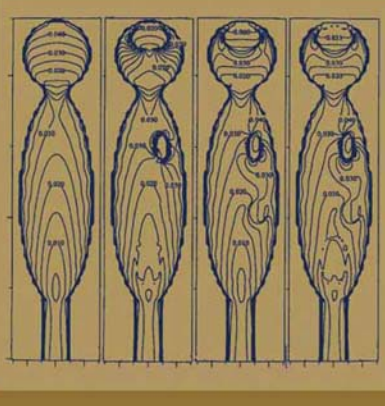
M. Kato (Ed.)

Electromagnetics in Biology



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With 80 Figures

Springer

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Preface

Bioelectromagnetics is a relatively new area of science that deals with the interaction of electromagnetic energy with biological systems. Therefore, studies usually are carried out jointly by researchers from both biological/medical sciences and engineering/physical sciences: expertise in both areas is necessary.

Given the complexity and newness of the discipline, it is no surprise that the results of published studies often appear to be inconsistent. Efforts to replicate are few, and they often involve differences from the original study; furthermore, the current knowledge is insufficient to know if the methodological differences among studies are critical or trivial. Often a phenomenon becomes "hot" for a few years, and different investigators try different experiments broadly related to the phenomenon of interest. As the situation becomes complicated, another hot effect emerges, and investigators chase what is believed to finally be the robust, unambiguous effect that will establish bioelectromagnetics. Examples of this pattern have included calcium efflux, neurite outgrowth, cellular proliferation, ornithine decarboxylase, reduced melatonin, and magnetic field blockage of melatonin's inhibition of MCF7 cell growth. Effect sizes often are small relative to the noise, and ability to replicate between and within labs, although not well documented, appears limited. Thus, the sad result often is inability to determine if an effect is real, limited to very unique circumstances, or otherwise.

There are many books attempting to provide comprehensive literature reviews. Examples include two books. One is *Research on Power-Frequency Fields Completed Under the Energy Policy Act of 1992*, by the National Research Council (NRC, 1997), and the other is *Health Effects from Exposure to Power-Line Frequency Electric and Magnetic Fields*, by the National Institute of Environmental Health Sciences (NIEHS, 1998). These sources include topical reviews of the published literature. For this book, papers published in peer-reviewed journals were scrutinized. Those papers with insufficient description of methodology, both biological/medical and/or physical/engineering, were not accepted when preparing this book. If the authors have a bias, it is slightly on the side that believes power-frequency and radiofrequency electromagnetic fields might have biological effects. If there are no effects, consideration of mechanism of action, which is the major objective of this book, is irrelevant. It is

much clearer that radiofrequency fields, if of sufficient magnitude, can have biological effects.

The extensive epidemiological literature is covered in the books cited above and thus is not reviewed here. The authors, who are engineers and scientists, lack the expertise needed for a critical review of the epidemiology. More fundamentally, epidemiology at best provides evidence only for association, i.e., correlation, not causation. The authors are most interested in what "is", not what "might be".

This book is intended for upper-level undergraduate students and/or lower-level graduate students with a beginning interest in bioelectromagnetics.

The authors are most grateful to Dr. Walter R. Rogers of San Antonio, our long-term friend, who made a painstaking effort to edit the manuscript. Without his kindness, this book would have never been published. Finally, I thank my wife Hisako for her understanding and patience for my writing the manuscript. This book has cost her something by distracting me from her for many, many hours over the years.

M. Kato, representing the authors.
2006

Contents

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Part I

Overview, Endpoints, and Methodologies

1

Introduction

Masamichi Kato, Tsukasa Shigemitsu

1.1 A Brief History of Research on Electromagnetic Field Effects on Organisms

All living organisms evolved on a giant magnet, the one called "Earth". The strength of the geomagnetic field is about 40 μT(see section 1.3.2.4.). The earth's magnetic field is quasi-static, varying only slightly with time and location. Natural static electric fields, under clear sky conditions, are about 0.1 kV/m on the earth's surface; field strengths of up to 30 kV/m are reached under clouds producing lightning.

In addition to these naturally existing electromagnetic fields, we live in an artificially created electromagnetic environment. Most commercial electrical systems operate at either 50 or 60 Hz. Electrical and electronic devices operating at this "power frequency"-such as hair dryers and refrigerators - are in everyday use. Furthermore, many of our daily activities are conducted near, and sometimes under, high-voltage transmission lines and lower-voltage distribution lines.

Even though the use of electricity began more than 100 years ago, the possibility that exposure in our daily activities to the electric and magnetic fields produced by various types of electrical equipment and facilities might have previously unrecognized adverse health effects. This topic has been a subject of concern, beginning about 1975.

At low frequencies, the electric and magnetic field components are independent, meaning there is no true electromagnetic field, as occurs at much higher frequencies. At these high frequencies, the electric and magnetic fields are coupled to each other, so there truly is an electromagnetic field. However, it has become the practice to talk about extremely low frequency (ELF, < 300 Hz) "electromagnetic fields". This phrase often is used indiscriminately to mean electric field, magnetic field, or electric plus magnetic field. Reluctantly, this text will follow the conventional practice and will, on occasion, use the phrase electromagnetic field in an ELF context.

Research on possible electromagnetic field effects on biological systems originated primarily from four different 'sources'. One focus was an interest in basic

neurophysiological function: the nervous system is fundamentally an electrical system. This area began with Galvani and Volta in the early 19th century, when they had their famous controversy about electrical stimulation and contraction of the frog legs. The second focus began in the 1930s among scientists interested in the effects of microwave irradiation on plant cells, animal sarcoma cells, and other targets. The third area was clinical and therapeutic study of the application of electric and magnetic fields to bone fractures: sometimes fractures do not heal properly, and application of currents or fields appears to promote healing. This success has led to an interest in other therapeutic applications. The fourth motivation was based on public concern about and scientific interest in possible adverse health effects. This area was triggered largely by the Soviet Union's governmental decree on electric workers in 1973. Because of concern about ill defined health effects, an occupational exposure standard was promulgated at a field strength far lower than what was considered hazardous in Western countries. Both public concern and scientific interest were strengthened by the epidemiological work of Wertheimer and Leeper (1979), who reported a possible association of power-frequency magnetic fields and childhood leukemia.

Although the former three research areas have been continued steadfastly by scientists and clinicians in each area, the fourth area has been studied most energetically in the last three decades, involving epidemiologist, engineers and scientists from around the world. Furthermore, as cell phones were adopted world-wide in the 1990s, similar concerns and research approaches were applied with these devices, which have much higher frequencies, such as 2 GHz in the newest phones.

1.1.1 Basic neurophysiologic research

Since the Braun tube oscilloscope was introduced to the study of neurophysiology (Gasser 1921), electrical stimulation of one point of the nervous system and recording of responses from a relevant area, either very close or distant, has been a very powerful research technique during the following decades. Surface electrical stimulation (cathodal) of the cerebral cortex was widely used, in animal and some human research, until about 1950. However, with this method, only tissues, such as dendrites and/or axons of neurons which are located near the surface and run parallel to the surface are excited; cell bodies and fibers which are located at some depth are not stimulated directly.

In order to overcome this drawback of this technique, magnetic stimulation techniques have been developed since 1985 in order to study human brain function. The technique was first proposed by Barker et al. (1985). Single coils were placed over the human head, and the motor cortex was stimulated by transcranial pulsed magnetic stimulation. Electromyographic responses were recorded from appropriate muscles. For example, if the motor area of the cortex controlling the arm and hand was stimulated, activity in the muscles, including gross movements, of the arm and hand could be induced. With this technique a wide area of the brain is stimulated. In order to stimulate a localized area of the brain Ueno et al. (1988; 1990) proposed to position a figure-eight coil over the head so as to produce a convergent eddy current so that only localized portion of the motor cortex is stimulated. This method now is used

widely for the study of brain function (e.g., Day and Brown 2001). Magnetic stimulation of other areas of the human brain also has been utilized in an effort to improve mental status (Pascual-Leone et al. 1996).

1.1.2 Biological research with microwave energy

During and after World War II, microwave technology was studied not only for military use, but also for civilian use. Communication technologies (e.g., wireless telephony) have advanced rapidly in recent years; hence, microwave energy now is ubiquitous in the atmosphere. In late 1940s, it was reported that clicking sounds could be heard near a radar station. The radiofrequency hearing effect was systematically studied about 15 years later (Frey 1961), concomitant with other studies of microwave effects on other organs and tissues, such as the eye and the nervous system.

1.1.3 ELF electric fields and bone healing

Since Yasuda (1954) measured piezoelectricity (pressure applied to bone produces a current) of bone, clinical attempts have been made to apply electric fields for the purpose of promotion of healing fractured bone, particularly tibia. Many clinical therapeutic studies have been published since then (e.g., Bassett et al. 1981). However, until the mid-1980s, most clinical studies did not use double-blind, randomized, placebo-controlled studies. Barker et al. (1984) first published a series of double-blind, randomized, placebo-controlled studies on bone healing. The results were generally positive. Since then, many clinical and laboratory animal experiments have been published, and it appears that the efficacy of electric and magnetic field therapy for this purpose has been established. Recently research interest has shifted to explore possible mechanisms for the bone healing induced by magnetic field exposure.

1.1.4 Public concern about exposure to electromagnetic fields

Up until the early 1970s, it was assumed that exposure to electromagnetic fields, at environmentally relevant field strengths, produced no harmful effect on human beings. The results of the few scientific studies completed on the question and the experience of nearly 100 years of successful use of electricity were reassuring. Rather electromagnetic field exposure had been thought to have some favorable effects on some kinds of plants. However, the report by researchers from the Soviet Union at the 1972 CIGRE Meeting, which indicated that workers exposed to high-voltage electric fields showed possible harmful effects, drew much attention worldwide. Apart from the studies of the Soviet investigators, there were almost no reports of harmful effects related to electric field exposure.

Published reports of negative outcomes from this early period included medical studies of ten workers exposed to energized 350 kV lines (Singewald et al.1973), medical studies of fifty-six maintenance workers at 735 kV substations (Roberge

1976), and medical studies of 53 workers over 5 years at 400 kV substations (Knave and Gamberale 1979).

Wertheimer and Leeper (1979) compared the incidence of childhood leukemia and brain tumor in case- and control-children living in the Denver area. Wertheimer and Leeper concluded that an association existed between cancer and exposure to magnetic fields, as their findings appeared to relate high current rather than voltages. The incidence of leukemia was roughly doubled in the exposed cases as compared to the control cases. Actual exposure was not measured; it was estimated based on a wire code classification scheme. After publication of this epidemiological study, many new research efforts related to the safety of ELF fields emerged in both epidemiological and biological areas.

1.1.5 The Bioelectromagnetics Society

The Bioelectromagnetics Society was founded in the United States in 1979, at a time when much of bioelectromagnetic study was motivated by concerns that exposure to anthropogenic electromagnetic fields or radiation might be a human hazard. (Since World War II, most of the bioelectromagnetic research had focused on microwaves.) The purpose of the Society, which now has world-wide membership, is to promote scientific study of the interaction of electromagnetic energy (at frequencies ranging from zero hertz through those of visible light) and acoustic energy with biological systems (Constitution of the Bioelectromagnetic Society, Article II - Purpose).

Although slightly deviant from this Article, however, it has been noted recently that "The history of The Bioelectromagnetics Society is a double-edged sword in that there is still a perception of The Society being focused on and concerned only with a biological threat from electromagnetic fields and waves" (BEMS Newsletter, No. 165, 2002). Under the changing situation in the last several years, particularly after the publication of National Research Council:NRC (1997) and completion of the NIEHS EMF-RAPID Program (1999), the Newsletter continues "For the long term health of The Society, however, emphasis should assume on important areas, such as understanding fundamental mechanisms and efforts to develop tools that can be applied by clinicians to improve human health".

1.1.6 Models used and topics investigated

A number of experimental animals have been used to investigate a variety of possible effects: animals used include chicks, cows, dogs, honey bees, mice, monkeys, pigs, rabbits, and rats. Human subjects also have been recruited for some specific experimental purposes. Experimental areas studied include behavior, development and growth, endocrinology, hematology, immunology, nervous system, and reproduction, along with a range of other areas. Besides these *in vivo* experiments, numerous *in vitro* experiments have been performed. Many of these studies have been published in *Bioelectromagnetics*, the official journal of the Bioelectromagnetics Society; others have been published in many other journals.

1.2 Bioelectricity and Biomagnetism

Bioelectricity is the study of electrical phenomena generated by living organisms and the effects of external electromagnetic fields on the living body. The electrical phenomena include inherent properties of the cells, such as membrane potential, action potential, and propagation of the potentials. Here the word "effects" of external electromagnetic field means how the cells in the body respond to the applied or exposed fields.

Because the brain is so important to human behavior, and because the function of the brain inherently involves a great deal of electrical activity, from the beginning of bioelectromagnetics it has been important to look for effects of electric fields and currents (and magnetic fields, which induce electric fields and currents) on the brain. Therefore, to understand this major research area, it is necessary to have a minimum background in electrophysiology. The following sections provide this needed overview. For advanced study readers are recommended to consult with textbook of neurophysiology (e.g., Kandel et al. 2000)

1.2.1 Bioelectricity

A difference in electrical potential exists between the inside of a cell and the extracellular fluid surrounding it, and this difference is called the membrane potential. The specialized function of the nervous system is to propagate changes in membrane potential within a cell (neuron) and to transmit them to other cells. Transmission of these changes in potential helps the body to coordinate the activity of all of the body's systems. The body can feed the information impinging on it from both the external and internal environments to the central nervous system, where it is processed, enabling the body to adapt in a suitable manner to both of its environments.

1.2.1.1 Membrane potential

The potential difference between the interior of a cell and the fluid surrounding the cell can be measured by connecting one pole of a voltmeter through a fine intracellular electrode inserted into the cell and the other pole to the extracellular fluid. Usually glass capillaries filled with a conducting solution are used as the intracellular electrodes. At the start of the measurement, both electrodes are located outside of the cell, and no potential difference exists between them. When the tip of the glass capillary is pushed through the membrane of the cell, the potential suddenly changes to approximately −75 mV (Fig. 1.1). Because this potential difference is recorded when the membrane is penetrated, it is called the membrane potential; it also is called the resting potential, because it is the potential recorded when the cell is at rest and not being stimulated.

1.2.1.2 Origin of the membrane potential

Both the intra- and the extra-cellular spaces are filled with aqueous salt solutions. In dilute salt solutions, the majority of the molecules dissociate into ions. In aqueous

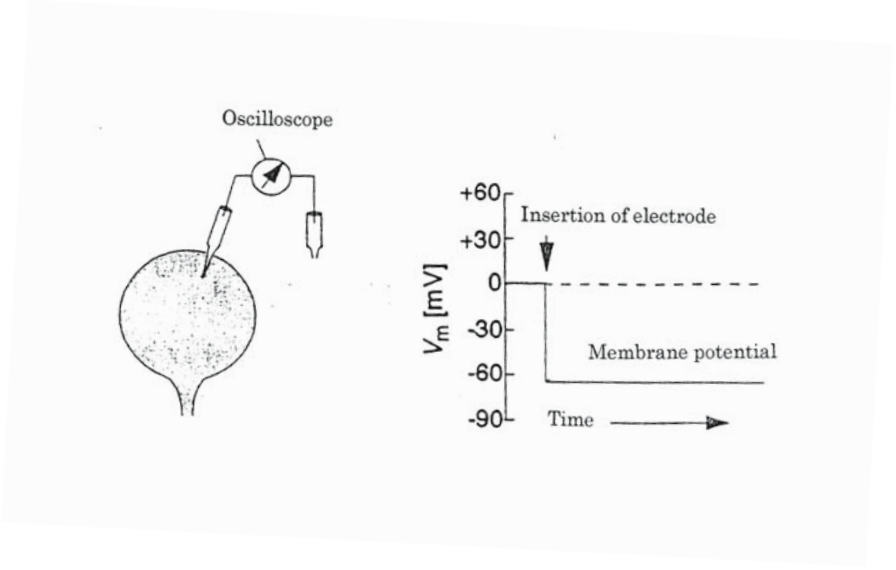

Fig. 1.1. Measuring the intracellular membrane potential.
A diagram of the measuring setup is shown at the left. When an intracellular microelectrode is inserted into the cell, the resting membrane potential is recorded.

solutions, the ions are the sole carriers of charge. Consequently, charge disequilibrium, which is expressed by the resting potential, indicates a certain excess of anions inside the cell and a corresponding excess of cations outside the cell. This disequilibrium is actively maintained by the cells, which use energy to pump ions against their concentration gradients. Thus, the electrical phenomena of the living body are generated by movement of ions, not by the movement of electrons. The source of the resting potential is the unequal distribution of several ions, particularly K^+ ions, inside and outside the cell. Na^+ and Cl^- also are important. The potassium concentration inside the cell is about 40 times higher than in the extracellular space, and the sodium concentration is about 12 times higher outside than inside.

