannabinoid receptors in the neonatal human brain to be present at similar levels to the adult.[48]

These results are in good general agreement with studies on the ontogeny of cannabinoid receptors in the rodent brain. The presence of cannabinoid receptors in the rat brain has been demonstrated from early postnatal ages.[53,64] The receptors exhibit a progressive increase in number, maximizing on postnatal days 30–40 and then subsequently decreasing to adult values. A role for cannabinoids in development has been suggested by several studies. Cannabinoid receptors exhibit a temporary presence in the subplate zone during development in kittens,[7] and prenatal exposure to cannabinoids has been found to markedly affect the maturation of several brain dopaminergic systems.[62,63] This study therefore confirms the presence of cannabinoid receptors within the developing brain, and suggests that further, more detailed studies are neccessary in order to elucidate the role of cannabinoids in the developing fetal brain.

CONCLUSION

The results of our detailed quantitative autoradiographic studies show that cannabinoid receptors are heterogeneously distributed in a similar pattern in the fetal, neonatal and adult human brains. The high concentrations of receptors in motor and cognitive regions of the brain correlates well with the known behavioural, psychomotor and psychological effects of cannabis. Further studies determining the distribution and action of putative endogenous ligands such as anandamide[11] for this receptor in the human brain should provide a better understanding of the physiological role of cannabinoid receptors in the CNS.

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REFERENCES

1. Bidaut-Russell M., Devane W. A. and Howlett A. C. (1990) Cannabinoid receptors and modulation of cyclic AMP accumulation in rat brain. *J. Neurochem.* **55**, 21–26.
2. Bidaut-Russell M. and Howlett A. C. (1989) Opioid and cannabinoid analgetics both inhibit cyclic AMP production in the rat striatum. *Adv. Biosci.* **75**, 165–168.
3. Braff D. L., Silverton L., Saccuzzo D. P. and Janowsky D. S. (1981) Impaired speed of visual information processing in marijuana intoxication. *Am. J. Psychiat.* **138**, 613–617.
4. Bruera E. (1993) Is the pharmacological treatment of cancer cachexia possible? *Support. Care. Cancer* **1**, 298–304.
5. Campbell K. A., Foster T. C., Hampson R. E. and Deadwyler S. A. (1986) Effects of delta-9-tetrahydrocannabinol on sensory-evoked discharges of granule cells in the dentate gyrus of behaving rats. *J. Pharmac. exp. Ther.* **239**, 941–945.