1.2.1.3 The action potential

It is the task of nerve cells to receive, process, and transmit information throughout the nervous system, thereby coordinating, integrating and regulating body functions. When a nerve cell fires, a short (about 1 msec), positive change in the membrane potential develops. These changes are called “action potentials”

Once generated, the action potential is propagated, i.e., conducted, along the nerve. It is characteristic of action potential conduction that the amplitude of the action potential remains constant along the propagation path, because the action potential is generated at every point on the membrane, obeying an “all-or-none” law.

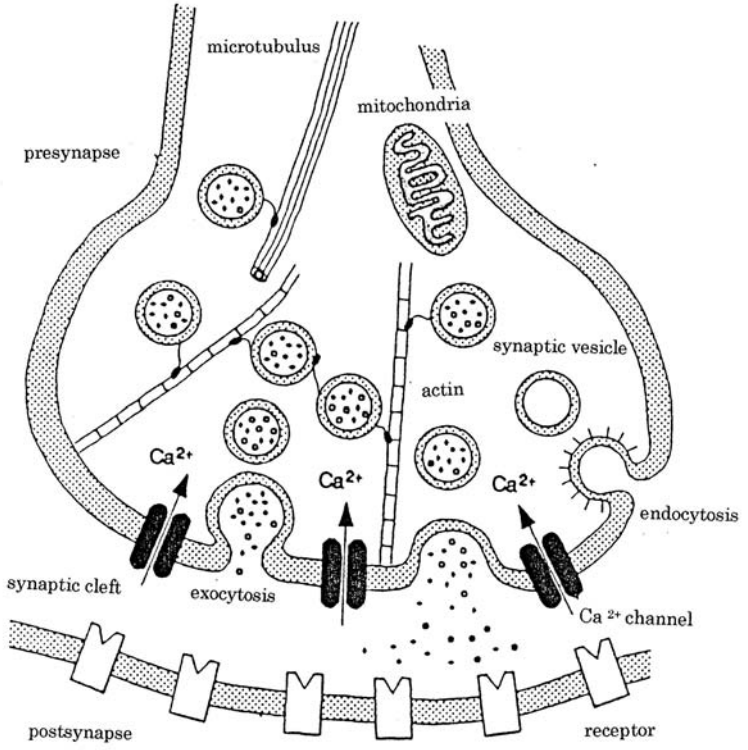

Fig. 1.2. Synapses and release of synaptic transmitter.
Synaptic transmitter is contained in the synaptic vesicles of synaptic knob, which is the tip of the axon. Synaptic vesicles are connected with microtubules and/or actin through synapcin I. With increased intracellular Ca^{2+} concentration synaptic vesicles are freed from the microtubules and/or actin. They then dock and fuse with the presynaptic membrane, from where the transmitter is released by exocytosis.

1.2.1.4 Synaptic transmission

The junction of an axonal ending with a neuron, a muscle fiber, or a glandular cell is called a synapse (Fig. 1.2). At a synapse, the propagated action potential is transmitted to the next cell or cells. There are two types of synapses. In one type of synapse the axonal ending releases a chemical substance that produces either an excitatory or inhibitory effect on the subsynaptic (i.e., post-synaptic; see below) membrane: this type of synapse is called a chemical synapse. The other type of synapse is called an electrical synapse (see below).

1.2.1.5 Structural elements of chemical synapses

Light and electron microscopic investigations have revealed that synaptic junctions contain a variety of elements. Functionally however, all the elements of the chemical synapses can be related to the basic elements illustrated in Figure, 1.2.

Light microscopy indicates that axons end in the presynaptic terminal, forming a spherical enlargement called a "synaptic knob". Electron microscopy shows the presynaptic terminal is separated from the postsynaptic side by a narrow cleft (space) that is, on the average, 10 to 20 nm wide. The subsynaptic membrane under a synaptic knob appears somewhat thicker than the other parts of the postsynaptic membrane, indicating it has a different function from the rest of the postsynaptic membrane.

The presynaptic terminal contains a large number of synaptic vesicles. They are about 50 nm in diameter and contain the transmitter substance that is released into the synaptic cleft upon arrival of an action potential. The transmitter binds to its specific type of receptor located in the external surface of the subsynaptic membrane, triggering responses of the subsynaptic membrane. Central neurons usually possess many dozens to several thousands of synapses. However, these synapses are classified into only two categories, excitatory and inhibitory. The continual integration of a huge number of excitatory and inhibitory signals is the basis of brain function.

1.2.1.6 Excitatory synapses

Stimulation of excitatory afferents to the neuron generates an excitatory postsynaptic potential (EPSP). EPSPs tend to reduce the membrane potential of the postsynaptic neuron. A change in this direction is called excitatory, because it increases the probability that the postsynaptic neuron will fire. The amplitude of the EPSP depends on the number of activated synapses: if this number is sufficiently large, the neuron is depolarized to the threshold, producing an action potential that propagates along the axon.

1.2.1.7 Inhibitory synapses

When inhibitory neurons are excited by some sources, a hyperpolarizing potential change can be recorded from a subsynaptic neuron. With hyperpolarization, the membrane potential is shifted away from the threshold for initiating action potential. For example, the membrane potential might be changed from −70 mV to −75 mV. Consequently the neuron is inhibited, i.e., the probability of firing is reduced. Such hyperpolarization recorded in a neuron is called an inhibitory postsynaptic potential (IPSP).

1.2.1.8 Synaptic receptors

The transmitter substances released from presynaptic terminals combine with receptors located on the surface of the postsynaptic membrane. Synaptic receptors have

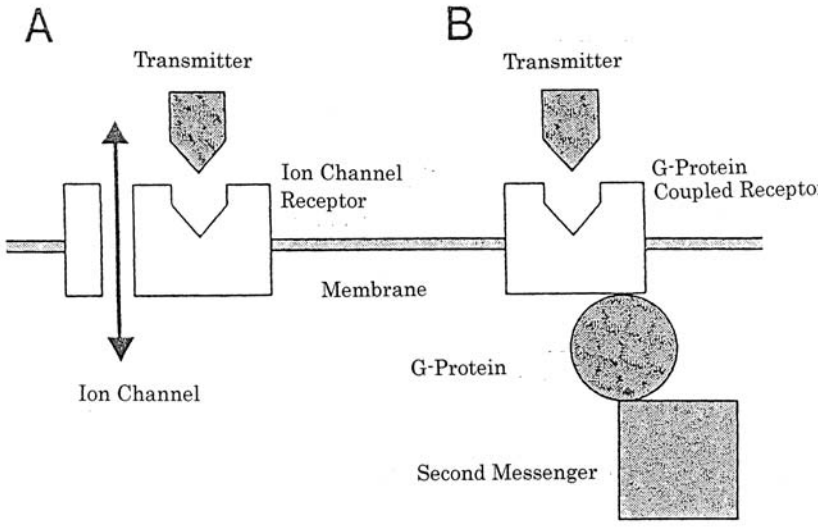

Fig. 1.3. Two types of synaptic receptors.
Transmitters and hormones are called first messenger. When the first messenger coupled with G-protein coupled receptor, second messengers such as cyclic AMP, GMP, Ca^{2+} or NO appear in the cytoplasma, triggering signal transduction pathways, eventually leading to changes of the cell.

two major functions: recognition of specific transmitter substances, and activation of effectors. The receptor first recognizes and binds a transmitter on the external surface of the cell; then as a consequence of binding, the receptor alters the cell's biochemical state.

Receptors for neurotransmitters can be divided into two major groups according to how the receptor and effector functions are coupled (Fig. 1.3). In one group — ionotropic receptors, i.e., those in which the receptors "gate" ion channels directly — the two functions are carried out by different domains of a single macromolecule (Fig. 1.3 A). In the second group — metabotropic receptors — recognition of the transmitter and activation of effectors are carried out by distinct and separate molecules (Fig. 1.3 B).

Activation of the effector component requires the participation of several distinct proteins. Typically the effector is an enzyme that produces a diffusible "second messenger", for example, cyclic adenosine monophosphate (cAMP) or inositol polyphosphate. The second messenger in turn triggers a biochemical cascade - either activating specific protein kinases that phosphorylate a variety of the cell's proteins or mobilizing Ca^{2+} ions from intracellular stores - thus initiating the reactions that change the cell's biochemical state.

There are perhaps 100 substances that act as transmitters, each of which activates its own specific receptors on the cell surface. As to transmitters more description is found in Chapter. 2.

1.2.1.9 Electrical synapses

The electrical synapses are the gap (or bridged junctions) where the zone of apposition is bridged by channels that run from the cytoplasm of the presynaptic neuron to that of the postsynaptic neuron. These junctions mediate electrical transmission, typically bidirectionally.

1.2.2 Biomagnetism

Biomagnetism deals with magnetic phenomena of the living body, which can be observed at different intensities and frequencies. For example, the so-called magnetophosphene is a visual sensation elicited by exposing the head to a low frequency (around 10–70 Hz) magnetic field at around 10–20 mT. The signal is generated in the retina. Magnetic stimulation of human brain and heart has been used for the purpose of both research and clinical treatment. Using SQUID (superconductive quantum interference devices) techniques, the weak magnetic fields from the brain, heart and lung can be measured from outside the body. Relation of biological organisms with the geomagnetic fields is discussed in 1.3.2.4. Geomagnetic fields and biological system in this chapter.

1.3 Environmental Electromagnetic Fields and Biosystems

We are exposed daily to electromagnetic fields, both from natural and human-made sources. The naturally occurring electromagnetic fields originate from properties of the earth's molten liquid core, electric discharges in the atmosphere (terrestrial sources), and solar and lunar activities (extraterrestrial sources). Anthropogenic electromagnetic fields come mainly from 50 or 60 Hz power transmission and distribution lines and from the electrical appliances driven at the power-line frequency and thus are classified as extremely low frequency (ELF).

1.3.1 Natural background fields

The natural sources of electromagnetic fields are associated with lightning discharges, and the resultant signals are called "atmospherics" or "sferics". They vary with time and location, and they have waves in ELF and very low frequency (VLF) ranges (ending at 300 kHz). The quasi-static (i.e., relatively constant or invariant) field consists of a negatively charged earth and a positively charged atmosphere. The ground-level electric field is about 0.1kV/m, but electric fields above 100 kV/m have been observed during thunderstorms. Atmospherics exhibit considerable amplitude

over a wide frequency band. Ground lightning with duration of few microseconds can generate global-scale, alternating-current electric fields.

Lightning discharges are considered as a source of electric currents, and it is believed that lightning discharges can generate to several kA to several hundred kA. The path of a lightning stroke (Fig. 1.4), which can be of various lengths, acts as a huge antenna. Electromagnetic waves, of frequencies determined by the length of the lightning stroke path, are emitted. The length of the discharge path can exceed several kilometers, and the frequencies range from several Hz to the GHz band. These are naturally emitted electromagnetic fields. The frequency spectrum of an electric field can be measured at a considerable distance from a cloud-to-ground lightning strike (Fig. 1.5).

Fig. 1.4. Photographic example of a typical lightning stroke (Courtesy of T. Shindo, CRIEPI). The lightning stroke in the upper was captured during summer season at Akagi Test Center, CRIEPI. The lower photo shows the upward lightning stroke occurred in the winter season, Hokuriku, Japan.

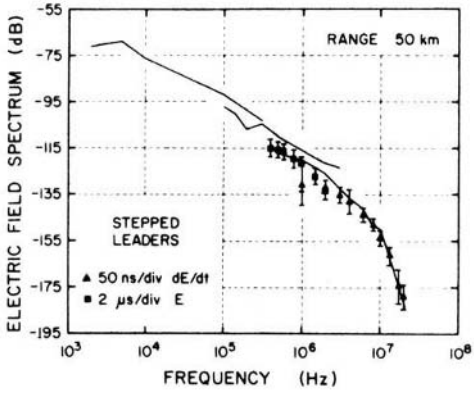

Fig. 1.5. Frequency spectrum of the electric field of cloud-to-ground lightning obtained from 9 leader steps between 20 and 50 km normalized to 50 km (Uman 1984).

The wave forms observed vary with the distance – which can be from 50 to 15,000 km – from the lightning strike (Fig. 1.6). When the distance is short, the waveform is a single pulse. However, at distances greater than 1,000 km, the waveform approaches an oscillating form with a definite periodicity. The changing of the waveform originates from the electromagnetic wave emitted by lightning, which propagates by reflecting between the ionosphere and the earth's surface, which acts as a perfect conductor. This phenomenon exhibits resonance at specific frequencies. The space between the earth and ionosphere serves as a large waveguide for atmospherics. Of the signals propagating from lightning discharges, low-frequency components have low attenuation and can circle the earth several times. Standing waves develop from the excitation of the spherical, surface-cavity resonator between the earth's surface and the lower boundary of the ionosphere. The fundamental frequency of this resonance is near 7.5 Hz, which is determined by dividing the propagation speed of the electromagnetic wave (3×10^5 km/sec) by the diameter of the earth (4×10^4 km).

More rigorously, as the electrical conductivity of ionosphere's boundary layer are finite and the special structure and form of the ionosphere, the fundamental resonant frequency is about 7.8 Hz. The harmonic resonant frequency can be obtained from following equation.

$$f = 7.8\frac{\sqrt{n(n+1)}}{2}Hz, \quad n = 1, 2, 3$$

Power spectrum of measured electric fields of natural signals has been described (Polk 1974). There is a fundamental resonant frequency at about 8 Hz (Fig. 1.7), and harmonic resonant frequencies are observed around 14, 20 and 26 Hz.

In 1952, Schumann theorized that the space between the earth and ionosphere forms a cavity, predicting that its resonant frequency was about 10 Hz (Schumann

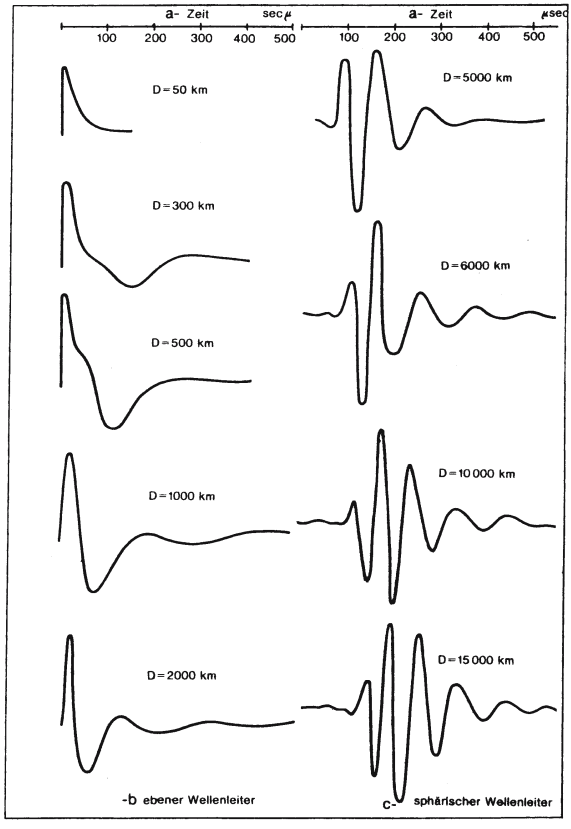

Fig. 1.6. Waveforms of atmospheric signals at various distances, between 50 and 15,000 km, from a lightning contact with the earth (König et al. 1981).

1952). This resonance phenomenon is called Schumann resonance. In 1954, König first reported the measurement of the resonance phenomena (König et al. 1981). In Figure 1.8, type I shows the Schumann resonance waveform. Type II is the wave form of naturally occurred electric fields at 3–6 Hz, and type III shows local variation at 0.7 Hz. In nature, ELF electromagnetic fields are mainly divided into these three variations.

When ELF electromagnetic fields are produced by lightning discharge, the electric field causes electric current flow in the atmosphere. Magnetic fields of the same frequency as the result of current flow are produced. The higher the frequency of the electromagnetic waves, the greater the attenuation and the lesser the propagation range. As described by Oehrl and König (1968), the electric field and the magnetic field intensity and frequency range 0–50 kHz measured at one point are inversely proportional in log-log plot (Fig. 1.9). This shows a relationship between electric and magnetic field and frequency. Natural electric and magnetic field strengths de-

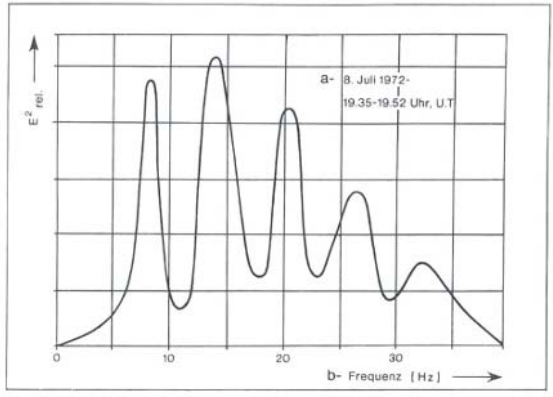

Fig. 1.7. Power spectrum of the naturally-generated electric field in the ELF ranges (Schumann resonance). (König et al, 1981).

creases rapidly with increasing frequency. The strength of the ultra low frequency electric and magnetic fields over a broad frequency range are about 10^{-3}~10^{-5} V/m and 10^{-12}~10^{-14} T, respectively.

1.3.2 ELF electromagnetic fields and biological systems

1.3.2.1 Circadian rhythms

Various periodicities in biological processes are coupled, to a certain extent, to geophysical cycles. Animals have 23~25 hour periods, and plants have 23~28 hour periods. However, the period lengths and phases of these internally generated (endogenous) daily rhythms of biological organisms are readily controlled by external environmental factors, such as the 24 hour day-night cycle produced by the earth's rotation. Biological organisms can adjust their rhythms (*Entrainment*) based on external environmental conditions, which are called synchronizing factors (a *Zeitgeber*): visible light is a very important *Zeitgeber*.

Among the natural conditions on the earth that could serve as a *Zeitgeber*, lightning and other electromagnetic phenomena are possible synchronizing factors. Human body temperature and activity have 24-hour periods and are synchronized by external environmental factors. If external environmental stimuli are removed, the free running cycle rhythm becomes 25.3 hours. During the 1960s, research using underground rooms or caves to isolate subjects from external information to investigate human circadian rhythms was popular (Fig. 1.10) (Wever 1974).

To investigate the effect of natural electric fields on the circadian rhythm of human activity, Wever (1968) conducted an experiment focusing on the effects of 10 Hz electric fields. To provide isolation from external sound, light, and other cues, two underground rooms were installed. One room was shielded from electromagnetic fields, while the other was not. The free-running circadian rhythms of activity and

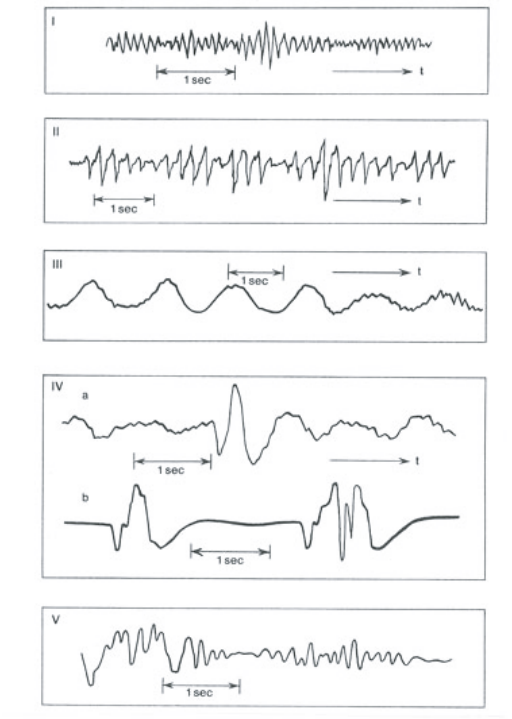

Fig. 1.8. An electromagnetic process of natural origins in the ELF ranges. (I) Schumann resonance, about 8 Hz, (II) Local variation of the electric fields, 3–6 Hz, and (III) Local variation of the electric fields, about 0.7 Hz, (IV) Electric field during thunderstorm, (V) Sunrise effect, electric field (König et al. 1981).

sleep, body temperature, urination and other rhythms of subjects living in the two rooms were studied. The results of the measurements showed that for subjects living in the shielded room, the circadian rhythm of activity was "split" into two rhythms, with periods of 25.3 and 33.4 hours; this is called internal desynchronization. The lengthening of the period for subjects in the shielded room was statistically significant (Fig. 1.11). During first 14 days, measured variables run synchronously to each other. After that time, the two rhythms ran separately, internal desynchronization occurred spontaneously. In about 20% of the experiments, internal desynchronization occurred. This phenomenon was not observed for subjects living in the unshielded room.

Next, a low frequency electric field of 10 Hz, 2.5 V_{p^-p}/m (square wave), which was equivalent to that in the natural environment (Schumann Resonance signal) was applied to the shielded room, and the same experiment was repeated (Wever 1968). The period of the free-running circadian rhythm became shorter and returned to its original length, when the electric field was terminated. As an example, Figure 1.12

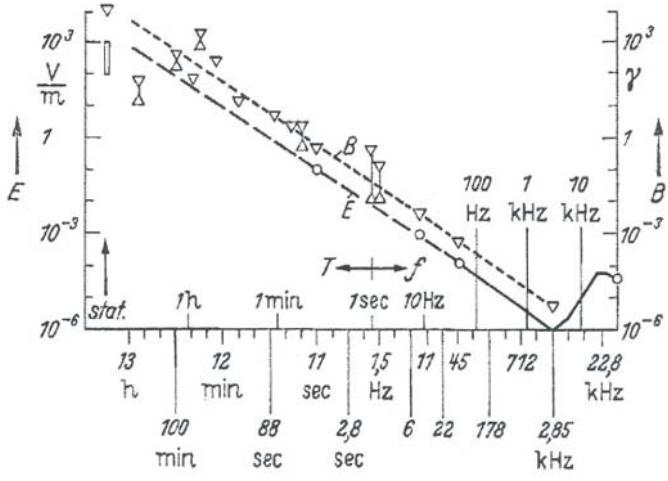

Fig. 1.9. Intensity of natural electric and magnetic fields in the frequency range 0–50 kHz. Circle: electric field (E) in V/m, triangles: magnetic field (B) in 10^{-9} T (Oehrl and König. 1968).

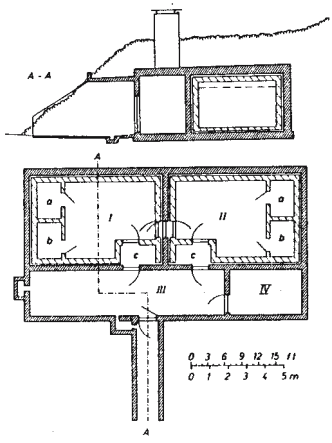

Fig. 1.10. Cross-section and floor plan of the isolated experimental room (Wever, 1974). I and II: experimental units (a: kitchen; b: bath; c: lock); III: control room, IV: experimental chamber.

demonstrated the change of free-running circadian rhythm. The 10 Hz field was off during first and third period in Figure. 1.12. When the field was turned on during second period, the period of free-running rhythm shortened. During third period with turn off, the period of rhythm lengthened and internal desynchronization occurred. Total 10 experiments shows that the period was shorter with the 10 Hz field than

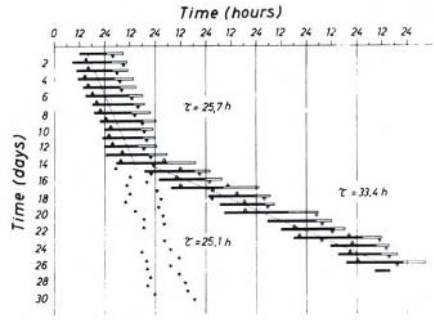

Fig. 1.11. Examples of deviation by internal cycles for sleep wake cycles and body temperature cycles. ■ sleep awake period, □ sleep period; ▲△ body temperature cycle. (Wever, 1974)

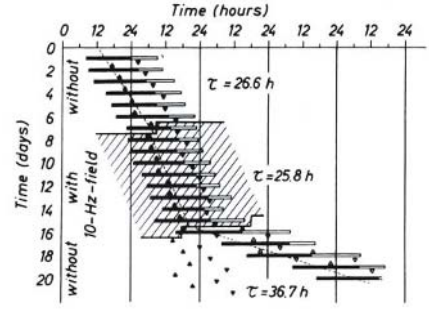

Fig. 1.12. Effect of presence or absence of 10 Hz electric field on changes in free-running circadian rhythms of experimental subjects (Wever 1968). The hatched areas are the period of field exposure. Activity rhythm is shown by bars (black filled: active period, white filled: resting period), body temperature rhythm, (▲) represent max and (▼) minimum. △ represents the temporal repetition of the maximum and minimum. Period (τ) represents the various phases of the experiment.

without it, with highly significance level ($p < 0.001$). The internal desynchronization was not observed when the electric field was applied. The application of the 10 Hz electric field reduced the length of the period by 1.3 hours. This showed that a 10 Hz electric field can affect circadian rhythms, including shortening the period and minimizing internal desynchronization.

Issues raised with regard to these results include (1) the lack of a clear cause and effect of the periodicity relationship between electromagnetic fields and organisms, (2) uncertainty regarding the mechanisms, (3) absence of measurements of electric field, and (4) inadequate explanation of the data analysis. However, Wever (1974) concluded that electromagnetic fields in the ELF range influence human circadian rhythms. Replicable data are not shown.

Since this pioneering report, effects of DC electric fields on circadian rhythm of mice (Dowse and Palmer 1969), effects of 10 Hz electric fields on activity of

green finches (*Carduelis chloris*) and other experimental results have been reported (Lintzen et al. 1989). Recent results reported for effects of exposure of fruit flies to 10 Hz, 1 and 10 kV/m electric fields on circadian locomotor activity (Engelmann et al. 1996). This experiment used 10Hz square-wave fields with 10,000 times stronger that natural generating field in the 8–14 Hz range. This provided support for the concept that electric fields can affect circadian rhythms and act as a weak *Zeitgeber*.

1.3.2.2 Similarity between EEG rhythms and Schumann resonance

When healthy adults relax with their eyes closed, brain waves of 8–12 Hz frequency and about 5–100 μV can be measured (α waves). α waves are the main component of brain waves of humans with β waves (13–30 Hz, 5–30 μV) being another component. δ wave activity declines during sleepiness, and 4–7 Hz low voltage slow waves (θ waves) appear. Under similar conditions, brain waves in the same frequency ranges are spontaneously observed for all vertebrates.

It has been noted that the form of brain waves are similar to the Schumann resonance waves. If one compares α and δ waves with the record obtained the electric field in ELF range, there are very similarity between α wave and type I signal, and between δ waves and type II signal (König et al. 1981).

1.3.2.3 Influences of natural electromagnetic processes on humans

As shown in Figure 1.8, there are three types of naturally occurring ELF electric fields, (1) Schumann resonance variation (type I) at 8 Hz, (2) local variation at about 3–6 Hz (type II) and (3) other local variation at about 0.5–2 Hz (type III). As these frequencies are in the same region as those of human brain waves, the possibility of correlation between these ELF electromagnetic phenomena and human activity has been considered. König et al. conducted an interesting experiment during an automobile traffic exhibition in Munich in 1953 (König et al. 1981). Using visitors as subjects, the correlation between naturally occurred ELF electric field and reaction time to light signal was investigated. The results showed that when type I signals were present, reaction time became shorter. When type II signals were present, reaction times became longer. These results were confirmed in experiments (König 1986) using artificially produced 8–10 Hz and 3 Hz (1 V/m). Although there are issues related to statistical analysis of the results, this was the first experiment to shown some type of relationship between atmospheric signals and human response.

1.3.2.4 Geomagnetic fields and biological systems

The earth is a huge magnet: its magnetic field is called the geomagnetic field. The intensity of geomagnetic field ranges from about 70 μT at the north and south poles to about 30 μT on the equator. The intensity in Japan is around 50 μT. The geomagnetic field is described by three components, (1) total magnetic intensity, (2) declination, and (3) inclination. There are solar and lunar diurnal variations of the geomagnetic

field. The diurnal variations may be pronounced during the day. Irregular pulsations and magnetic storms can be recorded in addition to these periodic variations.

It has been hypothesized that during the course of evolution, which has occurred within the earth's geomagnetic fields, organisms have used the geomagnetic field as a cue for directional orientation and migration. Magnetite (Fe_2O_3) that could be used as a sensor for the earth's geomagnetic field has been found in homing pigeons, migratory birds, honey bees and magnetotactic bacteria.

Kalmijin (1974) showed that fish use the geomagnetic field to maintain their orientation while swimming. Sharks and rays have the ampullae of Lorenzini, which are located near the front of their brains, that detect the extremely weak electric field induced by the geomagnetic field, i.e., earth currents (Matthes et al. 2000). There are various mechanisms for detecting electromagnetic fields (Fig. 1.13). In (a) the situation when a shark approaches the vicinity of a dipole field (0.2–0.5 μV/m) used to simulate prey is shown. As the shark swims through the geomagnetic field, in accordance with Faraday's Induction Law, a vertical electromotive force is induced. This induced electric field allows selection of direction relative to the direction of the geomagnetic field to be obtained. It has been conjectured that this is used to judge the direction that the fish is swimming. In (b) of Figure 1.13, the vector product of the flow of velocity (v) of the ocean stream (i.e., water current) and the geomagnetic field's vertical component (B_v) is equivalent to the electrical gradient created: current flow (ion flow) occurs, and detection of this current flow allows perception of the direction (up vs. down stream) of the flowing water; this provides a means of passive electro-orientation. In a slow ocean current, surface electric fields are 0.05–0.5 μV/cm. In a tidal current level, using a cross section of the Gulf Stream an example, total electric fields up to 0.5 μV/cm were predicted (Rommel and McCleave 1973).

In (c) of Figure 1.13, the shark is moving through the geomagnetic field: the electric field resulting from motion of the shark through the geomagnetic field gives it a magnetic compass heading; this is active electro-orientation. For a fish swimming at velocity (v) through the horizontal geomagnetic field component (B_h), an electric gradient is induced by the vector product. For example, a fish swimming at the speed of 1 m/sec through the geomagnetic field horizontal component of 25 μT will induce an electric field of 0.25 μV/cm. This electrical gradient passes through the ampullae of Lorenzini. Because sharks and rays can detect electric fields of 0.01 μV/cm, they can readily detect this field. Thus, the aquatic animal might perceive an electric voltage induced by water current or by its own motion in the geomagnetic fields.

Blakemore (1975) first found that bacteria change their swimming direction in muddy water by responding to magnetic fields (Fig 1.13(d)). Later, in marine and freshwater sediments from the southern hemisphere, bacteria that oriented towards the geomagnetic south were found. Where the magnetic-sensing capability of these bacteria was located was clarified, and the presence of magnetite was confirmed. The magnetic force lines of the earth's magnetic field are horizontal near the equator. However, as the north and south magnetic poles are approached, the vertical components become larger, and magnetic dip, the downward slant towards the earth's surface, increases. If the magnetic force lines are followed, northward in the northern hemisphere and southward in the southern hemisphere, it is possible to move in a

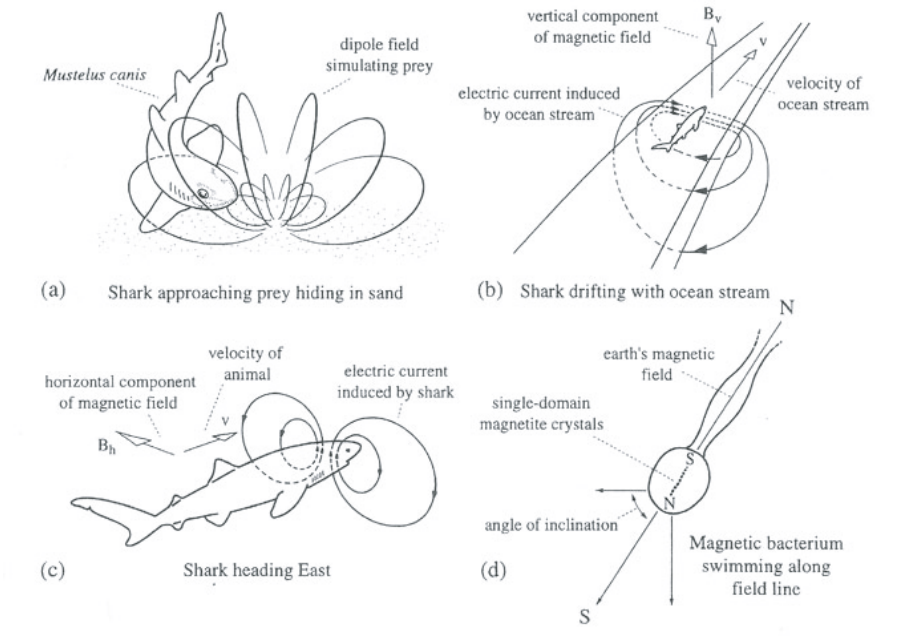

Fig. 1.13. Behavioral responses of organisms to electromagnetic fields in the aquatic environment (Matthes et al. 2000). (a) Behavior of a shark near a dipole imitating prey buried in the sand. (b) Sharks in a situation like a tidal current with the geomagnetic field. Electric field induction in fish oriented upstream and downstream in the flow is shown. (c) Sharks swimming in earth's geomagnetic field. (d) As magnetic bacteria are anaerobic, movement is toward the bottom, where conditions are most anaerobic, as directed by earth's geomagnetic field.

controlled direction within the low-oxygen environment of sediments. These bacteria are anaerobic.