6. Campbell K. A., Foster T. C., Hampson R. E. and Deadwyler S. A. (1986) Delta-9-tetrahydrocannabinol differentially affects sensory-evoked potentials in the rat detate gyrus. *J. Pharmac. exp. Ther.* **239**, 941–945.
7. Chen Z. and Cynader M. S. (1993) Transient concentration of cannabinoid receptors in the cortex subplate zone during kitten post-natal development. *Soc. Neurosci. Abstr.* **19**, 305.
8. Darley C. F. and Tinklenberg J. R. (1974) Marihuana and memory. In *Marihuana Effects on Human Behaviour* (ed. Miller L. L.), pp. 73–102. Academic, New York.
9. DeLong F. L. and Levy B. I. (1974) A model of attention describing the congnitive effects of marihuana. In *Marihuana Effects on Human Behaviour* (ed. Miller L. L.), pp. 103–120. Academic, New York.
10. Devane W. A., Dysarc F. A. III, Johnson M. R., Mevin L. S. and Howlett A. C. (1988) Determination and characterisation of a cannabinoid receptor in rat brain. *Molec. Pharmac.* **34**, 605–613.
11. Devane W. A., Hanus L., Breuer A., Pertwee R. G., Stevenson L. A., Griffin G., Gibson D., Mandelbaum A., Etinger A. and Mechoulam R. (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949.
12. Dewey W. L. (1986) Cannabinoid pharmacology. *Pharmac. Rev.* **38**, 151–178.
13. Dewey W. L., Jenkins J., O'Rourke T. and Harris L. S. (1972) The effects of chronic administration of *trans*-delta-9-tetrahydorcannibinol on behaviour and the cardiovascular system of dogs. *Archs int. Pharmacodyn. Thér.* **198**, 118–131.
14. Dornbush R. (1974) Marijuana and memory: effects of smoking on storage. *Trans. N. Y. Acad. Sci.* **36**, 94–100.
15. Edery H., Greenfeld Y., Porath G., Ben-Zri Z., Shani A. and Mechoulam R. (1972) Structure activity relationships in the tetrahydrocanabinol species. *Arzneimittel-Forshch.* **22**, 1995–2003.
16. Eichenbaum H. and Cohen N. J. (1988) Representation in the hippocampus: what do hippocampal neurons encode? *Trends Neurosci.* **11**, 244–248.
17. Faull R. L. M., Dragunow M. and Villliger J. W. (1989) The distribution of neurotensin receptors and acetylcholinesterase in the human caudate nucleus: evidence for the existence of a third neurochemical compartment. *Brain Res.* **488**, 381–386.
18. Faull R. L. M. and Villiger J. W. (1986) Heterogeneous distribution of benzodiazepine receptors in the human striatum: a quantitative autoradiographic study comparing the pattern of receptor labelling with the distribution of acetylcholinesterase staining. *Brain Res.* **381**, 153–158.
19. Faull R. L. M. and Villiger J. W. (1988) Multiple benzodiazepine receptors in the human basal ganglia: a detailed pharmacological and anatomical study. *Neuroscience* **24**, 433–451.
20. Foltin R. W., Fischman M. W., Brady J. V., Bernstein D. J., Caprioti R. M., Nellis M. J. and Kelly T. H. (1990) Motivational effects of smoked marijuana: behavioral contingencies and low-probability activities. *J. exp. analyt. Behav.* **53**, 5–19.
21. Gaoni Y. and Mechoulam R. (1964) Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am. chem. Soc.* **86**, 1646–1647.
22. Glass M., Faull R. L. M. and Dragunow M. (1993) Loss of cannabinoid receptor in the substantia nigra in Huntington's disease. *Neuroscience* **56**, 523–527.