When honey bees communicate the direction and distance to food to their companions, when food source is very close (within about 50 meters), they make a simple circular movement of the whole body while they also make a figure-8 movement with their posteriors. The direction of the wagging dance of honey bee shows the angle between the location of the food and the sun, and the angle is transposed with respect to gravity. The speed of turning is said to represent the distance to the food.

Lindauer and Martin, (1968) showed that this honeybee dance was affected by geomagnetic field. When the geomagnetic field is compensated to ± 4%, misdirection disappears. Later, it was found that there was magnetite in the abdomen of honey bees that was strongly magnetized laterally. But this visible iron-containing granule cells are in the form of magnetite. They are formed in hydrous iron oxides. The average magnetic moment is 1.7×10^{-5} emu (1.7×10^{-2} A·cm^2). It is thought that the magnetite is formed during the growth from larva to adult, with the magnetism being oriented with the geomagnetic field. Honeybees use this magnetite as a sensor

for geomagnetic field, and they are thought to use this to determine direction (angle) even when it is cloudy and the sun cannot be seen. However, this angle is affected by the magnetic field of 10^{-7} T (0.1μT)to 10^{-9} T (0.001μT).

The effects of geomagnetic field on bird migration have been investigated (Wiltschko and Wiltschko 1995). Tiny transmitters were attached to homing pigeons which were released 100 to 150 km from their home coops and tracked on their return. When first released, pigeons fly in circles, seemingly confused about which direction to fly, but they eventually accurately select the homeward direction. However, in areas with abnormal geomagnetic field, the selection is made erroneously.

It has been reported that the number of pigeons that do not return increases on cloudy days. On sunny days, the pigeons can use the sun compass, but it has been speculated that on a cloudy day pigeons fly while detecting geomagnetism. Thus, if a tiny magnet is attached to the head of a pigeon, on sunny days it is able to accurately return to its coop. The amount of magnetic material in the heads of pigeons is about 10^{-5} to 10^{-6} emu. It is thought that the iron-containing proteins are synthesized in their bodies.

A number of experiments have reported that geomagnetic field was being used for migration and orientation (Wiltschko and Wiltschko 1995). There have been (1) reports that the activity of gerbils is associated with changes in geomagnetic field, (2) experiments showing that mice have the ability to detect geomagnetic field, and (3) that the pineal gland of marmots and pigeons and some of the cells of pineal glands of rats respond to geomagnetic fields. Also, the sensitivity to geomagnetic field differs among rat species, and *Planaria* and *Paramecia* are thought to be able to distinguish between magnetic fields parallel and perpendicular to the long axis of their bodies.

The geomagnetic field has some effects on the growth of roots and leaves of plants (Phirke et al.1996). It has been reported that germination and growth is faster when seeds and rootlets of plants – including wheat, cucumbers, sunflowers and peas – are oriented in the geomagnetic north-south direction. It has also been reported that wheat seeds sprout faster when seeds oriented parallel to geomagnetic field south and north. In general, although many reports on the effect of magnetic fields on the growth enhancement of plants have been published, the consistent results were lacking. It is necessary in further research to specify the special environmental conditions for understanding the mechanism of magnetic field response in plants.

1.3.3 Anthropogenic electromagnetic fields

As stated in the previous section, the natural electromagnetic fields originate from the properties of the earth and the process of atmospherics. Sources can be divided into those that are natural in origin and those that artificial, i.e., anthropogenic. As briefly reviewed above, the possibility that effects in biological systems are influenced by the geophysical ELF electric and magnetic fields has been shown. However, in addition to the naturally produced ELF electric and magnetic fields in our environment, the extensive use of electrical equipment operating at a power frequency of 50 or 60Hz also produces ELF electric and magnetic fields. These anthropogenic electric and

Table 1.1. Representative Power Frequency Electric Field Values from Common Household Electrical Appliances (Miller 1974)

Electric fields (60Hz) measured 30cm from various electrical appliances, V/m			
Iacandescent light bulb	2	Toaster	40
Electric range	4	Phonograph	40
Clock	15	Hand Mixer	50
Vacuum Cleaner	16	Iron	60
Cofee pot	30	Refrigerator	60
Color TV	30	Stereo	90
Hair Dryer	40	Broiler	130
Vaporizer	40	Electric Blanket	250

magnetic fields are both ubiquitous and stronger, by many magnitudes, than those of natural origin.

1.3.3.1 Power-frequency electric fields in the environment

Artificial sources of ELF electric and magnetic fields are divided mainly into two types, DC and AC. Although DC power supply systems are not common, there are a few systems, such as high voltage DC transmission lines and DC-operated transportation systems. These systems produce small DC electric and magnetic field around them. Maglev systems use a high magnetic field, which produces a stray magnetic field inside the train. As a medical diagnostic tool, MRI uses strong DC magnetic fields. The magnets have field strength of 1–3 T, with the increasing use of higher magnetic fields to improve imaging capability a consistent tend.

Relatively strong electromagnetic field sources in the environments in which people live and work include electric equipment in homes, workplace, public facilities, etc, and transmission and distribution lines. Electric field sources in the home environment include indoor wiring and household electrical appliances. Electric fields from outdoor distribution lines and transmission lines are shielded by buildings. Because the voltage used by common appliances typically is c. 100 or c. 200 V, the electric field cannot become very large (Table 1.1). These values were measured extremely close to the sources, and the field strength decreases rapidly with distance from the source. At a few meters from the sources, the levels become equivalent to the background electric field.

For transmission line electric fields, which typically are measured near ground level, are the standard, the strength varies greatly with transmission voltage, conductor configuration, distance from the transmission, and other factors (Fig. 1.14). This gives a typical profile of electric field distribution at 1 m above ground beneath 500 kV vertical double-circuit transmission line in Japan. Japanese electrical equipment standards were established to prevent electrostatic induction perception, meaning electric field levels must be less than 3 kV/m underneath transmission lines (except for locations where humans are not usually present). Transmission lines with high

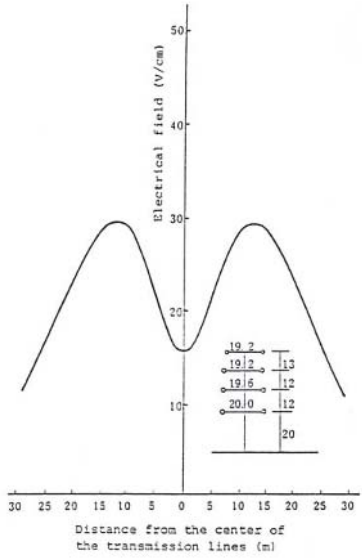

Fig. 1.14. Electric field distributions at 1 m above the ground beneath a 500 kV vertical double-circuit transmission line in Japan. Conductor height is 20 m above the ground and its arrangement is shown. Conductor used is 410 $mm^2 \times 4$ and the load condition is low reactance phasing.

operating voltage are designed with the wires high above the ground to meet the 3 kV/m near the ground standard. Also, because of the shielding effect of buildings, plants, trees, etc., the actual ground-level electric field strengths are even smaller. For substations, the actual electric field strength is reduced by the shielding effect of the equipment.

1.3.3.2 Power-frequency magnetic fields in the environment

The main sources of power frequency magnetic fields include household electrical appliances, industrial machine tools, and transmission lines. Transmission line magnetic fields, as with electric fields, generally are described by the ground-level field strength, which varies greatly depending on the current flowing through the conductors, configuration of the transmission line, and distance from the line. Also, magnetic field strength varies daily with the changes in electric current flow associated with the varying demand for electric power. Magnetic fields differ from electric fields in that (1) shielding by objects (other than large metallic devices) can almost be ignored, (2) changes with ground conditions do not occur, and (3) they have both horizontal and vertical components. Table 1.2 shows examples of U.S. measurement results (NRC 1997). The change in magnetic field strength with distance from the transmission line is shown. A transmission line's contribution to the ambient magnetic field disappears at distances greater than 100 meters from the transmission

Table 1.2. Magnetic Fields from Transmission Line (NRC 1997)

Transmission Lines, kV	Maximum Magnetic Field on Right-of-Way, μT(mG)	Representative Magnetic Fields at Different Distances from Lines, μT(mG)			
		15.24m (50ft)	30.48m (100ft)	60.96m (200ft)	91.4m (300ft)
115	3.0(30)	0.7(7)	0.2(2)	0.04(0.4)	0.02(0.2)
230	5.8(58)	2.0(20)	0.7(7)	0.18(1.8)	0.08(0.8)
500	8.7(87)	2.9(29)	1.3(13)	0.32(3.2)	0.14(1.4)

Source EPA 1992
mG values = $10 \times \mu$T values

line, meaning the environmental level becomes the same as the background magnetic field.

The level of magnetic field from various electric equipments is shown in Table 1.3. These values rapidly decline with distance from the source, meaning that they become the same as the background magnetic field within a few meters.

An example of magnetic fields profile under typical double circuit transmission line is shown in Figure. 1.15 (Yasui 1994). Figure. 1.15 shows the cross section of a 500 kV transmission line in Japan. This shows the magnetic field magnitude at 1 m above ground. Also shown are a number of magnetic field vector ellipses at each point 1 m above ground. Magnetic fields 1 m above the ground level was calculated by Bio-Savart's law and measured by Gaussmeter. The magnetic field of three phase-transmission line is a rotating field in a vertical plane at and near ground level. The degree of the ellipticity depends on the distance from each conductor as shown in Figure. 1.15. The values of voltage and current, number of conductors, are indicated. Gauger (1985), NRC (1997), NIEHS (1998), IARC (2002) and ICNIRP (2003) provide more information about the sources and levels of ELF electric and magnetic fields.

1.4 Summary

Research in bioelectromagnetics began nearly 200 years ago. In the 20th century, the "drivers" have been basic research in neurophysiology, basic and applied research with microwaves, medical applications directed primarily at enhancement of bone healing, and concern about possible adverse health effects from environmental exposures.

This chapter provides an initial background in three general areas: (1) basic neurophysiology, (2) the natural and artificial electromagnetic fields found in the environment, and (3) the biological effects of these fields. Because the function of the body, and much of the electric and magnetic field exposure from natural and artificial sources falls have frequencies of < 300 Hz, the emphasis is on ELF fields.

Portions of the chapter briefly describe the biological activity of organisms when they are exposed to naturally originated electromagnetic fields in the ELF ranges. Biological systems, including human beings, are exposed continually to naturally

Table 1.3. Representative Power Frequency Magnetic Fields from Common Household Appliances (NRC 1997)

Location and Appliance Type	Magnetic Field (μT) at 6 in (0.15 m)	Magnetic Field (μT) at 1 ft (0.3 m)
Bath room		
Hair dryers	0.1–70.0	Bkg[a] to 7
Electric shavers	0.4–60.0	Bkg to 10
Kitchen sources		
Blenders	3–10	0.5–2
Can openers	50–150	4–30
Coffee makers	0.4–1	Bkg to 0.1
Dishwashers	1–10	0.6–3
Food processors	2–13	0.5–2
Garbage disposals	6–10	0.8–2
Microwave ovens	10–30	0.1–20
Mixers	3.0–60	0.5–10
Electric ovens	0.4–2	0.1–0.5
Electric ranges	2.0–20	Bkg to 3
Refrigerators	Bkg to 4	Bkg to 2
Toasters	0.5–2	Bkg to 0.7
Laundry and utility-room sources		
Electric clothes dryers	0.2–1	Bkg to 0.3
Washing machines	0.4–10	0.1–3
Irons	0.6–2	0.1–0.3
Portable heaters	0.5–15	0.1–4
Vacuum cleaners	10–70	2.20
Office sources		
Air cleaners	11–25	2–5
Copy machines	0.4–20	0.2–4
Fax machines	0.4–0.9	Bkg to 0.2
Fluorescent lights	2–10	Bkg to 3
Electric pencil sharpeners	2–30	0.8–9
Video-display terminals	0.7–2	0.2–0.6
Workshop sources		
Battery chargers	0.3–5	0.2–0.4
Drills	10–20	2–4
Power saws	5–100	0.9–30

[a] The magnetic field of the device producing the lowest level could not be distinguished from background (Bkg) levels.
SOURCE: EPA 1992

originated electromagnetic fields. A modest number of experiments with animals and people indicate that these naturally occur fields can affect humans. In addition, ELF electric and magnetic fields from the generation and distribution of electric power exist commonly in nearly all human environments. Furthermore, the increased use of electricity accompanying new technologies, such as the superconductive magnet,

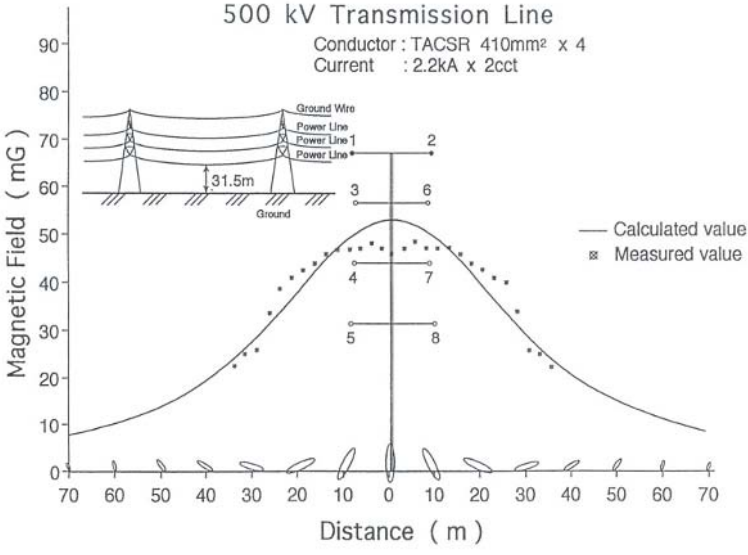

Fig. 1.15. An example of magnetic fields profile under double circuit transmission line. Magnetic fields 1 m above the ground level was calculated by Bio-Savart's law and measured by Gaussmeter. The magnetic field is a rotating field in a vertical plane at and near ground level. The degree of the ellipticity depends on the distance from the center of transmission line as shown. The values of voltage and current, number of conductors, are indicated. (Courtesy of M.Yasui, Tokyo Electric Power Company.)

energy storage systems (SMES), magnetic levitation (MAGLEV) systems, etc. have created many new sources of electromagnetic fields.

Thus, the possibility that biological effects might occur as a result of exposure to ELF electric and magnetic fields has increasingly become a subject of concern. In particular, the question of whether the electromagnetic fields in humankind's environment, both from natural and artificial sources, might cause previously unrecognized adverse health and biological effects continues to be raised. It should be noted that electrical power has been used widely for more than a century, with recognition of any significant adverse public health effects, other than well recognized and carefully controlled danger of electrocution.

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2

Endpoints and Methodologies

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Several research topics and endpoints are studied in both ELF and RF bioeffects research. This chapter describes the fundamental attributes and relationships of electric field, magnetic field, and electromagnetic wave. Then, the following sections provide a brief background explanations of nomenclature and methods used during performance of *in vivo* or *in vitro* experiments so as to aid understanding of the reviews of the experimental results given in Parts II (ELF) and III (RF). *In vivo*, i.e., in life, research refers to experiments conducted with whole organisms, and *in vitro*, i.e., in glass, research refers to experiments conducted with cells, tissues or isolated organs in tissue culture.