23. Gough A. L. and Olley J. E. (1978) Catalepsy induced by intrastriatal injectins of delta-9-THC and 11-OH-delta-9-THC in the rat. *Neuropharmacology* **17**, 137–144.
24. Graybiel A. M. (1984) Correspondence between the dopamine islands and striosomes of the mammalian striatum. *Neuroscience* **13**, 1157–1187.
25. Graybiel A. M. and Ragsdale C. W. (1978) Histochemically distinct compartments in the striatum of human, monkey and cat demonstrated by acetyl-thiocholinesterase staining. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5723–5726.
26. Graybiel A. M. and Ragsdale C. W. (1980) Clumping of acetylcholinesterase activity in the developing striatum of the human fetus and young infant. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1214–1218.
27. Grunfield Y. and Edery H. (1969) Psychopharmacological activity of the active constituents of hashish and some related cannabinoids. *Psychopharmacology* **14**, 200–210.
28. Herkenham M. (1980) Laminar organisation of thalamic projections to the rat neocortex. *Science* **207**, 532–535.
29. Herkenham M., Lynn A. B., de Costa B. R. and Richfield E. K. (1991) Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res.* **547**, 267–274.
30. Herkenham M., Lynn A. B., Johnson M. R., Melvin L. S., de Costa B. R. and Rice K. C. (1991) Characterization and localization of cannabinoid receptors in the rat brain: a quantitative *in vitro* autoradiographic study. *J. Neurosci.* **11**, 563–583.
31. Herkenham M., Lynn A. B., Little M. D., Johnson M. R., Melvin L. S., de Costa B. R. and Rice K. C. (1990) Cannabinoid receptor localization in brain. *Proc. natn. Acad. Sci. U.S.A.* **87**, 1932–1936.
32. Heyser C. J., Hampson R. E. and Deadwyler S. A. (1993) Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. *J. Pharmac. exp. Ther.* **264**, 294–307.
33. Hollister L. E. (1986) Health aspects of cannabis. *Pharmac. Rev.* **38**, 1–20.
34. Howlett A. C. (1984) Inhibition of neuroblasta adenylate cyclase by cannabinoid and nantradiol compounds. *Life Sci.* **35**, 1803–1810.
35. Howlett A. C. (1985) Cannabinoid inhibition of adenylate cyclase. Biochemistry of response in neuroblasta cell membrane. *Molec. Pharmac.* **27**, 429–436.
36. Howlett A. C. and Fleming R. M. (1984) Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response of neuroblasta cell membranes. *Molec. Pharmac.* **26**, 532–538.
37. Howlett A. C., Johnson M. R., Melvin L. S. and Milne G. M. (1988) Nonclassical cannabinoid analgetics inhibit adenylate cyclase: development of a cannabinoid receptor model. *Molec. Pharmac.* **33**, 297–302.
38. Howlett A. C., Qualy J. M. and Khachatrain L. L. (1986) Involvement of Gi in the inhibition of adenylate cyclase by cannabinoid drugs. *Molec. Pharmac.* **29**, 307–313.
39. Howlett A. C. (1987) Cannabinoid inhibition of adenylate cyclase: reactive activities of marihuana constituents and metabolites. *Neuropharmacology* **26**, 507–512.
40. Huang X. F., Törk I. and Paxinos G. (1993) Dorsal motor nucleus of the vagus nerve: a cyto- and chemoarchitectonic study in the human. *J. comp. Neurol.* **330**, 158–182.