2.1 Definition and Equations of Electric and Magnetic Fields

2.1.1 Electric field

An electric field is a region of space over which an electric charge exerts a force on charged objects in its vicinity. The units of the electric field are Newton/Coulomb (N/C) or Volt/Meter (V/m), both of which are equivalent. In a conductive material current density, which is defined as an electric current flowing at a particular point through a unit cross-section area of the material taken perpendicular to the current flow at that point, is often used in place of the electric field. The unit of the current density is Ampere/Squared-Meter (A/m^2). Let E and J be the electric field and the current density, respectively, in a conductive material such as a biological body we have

$$J = \sigma E \tag{2.1}$$

where σ is the conductivity in unit of Siemens/Meter (S/m) of material.

2.1.2 Magnetic field

A magnetic field is a region of space over which an electric force acts on moving charges. The units of the magnetic field are Newton/Weber (N/Wb) or Am-

peter/Meter (A/m), both of which are equivalent. Magnetic flux density, being defined as the amount of flux passing through a unit cross-section area, is often used in place of the magnetic field. The unit of the magnetic flux density is Wb/m or Tesla (T) which is equal to 10,000 Gauss (G). Let H and B be the magnetic field and the magnetic flux density, we have

$$B = \mu H \tag{2.2}$$

where μ is the permeability in unit of Henry/Meter (H/m). In biological materials μ is equal to the free-space's value, i.e., $\mu_0 = 4\pi \times 10^{-7}$ H/m.

2.1.3 Electromagnetic wave

Electromagnetic wave is a wave motion consisting of oscillating electric and magnetic fields. It is characterized by the wavelength λ in meter (m), the frequency f in Hertz (H), the photon energy U in Joule (J), and the absolute temperature T in Kelvin (K). Among them the following relationships hold

$$\lambda = \frac{c}{f}, \quad U = hf, \quad T = \frac{U}{k} = \frac{hf}{k} \tag{2.3}$$

where c ($= 3 \times 10^8$ m/s) is the speed of light, h ($= 6.626 \times 10^{-34}$ Js) is the Planck constant, and k ($= 1.381 \times 10^{-23}$ J/K) is the Boltzmann constant. With the increase of the frequency or the decrease of the wavelength, the photon energy and the temperature increase.

The spectrum is the distribution produced when the electromagnetic wave is decomposed into various frequency components. Fig. 2.1 shows the spectrum of electromagnetic wave. An electromagnetic wave is usually divided into ionizing and non-ionizing radiation. The former is the radiation when its frequency is higher than 3000 THz (1 THz = 10^{12} Hz), and the latter is the radiation when its frequency is lower than 3000 THz. This frequency corresponds to a wave with a wavelength of 100 nm (1 nm = 10^{-9} m), a photon energy of 12.4 eV (1 eV = 1.60×10^{-19} J), and a temperature of 143,666 °C.

The electromagnetic wave below 300 GHz (above 1 mm in wavelength) is usually called radio frequency (RF) wave. For RF wave the power density S in unit of watt/squared-meter (W/m^2) is often used in place of the electric or magnetic fields. The power density is defined in the far-field region where the ratio of the electric field E and the magnetic field H is equal to the free-space intrinsic impedance of ξ_0 ($\doteqdot 377\Omega$). In that region, we have

$$S = \frac{1}{2} E \cdot H = \frac{1}{2} \frac{E^2}{\xi_0} = \frac{1}{2} \xi_0 H^2. \tag{2.4}$$

2.2 Endpoints and Methodologies for *In vivo* Research

Because it reflects the nature of bioelectromagnetics, studies relating to the nervous system, are given the greatest emphasis. In addition to the nervous system, the body

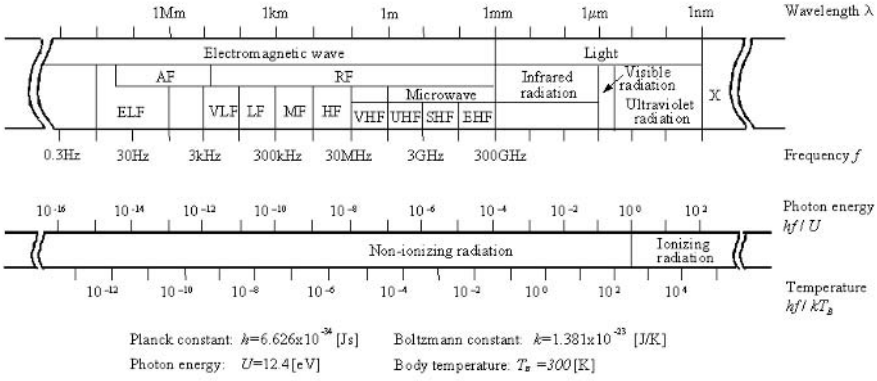


Fig. 2.1. The electromagnetic frequency spectrum. Energy can be described in terms of wavelength or frequency (top portion); it also can be described in terms of photon energy or temperature (bottom portion).

has two other great regulatory systems, the endocrine and the immune systems. Both interact with each other and with the nervous system; this is especially true for the endocrine system. Thus, these systems are described as well. Bioelectromagnetics is more extensive than these topics, but covering all bioelectromagnetics research is impossible, so the text in this chapter is focused on the most important parts.

2.2.1 Nervous system

The nervous system is the master regulatory system for the body. If it can cope successfully with an environmental agent, the organism will be successful. If an environmental agent degrades the performance of the nervous system, adverse effects are likely to result. Thus, research in bioelectromagnetics has placed great emphasis on the nervous system. The nervous system is the final common path for life or death.

2.2.1.1 Nervous system and behavior

Because the nervous system is so important, and because nervous system function is basically electrical, many studies of electromagnetic fields have focused on the nervous system. Because behavior is controlled by the nervous system, experiments using behavior as a dependent variable can provide information about nervous system function. Some scientists call this behavioral toxicology. The approach has been used frequently to study the effects of chemicals, drugs, and physical agents, and electromagnetic fields. Given the importance of behavioral studies in bioelectromagnetics, the following section provides a basic overview of behavioral research methods.

2.2.1.1.1 Outline of behavioral science

Living creatures can survive only by coexisting with many diverse environmental conditions. However, to do this, an individual must maintain relationships to interact

with other creatures and with the environment. Behavior can be defined as the mutual interaction of the individual and the surrounding environment. There are three main variables which affect behavior: (1) genetically determined variables, such as eating, locomotion, respiration, and the sexual drive; (2) variables developed from earlier environmental conditions, such as emotion, learning, memory, and others; and (3) variables relating to the present situation, like attention, perception, discrimination, decision-making, etc.

In a simplified view, the cerebrum consists of the neocortex and the limbic system. The cerebrum generates goal-directed behavior. In this process, the neocortex tends to regulate the precise spatiotemporal communication with the environment and the formal-intellectual and stereognostic activities. On the other hand, the limbic system, the phylogenetically old cortex surrounding the lateral ventricle, is more concerned with moods and incentives to action — that is, a motivational interactions and emotions — and the process of learning and memory. The limbic system endows the information derived from the internal and external worlds with its particular significance, and thus determines their characteristic purposeful behavior (Schmidt and Thews 1989). The limbic system consists of the hippocampus, parahipocampal gyrus, cingulate gyrus and old elements of the olfactory brain.

Learning is the process by which we acquire knowledge about the world, while memory is the process by which that knowledge is encoded, consolidated, stored, and later retrieved. Many important behaviors are learned. Molecular mechanisms of memory storage are highly conserved throughout evolution, and complex forms of learning and memory depend on many of the same molecular mechanisms used in the simplest forms. These molecular mechanisms contribute to our individuality by changing the connectivity of neurons in our brain (Kandel et al. 2000). Memory consolidation involves three processes (1) gene expression, (2) new protein synthesis, and (3) growth or pruning of synaptic connections.

Spatial working memory involves the hippocampus. The hippocampus contains a cognitive map of the spatial environment in which an animal moves (O'Keefe and Dostrovsky 1971). The location of an animal in a particular space is encoded in the firing pattern of individual pyramidal neurons, the very neurons that undergo long-term potentiation (LTP) when their afferent pathways are stimulated electrically. Long term potentiation is important for the formation and maintenance of place fields, and defects in long term potentiation interfere with spatial memory.

Maguire et al. (1996) describe a positron emission tomography (PET) study designed to investigate the regional cerebral blood flow changes associated with topographical memory formation in humans, i.e. the formation of representations of large-scale environments necessary for way-finding. Topographical learning of an urban environment, from viewing movies depicting navigation, was associated with activation of the right parahippocampal gyrus and hippocampus, with activation also of the left parahippocampal gyrus. In contrast, the encoding of non-navigation episodic memory in a similar real world context was not associated with activity in the hippocampus. These results shed light on the neural basis of the human representation of large-scale space, pinpointing a particular role for the human hippocampus in learning to find one's way.

Another aspect of memory was studied by Henke et al. (1999). Although the hippocampus is recognized as a crucial brain area subserving human long-term memory, its specific functions in memory are controversial. By measuring the regional cerebral blood flow by PET while healthy volunteers learned pairs of words with different learning strategies, the following functions were revealed; (1) the process of semantically associating items significantly activates the hippocampal formation, (2) associating originally unrelated items challenges the hippocampal formation more than does deep, single-item encoding and novelty detection, and (3) this effect is independent of the kind of stimulus material used.

One can infer functional changes in the brain by observing behavioral changes (output) following application of stimuli with chemical and/or physical agents (input). Behavioral changes include either inhibition or stimulation of normal behaviors, and/or induction of abnormal pathological behaviors. Stereotypy, for example, is an abnormal serial repetition of a behavior; it often occurs once a voluntary movement has occurred. Stereotypy (or stereotypical behavior) is evoked following administration of various pharmacological agents, and in many instances it appears dopamine is related to the development of stereotypy. Stereotypy which was evoked by administration of α-amphetamine or apomorphine is inhibited following destruction of bilateral neostriatum (Stolk and Rech 1970; Costall and Naylor 1973). From these and many other studies, it is believed that pathways from substantia nigra to striatum (i.e., the nigro-striatal, dopaminergic system in basal ganglia) play an important role in the development of stereotypy.

Once behavioral change is confirmed, further research is required to determine relevant neuronal system(s), including transmitter(s) and receptors. Altered behavior can occur as a result of environmental changes, alterations in motor or motor capability, disruption of motivation or memory, etc. Measurement of regional cerebral blood flow (rCBF) has recently been employed in bioelectromagnetic research on various topics, particularly on human subjects.

Several laboratories have used this approach, behavioral toxicology, to examine whether magnetic field exposure at ELF or electromagnetic field exposure at RF affects any brain function.

2.2.1.1.2 Activity and attention, learning and memory, and task performance

The most basic way to examine behavior is to measure the presence or absence, or the amount and pattern of, activity. Most often, general locomotor behavior is determined, usually with automatic systems. A slightly more sophisticated approach is to assess the ability of a subject to attend to stimuli in the environment and to respond appropriately. In some cases, the ability of a subject to perform a previous learned task, acquired prior to field exposure, is assessed during or after field exposure. This is an examination of effects on task performance. However, in the study of electromagnetic field (EMF), effects of exposure on learning of – and memory for – a new task have received the most attention.

In animal experiments, the memory system is considered to be composed of two components: (1) reference memory (or long-term memory), which measures

the transfer of information over trials, and (2) working memory, which is one form of short-term memory, which refers to coping with intra-trial information (Olton, 1977). The term 'working memory' is defined differently in the area of clinical neuropsychology (Baddeley 2000). Therefore, one must be cautious when evaluating experiments on the topic of working memory.

2.2.1.1.3 Behavioral methodologies employed in bioelectromagnetics

Innate behavior, or genetically determined behavior, is a naturally emitted response of a particular animal species. It is 'hard-wired', occurring automatically when the appropriate environment occurs, rather than acquired, through some form of learning, by interacting with the environment. Rodents, such as mice, rats and hamsters, and non-human primates often are used for studying the effects of EMF exposure. Many kinds of motor activity – including grooming, locomotion, nest building, rearing, scratching, and sniffing – are innate behaviors. Motor activities that occur individually or in combination comprise an animal's normal and spontaneous behavioral repertoire.

For many years, two methods have been used to assess such behavior, both in the laboratory and in the field. One is direct observation of individual and well defined components of behavior; typically a variety of basic behavioral units are scored over time by one or more observers. Typically the animals are in a naturalistic environment. The second approach, involving automated techniques, quantifies behavior with the help of mechanical or electronic devices designed to record certain behavioral components, which typically are artificial behaviors learned in the laboratory.

Learned behaviors involve the strengthening of particular behavioral responses by scheduled (delivered by an automatic rule) rewards, i.e., reinforcements. Two paradigms traditionally have been used to characterize learned behavior: respondent, and operant conditioning. Pavlov's dogs are a famous example of respondent conditioning. When a tone is repeatedly paired with food, the dog eventually learns, or becomes conditioned, to salivate to the sound of the tone alone. This type of learning is variously called respondent, classical, or Pavlovian conditioning. The response itself, e.g., salivation at sight of food, is unlearned; what is learned is the relationship of the unconditioned response and the conditioned stimulus. Before repeated pairing of the stimuli, dogs not salivate when they hear a buzzer.

Operant behavior operates on the environment to produce an effect, such as delivery of a food reward. When a reinforcer – something desirable to the animal, such as food or water, or access to nesting material or the opposite sex – is delivered close in time after an emitted behavior, the probability of a subsequent emission of the behavior will increase. If this occurs repeatedly, the probability that the behavior will reoccur increases considerably; the animal has learned that if does X, a reward will follow. This type of learning is called operant or instrumental conditioning. A large variety of operant behaviors have been conditioned, including lever pressing, chain and rope pulling, key pecking, and breaking a photocell beam with the nose. When emitted at the appropriate time, which often is signaled by the presence of a cuing stimulus, the operant response changes the animal's environment by producing a reinforcer such as food or water (positive reinforcement), or absence of electric shock

(negative reinforcement). A stimulus, often visual or auditory, used to signal that the reinforcement contingency is in effect, is called a discriminative stimulus.

The temporal or numerical rule by which rewards are delivered for the desired behavior sequence of stimuli is called a reinforcement schedule. For example, a fixed interval (FI) refers to delivering a reinforcer for the first response that occurs after fixed temporal interval; for example, on an FI10 sec schedule, the first barpress made more than 10 seconds after the previous response produces a reward. Responses that are "too soon" are not rewarded, meaning total number of responses does not matter. A fixed ratio (FR) schedule refers to delivery of reinforcement after a fixed number of responses is emitted. On an FR10, the reward occurs after 10 responses. The 10 responses can be made over a period of 2 seconds or 2 minutes; time does not matter.

A key point is that use of a given schedule produces a certain pattern of responding; these patterns are remarkably similar across species. If the pattern of responding to a given schedule is altered, it means that some aspect of brain function has been altered. Thus, operant behavioral approaches are used often to study the effects of chemical, drugs and physical agents.

FI schedules can produce high rates of responding if the interval is small (e.g., FR 1 sec) or slow rates of responding if the interval is long (e.g., FR 1 min). FR schedules produce rapid rates of responding. Variable interval (VI) and variable ratio (VR) schedules produce more steady patterns of responses that are distributed around the schedule parameter. On a VI 10 sec, for example, some response will occur 1 sec apart and some will occur 30 sec apart, but most will occur at 9–11 second intervals.

For investigating a working memory task, the maze paradigm is commonly used in animal studies, especially with rodents because running through tunnels is a part of these animals normal behavioral repertoire. Although the T-maze and water-maze devices differ, spatial learning in a Morris water-maze and reversal learning in a T-maze both require a common memory component. In a T-maze, the subject learns to run left (or right) to find food. This behavior is learned rapidly, e.g., in 10 trials, or less. The location of the food can be reversed to the right side, meaning that the subject must 'unlearn' the old behavior (this is relatively difficult) and learn a new behavior.

In the Morris water-maze, which is regarded as a spatial reference memory task, the subject swims in a tank of opaque water. Hidden just below the surface is a platform on which the subject can rest. Visual cues placed in the device allow the subject orient towards and locate the platform. Rats and mice also learn this task in a small number of trials. Once again, the platform can be moved, forcing the subject to extinguish the old response (swim towards back left corner) and learn a new response (e.g., swim to front right center area).

Liu and Bilkey (1998) reported that perirhinal cortex contributes to storing information regarding the cue-platform relationship during Morris water-maze performance and transfers this information to related structures. They carried out experiments to compare initial acquisition in the Morris water-maze between rats with bilaterally lesioned perirhinal cortex and sham-control rats. Creation of brain lesions typically involves placing an electrode into a specific area of the brain and applying an electrical current to destroy some brain tissue by heating. Because this procedure

involves surgery, the control group receives a "sham" operation in which the procedure is conducted, but the current is not applied. When the performance of sham- and perirhinal cortex-lesioned rats was tested with variable memory delays inserted between training and probe trials, lesioned rats displayed an increase in the rate of forgetting for information made available during the training trial.

Besides mazes, shuttle boxes also are used to study learned behavior. Here the learned response is locomotor, rather than manipulative. The paradigms are somewhat different in their details, but the general idea is to present a warning stimulus, e.g., a light or a buzzer, a few seconds before an electrical shock is applied to the floor grid. The subject soon learns to rapidly jump to the other side of the shuttle box to escape (shock has started) or avoid (jumps before shock starts) the shock. Measurement of response include response time, correct responses, and errors made.

The radial arm-maze is widely used for studying the effects of electromagnetic field on learning and memory. This maze consists of a central start box and a set of 8–12 alleys (i.e., arms) extending outward from the center. A cup is located at the end of each arm, and food is placed in some of these food cups. The test animal, usually a rat, is allowed to explore the maze to acquire the food. They soon learn to rapidly run to for example, arms 3, 6, 7, and 10, to get the food and to avoid the other arms where food is not present. This mimics the natural foraging behavior of a rat. An error is scored whenever the rat enters a wrong arm, or a given arm more than once, within a prescribed time period.

2.2.1.2 Electroencephalogram

The electroencephalogram (EEG) is a recording of fluctuating electrical potentials acquired from electrodes placed outside the intact skull and scalp. The waveforms and amplitudes of this electrical activity vary from second to second. Its frequency components cover 0–60 Hz, and the amplitudes are from several μV to about 200 μV. Based on frequency analysis, the EEG is usually classified to four classic frequency bands: δ wave (0–4 Hz), θ wave (4–8 Hz), α wave (8–12 Hz), and β wave (13–60 Hz). Typically about 20 EEG electrodes (metal discs 8 mm in diameter, which are filled with a conductive paste) are placed over the top and side of the scalp according to an internationally standardized location scheme. Each electrode records the total activity of a large number of neurons, say about 10^6 neurons, located near the electrode.

Substantial fluctuation in the mass potentials can occur when a major fraction of the neurons beneath an electrode is activated synchronously. Therefore, the EEG reflects the mass potentials of neuronal activities beneath the recording electrodes. Synaptic potentials of cortical pyramidal neurons also are considered to contribute to the waveform. However, the most basic question - which nuclei's or pathway's neuronal activities are reflected in the waveform? - is still unanswered, more than 70 years after Hans Berger (1929) first popularized the EEG. Thus the EEG is of limited value as an analytical tool.

However, the EEG is used widely because of its advantages, which include the fact that its noninvasive character makes long-term recording possible, with animal

or human subjects. Furthermore, with modern computers, data collection and analysis is convenient and relatively inexpensive. The importance of the EEG has been evaluated from empirical, experimental and statistical viewpoints, and the method has been widely applied for diagnosis of epilepsy, determination of level of consciousness and depth of anesthesia, study of sleep, development of childhood nervous system, etc.

D'Arsonval reported in1896 that a human could see 'light' when the head was exposed to a strong magnetic field; he called this phenomenon a magnetophosphene. Data from animal experiments indicates that this phenomenon is triggered by stimulation of the retina (Lövsund et al. 1981). The retina is a part of brain, because it develops from neural tissue.

Primarily for two reasons, it long has been assumed that the brain is susceptible to magnetic fields through the induction of electric fields or currents: the brain's function is inherently electrical, and it can be stimulated by strong magnetic fields. However, the site of action and the mechanisms of action of magnetic fields on the living body are not known. The intrinsic electrical activity of neurons and the occurrence of magnetophosphenes suggest that the nervous system is a good candidate for the site of interaction. Therefore, one might be able to detect changes in EEG in association with exposure to magnetic or electromagnetic field exposure. Based on this assumption, several EEG studies have been performed.

The human EEG changes according to a circadian (24 h) rhythm of behavior in response to the 24 h day-night cycle. Sleep states, lasting approximately 8 h during the night, form the unconscious part of that cycle. A basic principle of sleep cycle control in human is articulated in Borbely (2001): In the two-Process model, sleep-wake state transitions result from the combined effects of circadian factors (process C) and homeostatic factors (process S). During sleep, a third regulator, the ultradian, REM-NREM oscillator comes into play (Pace-Schott and Hobson, 2002). (REM indicates "rapid eye movement sleep" and NREM indicates "non rapid eye movement sleep. Dreaming occurs, and bodily movement is suppressed during REM sleep.) The 90 min REM-NREM cycle of adult human sleep is an ultradian rhythm.

2.2.1.3 Evoked potentials

Several types of evoked potentials, electrical responses elicited by various kinds of external or internal stimuli, can be recorded from the scalp. They are classified into two groups. The first one is the potentials evoked from the relevant cortical area following external sensory stimulation, such as visual or auditory; these are called a sensory evoked potential (SEP). The other type of evoked potential is called the event-related potential (ERP). For example, if a subject viewing a screen is asked to press a button every time a given symbol appears, a specific pattern of potential changes occurs with the event of recognizing the event of seeing the symbol. This electrical response is different from a motor potential recorded in association with movement of a subject's hand or finger to push a button.

2.2.1.3.1 Sensory-evoked potential

The SEP is the electrical activity recorded from the brain as it processes sensory input. A visual evoked potential (VEP) is obtained when the EEG recorded from the occipital region (over the visual cortex) is measured for about half a second following a brief flash of light directed to the eyes. Because the response to a single flash is small and buried in the ongoing - and therefore random with respect to the flash, activity is computer-averaged for 1,000 to 3,000 repetitions. Through this process, the regularly occurring aspects of the signal tend to 'add up' so they can be separated from the varying, and thus self-canceling background parts of the signal. Other SEPs can be obtained from the corresponding cortical areas by applying physiological stimulation to auditory or somatic receptors or by applying electrical stimulation to the peripheral sensory nerves.

An SEP is composed of several potentials that are designated according to the polarity and latency of their peak voltages. Thus, N_{100} is a negative potential that is maximum in amplitude, i.e., its peak, occurs with latency of 100 msec, and P_{300} is positive potential with a peak amplitude occurring with a latency of 300 msec. Because the analysis of recorded waveforms has advanced greatly during the last several decades, some information on the meaning of these peaks is available. For example, the N_{100} wave is evoked by nervous impulses traveling through the shortest route from the periphery to the cortical area; the long-latency P_{300} is associated with higher brain functions involving additional signal processing, such as decision making. Inability to record the N_{100} potential indicates the shortest relay pathway from the periphery is not functioning. In the visual system, for example, the short-latency components reflect the brain's electrical activity as the optic nerve and subcortically located lateral geniculate nucleus relays the signal to the visual cortex; the long-latency components reflect cortical receipt and processing, often involving some sort of decision making, of the sensory input.

2.2.1.3.2 Event-related potential

ERPs reveal intracerebral information processing following sensory or other forms of stimuli. The latency of ERP characteristically is long; it can be several hundred milliseconds or a few seconds, depending on the situation being studied. The contingent negative variation (CNV) is a slow negative shift after a warning stimulus (S1) which prepares the subject to a second stimulus (S2). CNV has a widespread anteroposterior and bilateral distribution over the scalp.

2.2.1.4 Neurotransmitters

Neurotransmitters can be classified into four major groups: (1) cholinergic, i.e., using acetylcholine, (2) amino acid, (3) monoamine and (4) peptides. Important amino acid neurotransmitters are glutamate, gamma amino butyric acid (GABA), and glycine. Monoamine neurotransmitters include dopamine, noradrenaline, adrenaline, and serotonin; these often are referred to as adrenergic neurotransmitters. Some peptides, e.g., substance P and opioid peptides, like enkephaline, that exist in the nervous

system function as transmitters. The former three groups are sometimes called classical transmitter substances.

Upon arrival of an action potential to the nerve terminal, Ca^{2+} channels at the terminal open and Ca^{2+} flows into the terminal from outside; hence intracellular calcium concentration $[Ca^{2+}]_i$ increases. Increased $[Ca^{2+}]_i$ induce exocytosis of the transmitter substance. The released transmitter combines with the receptors located on the surface of the postsynaptic membrane, as illustrated in Figs 1.2 and 1.3.

The binding of a transmitter to the receptor dissociates sometime during the process of channel activity or the second-messenger action. Then the transmitter molecule is removed from the synaptic cleft to terminate synaptic transmission. The way in which a neuron disposes of transmitter to end the signal is critical to synaptic transmission, because if a released transmitter persisted for a long period of time, the next signal could not get through.

There are three mechanisms by which nervous tissue disposes of transmitter substances: 1) diffusion, 2) enzymatic degradation, and 3) reuptake. By diffusion some fraction of all chemical transmitters will be removed. Enzymatic degradation of transmitter substance is used primarily by the cholinergic system, and the extracellular enzyme involved is cholinesterase. There are many other enzymatic pathways that degrade transmitter substance within both neurons and in non-neuronal tissues. These enzymes can be important for controlling the concentration of the transmitter within the neuron or in detoxifying transmitters that have escaped.

The slow rate of removal is another synaptic feature that distinguishes neuroactive peptides from classical transmitters. Their relatively slow removal contributes to the long duration of the action of the peptides and makes their metabolism seem more akin to that of hormones. For example, the ultra-slow EPSPs evoked by luteinizing hormone releasing hormone (LHRH) hormone can be recorded from certain neurons in sympathetic ganglia for 10 min.

Reuptake of the transmitter substance from the synaptic cleft is probably the most common mechanism used for inactivation. At nerve endings, there are high-affinity uptake mechanisms for the released transmitter. Choline, for example, is taken up specifically. Biogenic amines also are taken up into the presynaptic terminal by specific concentrating mechanisms.

2.2.1.5 Receptors for neurotransmitters

The cell membrane is composed of a lipid bilayer through which are folded intramembrane proteins (IMP) that function as ion channels, enzymes, and receptors. Under electron microscopic observation, the distribution of IMP shows some regular patterns.

Receptors are often classified as fast and slow. This distinction refers to the speed of onset of the postsynaptic effect. The opening of ion channels is usually a rapid process. Therefore, ionotropic receptors usually act rapidly - on the order of tens of milliseconds. The effects of some receptors for small molecules and for most peptides typically are slow in onset - hundreds of milliseconds to seconds, even though the action mediated is a conductance change in the postsynaptic membrane. This group

Table 2.1. Classification of Some of the Major Neurotransmitter Receptor Types

Transmitter	Ionotropic Receptor	Metabotropic Receptor
Acetylcholine	nicotinic type	Muscarinic type
	neuron, muscle type	M1M5
Glutamate	AMPA, NMDA, Kianate	mGluR1mGluR8
GABA	$GABA_A$	$GABA_B$
Glycine	$\alpha 1$–$\alpha 3, \beta$	
Dopamine		D1D5
Noradrenaline		
Adrenaline		$\alpha 1$, $\alpha 2 \beta 1 \beta 3$
Serotonin (5HT)	$5HT_3$	$5HT_1$, $5HT_2$, $5HT_4$
Opioid (enkephaline *et al.*)		μ, δ, κ
Tachiquinin (Substance P *et al.*)		NKR1, NKR3

contains all of the receptors that the recognition domain directly gates an ion channels. It includes nicotinic acetylcholine (ACh), gamma amino butyric acid (GABA), glycine, AMPA, and the NMDA class of glutamate receptors. In contrast, receptors that bring about biochemical changes in the postsynaptic cell typically are slow: this might be expected, because the several molecular steps involved each take time. This group includes (1) α- and β-adrenergic receptors, (2) serotonin, (3) dopamine, (4) muscarinic ACh receptors, and (5) receptors for neuropeptides. In each member of this group, the receptor molecule is coupled to its effector molecule by a guanosine nucleotide-binding protein (G-protein).

Some transmitters such as ACh and GABA can couple with both ionotropic and metabotropic receptors, and some other transmitters, like dopamine and glycine, appear to combine with either type of receptor (Table 2.1).

2.2.1.6 Opioid system

Opioids are transmitters, and three types of receptors have been identified (Table 2.1). However, the present author deems it necessary to describe opioid system separately, because EMF effects on opioid system are discussed frequently by several research groups.

Ever since discovery of the opium poppy, it has been known that opiates, such as morphine, are potent analgesic agents. This finding suggested that the brain contains specific receptors for opiate. Three major classes of opioid receptors have been identified: μ, δ and κ. These receptors originally were defined on the basis of their affinity for binding agonists. Opiate alkaloids, such as morphine, are potent agonists at the μ receptor. The μ-receptors are highly concentrated in areas which are important in

the regulation of pain. Three major classes of endogenous opioid peptides that interact with the opioid receptors have been identified: enkephalins, β-endorphine, and dynorphins.

Opioid receptors also are located in regions of the nervous system other than those mediating pain, and their high density in regions not associated with analgesia suggest that the endogenous opioids operate in a variety of physiological roles in addition to pain modulation. For example, opioids in amygdala are involved in emotional behavior and fear, and those in caudate or putamen play roles in motor coordination.

2.2.2 Endocrine system

The classical definition of hormone is a chemical substance that is synthesized and released from a specifically developed endocrine gland, conveyed throughout the body in the blood stream, and acts on specific target organs with extremely small dose to modulate the physiological functions of the target cells. However, recent studies have revealed that some hormones, such as digestive hormones or hypothalamic hormones, are released from tissues without specific endocrine structures. Noradrenaline is released not only from the adrenal medulla, which is a classical endocrine gland, but also from sympathetic nerve terminals, where it acts as a neurotransmitter. A parahormone is released from diffuse endocrine cells that do not form a clear gland structure, and act on nearby cells. This is called paracrine secretion.

Neuronal information originating from either the external or internal environments of an organism is processed via appropriate pathways and nuclei in the central nervous system, and then necessary messages can be sent to the hypothalamus. From there, there are two main routes by which the neuroendocrine control system can manifest hormonal actions. The first is via the small neurosecretory cells in the tuber cinereum and infundibulum of the hypothalamus, which excrete neurohormones into the hypophyseal portal vein system and thereby modulate anterior pituitary hormones. The anterior pituitary hormones control the hormonal actions of the thyroid, adrenal cortex, etc.

The second route is via large neurosecretory cells in the supraoptic and paraventricular nuclei which produce vasopressin and oxytocin, which are secreted from their axon terminals into the posterior pituitary lobe from which the hormones are released into the blood stream. Target cells that receive the hormones react, and their functions are modulated, thus completing the long-chain reactions that were triggered from the nerve impulses (Fig. 2.2).

In addition to these ‘classical’ routes, research on melatonin, which is secreted from the pineal gland, has made considerable progress in the last three decades (see below).

2.2.2.1 Pineal gland

The pineal gland synthesizes and secretes melatonin. Other tissues, such as brain, retina, Harderian gland, and intestine also produce melatonin, although the amounts

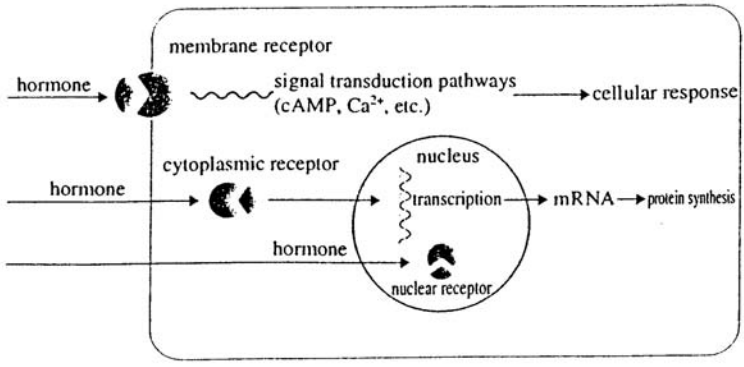


Fig. 2.2. When a hormone molecule arrives at a target cell, it initiates a series of steps that produce specific cellular actions. Receptors are located on cell membrane, in cytoplasm, and in the nucleus. When the receptor is an ionic-channel, the membrane potential of the cell is changed by hormone binding. Receptors for peptide-type hormones and catecholamines, which cannot penetrate the cell membrane, are located at the cell membrane. When these hormones combine with their membrane receptors, they activate signal transduction pathways, a long series of steps resulting in modulation of cellular functions. Lipid-soluble steroid hormones can penetrate the cell membrane and combine with cytoplasmic receptors; these affect nuclear receptors, causing DNA transcription, a process that eventually results in modulation of protein synthesis. The new proteins change the functions of the cell itself, which can, in turn, affect function of other cells (Kato 1999).

are insignificant compared with that produced from the pineal gland. Recently it was reported that mouse and human bone marrow cells contain high concentration of melatonin which was synthesized by the bone marrow cells and it was suggested that melatonin may have intracellular and or paracrine functions (Tan et al. 1999, Conti et al. 2000).