41. Johnson M. R. and Melvin L. S. (1986) The discovery of non-classical cannabinoid analgesics. In *Cannabinoids as Therapeutic Agents* (ed. Mechoulam C. R.), pp. 121–145. CRC Press, Boca Raton, FL.
42. Jones E. G. (1985) *The Thalamus*. Plenum, New York.
43. Kolansky H. and Moore W. T. (1972) Toxic effects of chronic marijuana use. *J. Am. med. Ass.* **222,** 35–41.
44. Kujtan P. W., Carlen P. L. and Kapur B. M. (1983) Delta-9-tetrahydrocannabinol and cannabidiol: dose dependent effects on evoked potential in the hippocampal slice. *Can. J. Physiol. Pharmac.* **61,** 420–426.
45. Lehrman S. (1995) US stalls over tests of marijuana to treat AIDS patients. *Nature* **374,** 7–8.
46. Little P. J., Compton D. R., Johnson M. R. and Martin B. R. (1988) Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J. Pharmac. exp. Ther.* **247,** 1046–1051.
47. London S. W., McCarthy L. E. and Borison H. L. (1979) Suppression of cancer chemotherapy induced vomiting in the cat by nabilone, a synthetic cannabinoid. *Proc. Soc. exp. Biol. Med.* **160,** 437–440.
48. Mailleux P. and Vanderhaeghen J. J. (1992) Localization of cannabinoid receptor in the human developing and adult basal ganglia. Higher levels in the striatonigral neurons. *Neurosci. Lett.* **148,** 173–176.
49. Mailleux P., Verslijpe M. and Vanderhaeghen J. J. (1992) Initial observations on the distribution of cannabinoid receptor binding sites in the human adult basal ganglia using autoradiography. *Neurosci. Lett.* **139,** 7–9.
50. Manno J. E., Kiplinger G. F., Haine S. E., Bennett I. F. and Forney R. B. (1970) Comparative effects of smoking marihuana or placebo on human motor and mental performance. *Clin. Pharmac. Ther.* **11,** 808–815.
51. Matsuda L. A., Lolait S. J., Brownstein M. J., Young A. C. and Bonner T. I. (1990) Structure of cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346,** 561–564.
52. McGlothin W. H. and West L. J. (1968) The marijuana problem: an overview. *Am. J. Psychiat.* **125,** 370–378.
53. McLaughlin C. R. and Abood M. E. (1993) Developmental expression of cannabinoid receptor mRNA. *Devl Brain Res.* **76,** 75–78.
54. Mello N. K. and Mendelson J. H. (1985) Operant acquisition of marijuana by women. *J. Pharmac. exp. Ther.* **235,** 162–171.
55. Mendelson J. H., Kuehnle J. C., Greenberg I. and Mello N. K. (1976) Operant acquisition of marijuana by men. *J. Pharmac. exp. Ther.* **198,** 42–53.
56. Miller L. L. and Braconnier R. J. (1983) Cannabis: effects on memory and the cholinergic limbic system. *Psychol. Bull.* **93,** 441–456.
57. Moss D. E., Manderscheild P. Z. and Montgomery S. P. (1989) Nicotine and cannabinoids as adjuncts to neuroleptics in the treatment of Tourette syndrome and other motor disoders. *Life Sci.* **44,** 1521–1525.
58. Myers R. H., Vonsattel J. P., Paskevich P. A., Keily D. K., Stevens T. J., Cupples L. A., Richardson E. P. and Bird E. D. (1991) Decreased neuronal and increased oligodendroglial densities in Huntington's disease caudate nucleus. *J. Neuropath. exp. Neurol.* **50,** 729–742.
59. Pacheco M. A., Ward S. J. and Childers S. R. (1993) Identification of cannabinoid receptors in cultures of rat cerebellar granule cells. *Brain Res.* **603,** 102–110.
60. Paule M. G., Allen R. R., Bailey J. R., Scallet A. C., Ali S. F., Brown R. M. and Skinner W. J. (1992) Chronic marijuana smoke exposure in the rhesus monkey. II: Effects on progressive ratio and conditioned postition responding. *J. Pharmac. exp. Ther.* **260,** 210–222.
61. Richfield E. K. and Herkenham M. (1994) Selective vulnerability in Huntington's disease: preferential loss of cannabinoid receptors in lateral globus pallidus. *Ann. Neurol.* **36,** 577–584.
62. Rodriguez de Fonseca F., Cebeira M., Fernandez-Ruiz J. J., Navarro M. and Ramos A. J. (1991) Effects of pre- and perinatal exposure to hashish extracts on the ontogeny of brain dopaminergic neurons. *Neuroscience* **43,** 717–723.
63. Rodriguez de Fonseca F., Cebeira M., Hernandez M. L., Ramos J. A. and Fernandez-Ruiz J. J. (1990) Changes in brain dopaminergic indices induced by perinatal exposure to cannabinoids. *Devl Brain Res.* **51,** 237–240.
64. Rodriguez de Fonseca F., Ramos J. A., Bonnin A. and Fernandez-Ruiz J. J. (1993) Presence of cannabinoid binding sites in the brain from early postnatal ages. *NeuroReport* **4,** 135–138.
65. Shulze G. E., McMillan D. E., Bailey J. R., Scallet A., Ali S. F., Slikker W. J. and Paule M. G. (1988) Acute effects of delta-9-tetrahydrocannabinol in rhesus monkeys as measured by performance in a battery of complex operant tests. *J. Pharmac. exp. Ther.* **245,** 178–186.
66. Thompson R. F., Berger T. W. and Madden J. I. (1983) Cellular processes of learning and memory in the mammalian CNS. *A. Rev. Neurosci.* **6,** 447–491.
67. Vincent B. J., McQuiston D. J., Einhorn L. H., Nagy C. M. and Brames M. J. (1983) Review of cannabinoids and their antiemetic effectiveness. *Drugs* **25,** 52–62.
68. Waldvogel H. J. and Faull R. L. M. (1993) Compartmentalization of parvalbumin immunoreactivity in the human striatum. *Brain Res.* **610,** 311–316.
69. Walton R. P., Martin L. F. and Keller J. H. (1938) The relative activity of various purified products obtained from American grown hashish. *J. Pharmac. exp. Ther.* **62,** 239–251.
70. Weil A. T. and Zinberg N. E. (1969) Acute effects of marijuana on speech. *Nature* **222,** 434–437.
71. Weisz D. J., Gunnell D. L., Teyler T. J. and Vardaris R. M. (1982) Changes in hippocampal CA1 population spikes following administration of delta-9-THC. *Brain Res. Bull.* **8,** 155–162.
72. Westlake T. M., Howlett A. C., Bonner T. I., Matsuda L. A. and Herkenham M. (1994) Cannabinoid receptor binding and messenger RNA expression in human brain: an *in vitro* receptor autoradiography and *in situ* hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience* **63,** 637–652.

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