Phylogenetically, the pineal gland of fishes and amphibians possess visual functions; hence, it sometimes is called a 'third eye'. Reptiles and birds maintain function as a light receptor in the pineal gland. However, in mammals the visual function is lost, and the endocrine function is increased to synthesize and secrete melatonin.

Production of melatonin in the mammalian pineal gland is controlled by the nervous system through a chain from the retina: 1) retinohypothalamic fibers that synapse in the suprachiasmatic nuclei (SCN, which is the site of circadian clock) of the hypothalamus, 2) projections from the SCN to the area of the paraventricular nuclei (PVN), 3) long descending fibers from the PVN region that synapse on preganglionic sympathetic cell bodies in the intermediolateral column of the thoracic spinal cord, 4) axons of these neurons that leave the spinal cord to synapse neurons in the superior cervical sympathetic ganglia, and 5) peripheral postganglionic sympathetic neurons that terminate within the pineal gland (Fig. 2.3). In the pineal gland, the

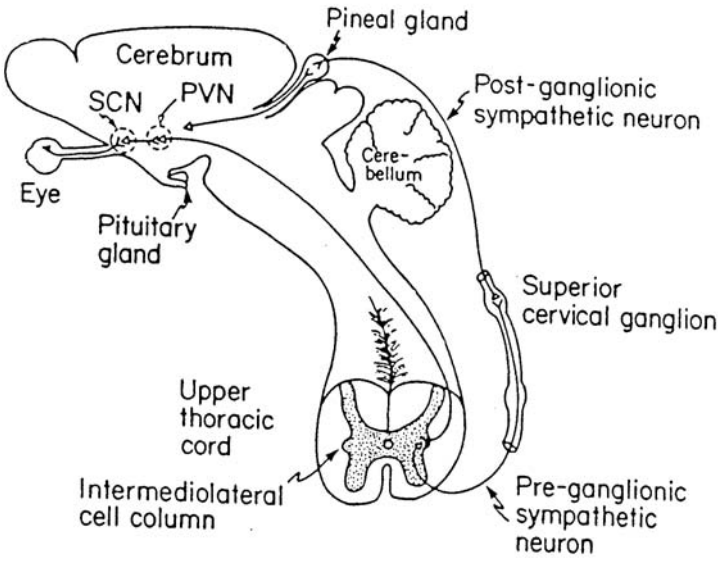


Fig. 2.3. The neural pathway that connects the eyes to the pineal gland in mammals, including humans (Henshaw and Reiter 2005). See text for details.

postganglionic sympathetic fibers end in the vicinity of pinealocytes, the hormone-producing cells. The postganglionic sympathetic neurons release a catecholaminergic neurotransmitter (noradrenaline) primarily during darkness. Thus, melatonin synthesis shows a clear circadian rhythm, occurring during the night.

The circadian rhythm is generated by neurons in the SCN. The pineal gland is a unique endocrine organ that is innervated directly by nerve fibers, as mentioned above: thus, it functions to transduce visual nervous information into humoral hormonal information. It should be noted that postganglionic sympathetic fibers from the superior cervical ganglia are not the sole source of pinealocyte enervation. The pineal gland of different mammalian species, such as rodents, tree shrew, monkey, and cow receive abundant pineal innervations from such brain portions as hypothalamus, lateral geniculate nucleus, habenula and posterior commissure (Matsushima et al. 1999, Kado et al. 1999, and Sakai et al. 2001).

Melatonin is synthesized from tryptophan through four enzymatic actions. The key, i.e., rate-limiting, enzyme in this system is serotonin N-acetyltransferase, which acetylates serotonin to N-acetyl serotonin. Thus, something that affects serotonin N-acetyltransferase would affect the pineal gland's melatonin output. There is general

consensus that melatonin produced in pinealocytes is directly released into blood stream without being stored inside the cell.

Recent extensive research has been revealing a variety of functions of melatonin (Vollrath 2001). Several of the functions relevant to this book are briefly mentioned below.

2.2.2.1.1 Measurement of melatonin

Melatonin production is measured by determining (1) content in the pineal gland (pg/mg of gland), (2) concentration in blood (pg/ml), as the produced melatonin is quickly released into the blood stream, (3) concentration of the melatonin metabolite, 6-sulfatoxymelatonin aMT6s or 6-OHMS) in urine (ng/ml), or (4) concentration of melatonin in saliva. About 70–80% of the circulating melatonin is metabolized in the liver, first to 6-hydroxymelatonin and then to 6-hydroxymelatonin sulfate followed by excretion through the kidney (Young et al. 1985). The urinary measures correlate highly significant ($P < 0.0001$) with plasma melatonin concentration (Graham et al. 1998). Rodents, humans, baboons, sheep, cows, and birds have been studied to assess the effects of electric, magnetic or electric plus magnetic field exposure on melatonin production.

2.2.2.1.2 Melatonin effects on the endocrine system

Melatonin has an inhibitory effect on the hypothalamo-pituitary-gonadal axis. Melatonin inhibits release of LHRH from the hypothalamus as well as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary and thus controls ovarian functions in several species of animals. However, in humans the endocrine system is relatively little affected by melatonin (Vollrath 2001).

2.2.2.1.3 Oncostatic action of melatonin

Nakatani et al. (1940) first demonstrated that an unidentified compound extracted from the bovine pineal gland could inhibit the growth of cancer cells in culture. Melatonin has been proven to be responsible for this effect, and subsequent reports indicate that melatonin inhibits tumorigenesis in an apparently circadian dependent manner (Shah et al. 1984). Furthermore, evidence from a number of clinical trials suggests that melatonin might be an effective therapeutic agent (Panzer and Viljoen 1997, Hrushesky 2001).

2.2.2.1.4 Melatonin effects on immune function

Most of the hormones and neurotransmitter substances influence immune function (Maestroni, 1993). Melatonin has been reported to have an immuno-regulatory action (Maestroni and Conti 1990, Liebermann et al. 2001).

2.2.2.1.5 Analgesic action of melatonin

Melatonin exerts analgesic effects including snails and humans (Golombek et al. 1991, Ebadi et al. 1998). The activity of melatonin can be inhibited by the opiate antagonist naloxone. Shavali et al. (2005) proposed that melatonin exerts its analgesic actions by increasing the release of β-endorphin, an endogenous opioid.

2.2.2.1.6 Other actions of melatonin

Other actions of melatonin, such as providing an effective aid for sleep (Wurtman and Zhdanova 1995) and serving as a potent free radical scavenger have been reported. However, the antioxidant effects require a concentration of melatonin that is much higher than peak nighttime serum concentration. Thus, the antioxidant effects of melatonin in humans probably occur at pharmacologic rather than physiologic concentrations (Reiter et al. 1997).

Because melatonin has these multiple actions, the hypothesis that alteration of melatonin secretion is a possible mechanism for some of the biological effects of magnetic field exposure was proposed first by Stevens (1987).

2.2.2.1.7 Melatonin in humans

When discussing possible biological effects of electromagnetic field exposure, we usually are concerned most about possible effects on humans. Therefore, the characteristic features of human melatonin secretion are summarized below.

Among humans, who do show the basic circadian rhythm in melatonin concentration in plasma, there are strong inter-individual differences in night-time melatonin secretion (Fig. 2.4). However, within individuals the rhythm is rather constant. About 5% of the general population is thought to not synthesize appreciable amounts of melatonin (Langer et al. 1997).

Nighttime serum melatonin peak concentrations in humans change with growth and aging. Waldhauser et al. (1988) measured melatonin levels in a large number of human subjects of all ages. Melatonin levels are low, less than 50 pg/ml, during the first 3 months of life. They begin to increase between 3 and 6 months of age. The highest concentrations are found in children aged 1–3 years (329.5 ± 42 pg/ml). There is a rapid exponential decrease of approximately 80% from age 1–3 years to age 15–20 years (62.5 ± 9.0 pg/ml). Thereafter (20–90 years) there is an additional moderate decline (Fig. 2.5). Humbert and Pevet (1994) presented morphological data that the number of melatonin producing pinealocytes decreased during the process of aging in Wistar rats.

Melatonin production is influenced by several other factors, such as alcohol consumption (Ekman et al. 1993), gonadal cycle, season of the year (Bartsch et al. 1994) and beta blockers, calcium channel blockers and some other kinds of drugs (Lambrozo et al. 1996).

Research on the action of melatonin relating to human health is still in an early stage, and the many potential effects of this hormone might contribute to the diverse reported effects of electromagnetic field exposure.

2.2.3 Immune system

The immune system is the natural defense mechanism of the body for large molecular weight compounds, often other living organisms with complex protein structures. Small molecules, typically chemicals, are handled by the liver, kidney and other

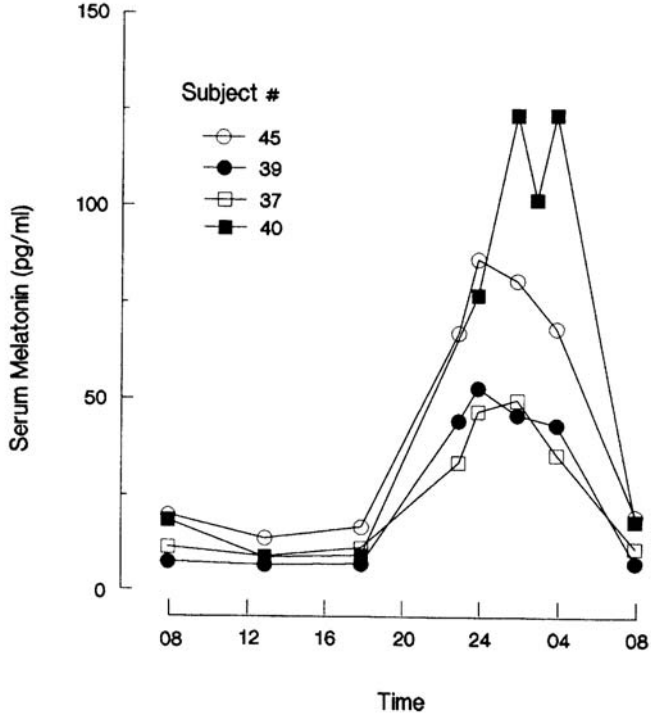


Fig. 2.4. Humans show appreciable individual differences in their patterns of melatonin secretion. The circadian rhythms of four adults are shown for 24-hour periods. Even in constant laboratory conditions, there are large differences in the peak amplitudes of melatonin in blood (Reiter 1997).

organs. The immune response is a reaction of the body which starts with recognition of a foreign body (none-self), followed by a chain reaction intended to eventually destroy and to expel the pathogen.

2.2.3.1 Acquired and innate immunity

The immune response is classified into two categories of reaction: (1) natural or non-acquired immunity, and (2) adapted or acquired immunity. The biggest difference between these two categories is that acquired immunity entails a specific reaction to an individual type of antigen, and the "memory" of the antigen structure is stored in the immune system. Only after an initial exposure can an acquired immunity response be mounted. This happens, for example, with poison ivy. In a sense, this is a learned response. With non-acquired immunity, the response is "innate"; the same response can be mounted on first exposure or 100th exposure to the same antigen.

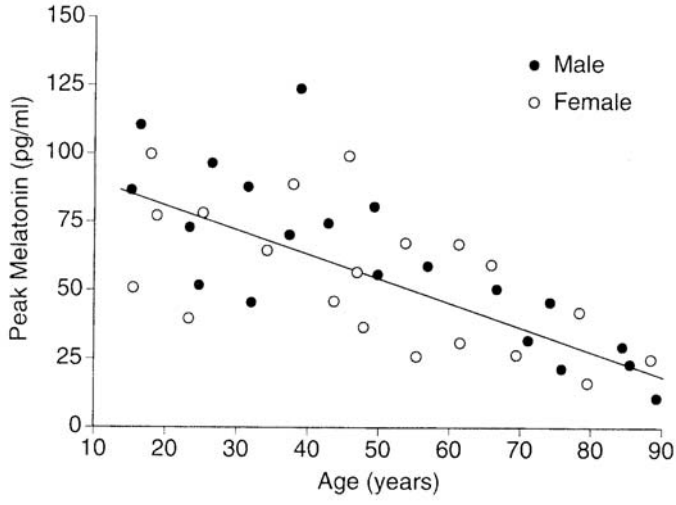


Fig. 2.5. Gradual reduction in nighttime melatonin concentrations in the blood of humans throughout life. Males and females exhibit a similar decline.(Reiter 1997)

Non-acquired immunity deals primarily with pathogens; acquired immunity deals with a wide variety of agents and molecules.

2.2.3.2 Types of immune cells

Immune responses are carried out mostly by leukocytes. For natural immunity, phagocytitic cells - such as monocytes, macrophages and neutrophiles - play important roles. These cells bind with microbes; once "tagged", they are destroyed. The phagocytes "catch" various microbes, based on non-specific, primitive recognition of the foreign bodies; hence, the phagocytes play the front-line role in the immune defense system.

Another important class of leukocytes is lymphocytes; these cells recognize pathogen microbes specifically and reveal various types of acquired immunity. Lymphocytes are classified first into T-lymphocytes and B-lymphocytes. (Each class is further characterized by the complex patterns of cell surface markers they display). The organs that play important roles for immune action, principally for manufacture and storage of cells immune cells are bone marrow, thymus, lymph nodes, and spleen.

2.2.3.3 Immune modulators

There is a group of soluble factors (proteins) known as cytokines that acts as information-transmitting-substances among cells involved in immune responses and/or inflammatory responses. Inflammation is responses of the body to infectious microbes, antigen stimulation, or damage of normal cells and tissues. It is characterized by an increased cellular infiltration, caused by increased local blood flow, and exudation of serum proteins, caused by increased permeability of capillary walls. Proteins – such as antibodies, complements, and enzymes – infiltrate into the inflammatory focus. Clinically, inflammation is characterized by redness, fever, swelling and pain. Cytokines produced by a variety of cells are important regulators of immune function. Specific cytokines, such as the many interleukins, combine with specific receptors on cell surfaces to convey information quite efficiently with extremely small doses, thereby intermediating or modulating immune function.

There are similarities in function between hormones and cytokines. However, cytokines differ considerably from hormones in three ways: (1) Several different cells produce the same kinds of cytokines, (2) a cell produces several kinds of cytokine, and (3) each type of cytokine exerts a variety of functions. It is called an autocrine phenomenon when a cell releases a chemical messenger and subsequently the originating cell is modulated by the common messenger. With cytokines, the cells are activated by their own cytokines, following production of receptors for the cytokine on the cell membrane. This produces a form of positive feedback to upregulate cytokine production as an immune response to injury.

Various types of cytokines are known: for example, there are monokines (related mainly to non-specific immunity), lymphokines (mainly to specific immunity), etc. The interleukin family is very important for immunity, and by now more than 25 interleukins are known. (A few of them are described here.) Interleukin-1 (IL-1) – a product of activated B lymphocytes, as well as numerous other cell types – exerts a range of biological activities, including co-stimulation of T lymphocytes and enhancement of synthesis of other cytokines (e.g., IL-2) involved in growth stimulation of T cells. IL-2, originally described as T-cell growth factor, is produced primarily by activated $CD4^+$ T lymphocytes, although some $CD8^+$ T cells and B cells can produce small amounts as well. IL-2 plays a central role in the immune response: it (1) stimulates the proliferation and differentiation of T lymphocytes, in both autocrine and paracrine fashions, (2) increases the cytolytic activity of natural killer (NK) cells, and (3) promotes the development of lymphokine-activated killer cells. Activation of T lymphocytes is a pivotal event in the generation of immune responses to most antigens, including tumor antigens (Szamel and Resch 1995).

NK cells are large granular lymphocytes phenotypically dissimilar to either T or B lymphocytes. NK cells can non-specifically destroy a variety of target cells, including parasites, virally infected cells, and certain tumor cells (Pross and Lotzova 1993, Whiteside and Herberman 1994). T lymphocytes are mediator of cellular immunity, and regulate immune response. B lymphocytes are mediators of humoral immunity and produce antibodies.

Any interference of magnetic field exposure with IL-1 or IL-2 production or function would have significant effects on the immune system. Based on this assumption, attempts have been made in several laboratories to examine the effects of EMF exposure on cytokine activity. These studies vary considerably, both in (1) field strength and ELF exposure parameters (i.e., pulsed, sinusoidal, static, etc.) and in (2) outcome.

2.2.4 Summary

The physiologic processes by which the body regulates both its internal environment and its interactions with the external environment are exceedingly complex. The primary integrative systems are nervous, endocrine and immune, all of which interact. For an environmental agent to adversely affect an organism, it must disrupt these systems. Conversely, if an environmental agent has no discernable effect on these systems, the exposure is likely to be safe. Research in bioelectromagnetics requires the collaboration of experts familiar with a wide variety of biology and medicine. Furthermore, as seen in Chapters 5–9, a wide variety of expertise in engineering and mathematics also is required. And these experts form diverse disciplines apply their wide variety of techniques to the study of bioelectromagnetics. This complexity represents a daunting challenge to both the "newcomer" and the "old hand" in this field of endeavor.

2.3 Endpoints and Methodologies for *In vitro* Research

2.3.1 Cell growth

Cell growth is a basic cell process that can be measured easily using *in vitro* methods. Furthermore, it is an integrative process: all cellular components and processes must be working properly to maintain normal cell growth. Thus, it is a good screening endpoint, for it will detect a wide variety of defects.

2.3.1.1 Basic characteristics of cell growth *in vitro*

Cultured cells in dishes usually grow exponentially, where the number of cells reaches 2-fold, 4-fold, 8-fold, etc. over successive time intervals of equal lengths. Immediately after cells are placed ("plated") into culture dishes, the cells grow slowly for several hours; this is called the lag phase. However, after that the cells enter into the exponentially growing phase. Different cells have their own, cell-specific doubling time. Growth continues until the cells in a dish reach a confluent state. Normal cells grow in a monolayer, and the growth is arrested by cell-cell contact inhibition. However, on the contrary, some cancer-derived cells grow without cell-cell contact inhibition and grow even in a confluent state: thus, they pile up into multilayer. This is called a focus. When cell growth rate is changed by internal or external factors, it can be indicated as a change in the doubling time during the exponentially growing phase (Miyakoshi et al. 1994).

2.3.1.2 Cell cycle and DNA synthesis

The cell cycle is divided into four phases: (1) mitotic phase (M-phase), from the beginning to the completion of a round of cell division; (2) gap 1 phase (G_1 phase), after the completion of cell division and before the beginning of DNA synthesis; (3) DNA synthesizing phase (S-phase), from the beginning to the completion of DNA synthesis, and (4) gap 2 phase (G_2 phase), after the completion of DNA synthesis and before resumption of cell division.

The duration of the cell cycle is specific to specific types of cell lines. However, generally no important differences are found in M-phase and S-phase among various cell lines. Therefore, it is considered that differences in the duration of the whole cell cycle are dependent on differences in the G_1 and G_2 phases.

When cells are damaged by stimuli, i.e., external factors, repair of the damage requires time; thus, the durations of normal cell-cycle phases often are changed. Usually, cell cycle progression is delayed (for example, prolonged G_1 phase), meaning the cell-cycle distribution is changed. The cell-cycle distribution usually is determined (Nakahara et al. 2002) using with collected and fixed cells whose DNA has been stained with propidium iodide.

During DNA synthesis (S-phase), the synthesis rate is almost constant in normal cell growth for most types of cells. The DNA synthesis rate usually is determined as the specific activity of the radioisotope tritium (^{3}H)-labeled thymidine uptake per unit time. As one of the four fundamental bases of DNA (adenine paired with thymine, and guanine paired with cytosine), thymidine necessarily is used during DNA synthesis, making it an appropriate marker. Also external stimuli or internal factors occasionally change DNA synthesis rate with cell characteristics. Changes in DNA synthesis rate depend on various synthesis-related enzymes, and damage repair procedures are complex.

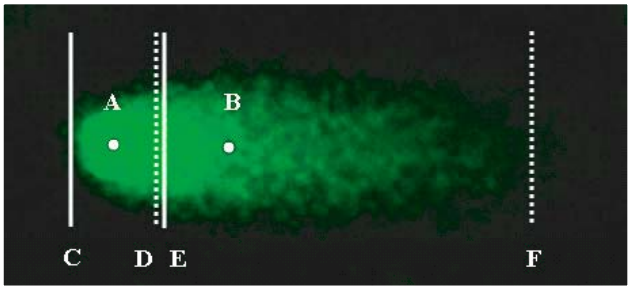
2.3.2 Genotoxicity

Damage to genes is called genotoxicity. Contemporary experimental methods for assessment of genotoxicity emphasize four basic methods: chromosomal aberration, DNA strand breaks, micronucleus (MN) formation, and mutation.

2.3.2.1 Chromosomal aberration

Chromosomal aberration is produced by DNA damage (and also by other factors) in condensed chromosomes during the mitotic phase. For example, it is well known that ionizing radiation breaks DNA strands of cells and thereby induces chromosomal aberration. In cultured cells, chromosomal aberration can occur spontaneously, but this is extremely infrequent.

To observe chromosomal aberration, cell division is arrested (stopped) using colcemid when chromosomes are condensed in metaphase of M-phase. Next, cells are suspended in hypotonic solution and centrifuged; then they are placed into a solution for fixation. Finally, fixed cells are plated onto a glass microscope slide, stained using Giemsa stain, and observed using a light microscope.



Picture of a Sample Comet

A: Head center
B: Tail center
C: Head top
D: Head end
E: Tail top
F: Tail end

Fig. 2.6. Picture of a sample result from a comet assay. DNA strand breaks were analyzed using IDL Comet 6.0 (Adamnet; Tokyo, Japan). A = head center, B = tail center, C = head top, D = head end, E = tail top, and F = tail end, as determined by image analysis software. Three parameters are computed: (1) Tail length = F – E; (2) Tail percent = (Tail (F – E) content/comet (F – C) content) ×100; and (3) Tail moment = [Tail percent × (B – A)]/100. At least 100 comets were analyzed from each of two replicate cultures, and this experiment was repeated at least three times. (J. Miyakoshi, unpublished)

Various types of chromosomal aberration can be observed. Some are severe, such as (1) chromosomal break, (2) ring, (3) dicentric chromosome, (4) large fragment, (5) rearrangement, (6) loss, and (7) amplification. Other chromosomal aberrations, like gap, are slight. However, it should be noted that, when an extremely important gene is included in an aberration site, the severity of chromosomal aberration is not consistent with the severity of the type of chromosomal aberration (Yaguchi et al. 2000).

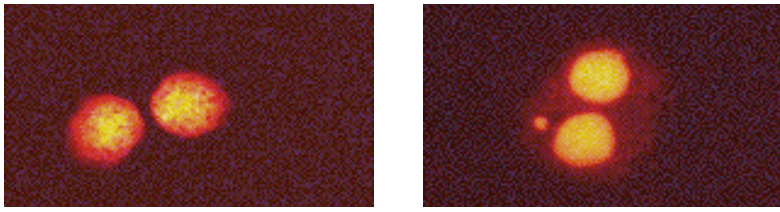
In a dividing cell, the chromosomes are divided into two chromatids, each consisting of one DNA molecule. Two major classes of chromatid damage are recognized: chromatid-type aberration, and sister chromatid exchange (SCE). In a broad sense, chromatid-type aberrations are included as a form of chromosomal aberration.

It is known that SCE occurs infrequently in normal cultured cells. However, it also is well known that SCE and chromatid-type aberration are very frequently observed in cells treated with mitomycin-C (MMC). However, it has not been confirmed whether or not SCE alone causes great damage to cells, and the severity of the consequences of genetic damage depends on the genes that are involved with SCE (Yaguchi et al. 1999).

2.3.2.2 DNA strand breaks

DNA strand breaks are an index to show whether or not DNA strands in cells are directly broken by genotoxic agents. DNA strand breaks usually are examined using the comet assay (Miyakoshi et al. 2000a: 2002). A brief explanation of the comet assay follows:

(1) Cells treated with exposure to a magnetic field or to another external stimulus, including chemical agents, are collected.



A binucleated cell which has no micronucleus and a binucleated cell with a micronucleus of CHO-K1 cell.

Fig. 2.7. A binucleated CHO-K1 cell which has no micronucleus (left), and a binucleated CHO-K1 cell with a micronucleus (right). (J. Miyakoshi, unpublished)

(2) A sample of cell suspension is mixed with agarose and fixed on a glass microscope slide.
(3) The slide is soaked in Lysis solution, and cells are melted.
(4) After electrophoresis under alkaline or neutral conditions, the slide is soaked in 70% - 80% ethanol to fix the cells. Am alkaline condition is used during electrophoresis for analysis of single strand breaks; neutral conditions are used in assessment of double strand breaks.
(5) The slide is dried in air and then stained using SYBR Green I.
(6) Using a fluorescent microscope, the stained DNA is observed and photographed. Images are analyzed using a data analysis program for the comet assay.

Figure 2.6. is a photograph of the result of a comet assay, with a summary of the analysis procedure. Usually, three image analysis values – tail length, tail percent, and tail moment – are compared with those of untreated controls to evaluate effects on DNA strand breaks.

2.3.2.3 Micronucleus formation

MN formation frequently is used also as an index for genotoxicity evaluation. MN is a phenomenon that occurs when DNA in a dividing cell is damaged. A part of the DNA becomes isolated from the original nucleus (or the paired nuclei in a binucleated cell that has not yet separated into two cells) and appears as a small, separate nucleus (Koyama et al. 2003: 2004). A brief explanation of the MN analysis method follows:

(1) After exposure to a magnetic field or treatment with a chemical agent, cells are cultured in medium with cytochalasin B for 18–36 hours. (usually for 1.5-times the doubling time) to arrest the cell-division cycle immediately after cell division, when cells are binucleated.
(2) Cells are collected and centrifuged; then they are plated onto a glass microscope slide and fixed with ethanol.

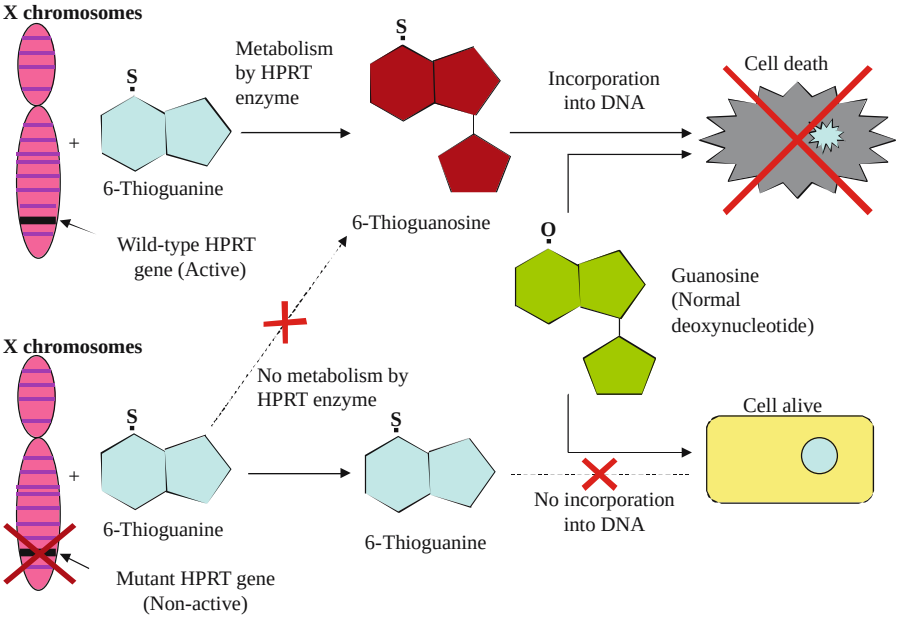


Fig. 2.8. An outline of the method detecting mutation at the HPRT gene on X chromosomes. (J. Miyakoshi, unpublished)

(3) Cells are stained using propidium iodide, and stained nuclei are observed with a fluorescent microscope.
(4) At least 1,000 images of binucleate cells are examined per experiment, and the numbers of cells including one, two, or three or more MNs are determined.

Figure 2.7. shows a cell with MN formation. Although MN formation very rarely occurs spontaneously, it can occur. Therefore, MN formation is evaluated in both untreated and treated groups, and an appropriate statistical method is used to evaluate differences.

2.3.2.4 Mutation

Mutation, a change in the DNA base sequence, is a form of genotoxicity in cells that cannot be confirmed in criteria of chromosomal aberration, DNA strand breaks, or MN formation. Human cells have about thirty thousand genes; therefore, it is impossible to examine for mutation in all genes. A gene is incredibly long with thousands to millions of base pars, and it is impossible to check for alterations in this entire sequence. The idea becomes not to search for some or all specific changes. Rather, the strategy is to check the frequency of a specific mutation used as a marker. If a chemical or physical agent increases the frequency of this mutation, it probably increases the frequency of other mutations as well.

Cells containing the normal HPRT gene cannot grow in a specific culture medium lacking a certain substrate. However, when a certain mutation occurs with the HPRT gene, the enzyme that produced by this gene shows abnormal status, and some of the abnormal forms can grow in the deficient medium. Thus, by addition of a specific agent, only cells producing the abnormal enzyme are capable of surviving and so the mutant is detected (Miyakoshi et al. 1997: 1999). A summary of the mutation analysis procedure using HPRT gene locus on the X chromosome follows:

(1) First, to exclude cells with spontaneous HPRT mutations, cells are cultured in medium containing hypoxanthine-aminopterin-thymidine.
(2) After being transferred to a normal cell culture medium, the cells are exposed to a magnetic field.
(3) After exposure, cells are cultured for a duration equivalent to 6- to 10-fold of the doubling time; this is the mutation expression time.
(4) After that, cells are cultured in a medium containing 6-thioguanine, where only cells with HPRT gene mutation can survive until colony formation occurs.
(5) Colonies are stained and counted to calculate mutation frequency.

Figure 2.8. shows the detection procedure for HPRT mutants. Mutation consists of various types of mutation, including base change, deletion, frame shift, etc. It also is known that there are gene sites exist where mutation occurs relatively frequently extremely. (These locations can be called “hot spots”.)

2.3.3 Gene expression

Gene expression is the process by which a DNA base sequence (exactly speaking, the exon portion of the gene) is interpreted. Messenger RNA (mRNA) specific to this gene is produced in the cell nucleus and travels outside the nucleus to the cytoplasm. Then the gene-product, i.e., the protein, is produced as a polypeptide chain assembled from the mRNA template.

As the endpoint for evaluation of gene expression, the existence/absence of gene expression is examined using either mRNA or the production of a protein that is derived from a specific gene. (Usually the latter approach is used.) Contemporary analysis procedures for protein expression are given by Miyakoshi et al. (2000b, 2005), Tian et al. (2002) and Hirose et al. (2003).

Various “kits” have been developed so that researchers easily can carry out many kinds of cellular and molecular biology procedures, including assessment of gene expression. The Western blot procedure, which generally is used in protein expression analysis, is summarized below:

(1) Cells are exposed to a magnetic field, heated, or treated with chemical agents.
(2) Immediately after treatment, or after an appropriate expression time (depending on cell lines being used, treatment administered, and protein to be measured), cells are collected using trypsin treatment and the scratching method.
(3) Collected cells can be stored at −80°C until the next step is performed.

(4) Cells are treated with a surfactant to isolate protein; then protein density and volume are determined.
(5) A certain of protein, including 20~30 μg of protein, is applied to gel for the Western blot procedure to isolate protein by molecular weight with electrophoresis.
(6) Isolated protein in gel is transferred onto the membrane.
(7) The protein-transcribed membrane and antibody specific to the gene to be analyzed are mixed (and shaken slowly) to bind protein with antibody.
(8) Using a coloring reagent, protein production volume is estimated based on the volume of the colored part.
(9) Protein expression volume is estimated using image analysis software.

Figure 2.9. shows examples of Western blots. In this case, heat shock protein (HSP 70) and β-actin were assayed. It is extremely important to select an appropriate antibody.

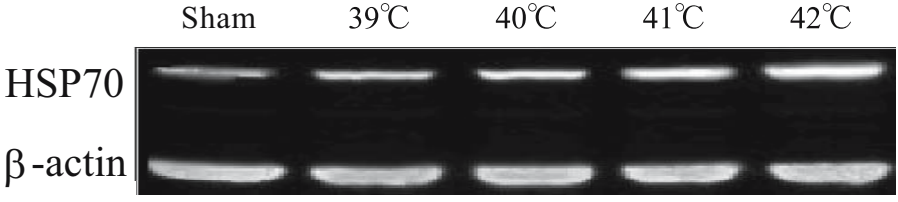


Fig. 2.9. Expression of HSP70 by Western blot in A172 cells after exposure to hyperthermia at 39°C, 40°C, 41°C or 42°C for 3h. The amount of HSP70 is increased by hyperthermia. (J. Miyakoshi, unpublished)

2.3.4 Transformation

Transformation means cell transformation, i.e., characteristics of normal cells are changed and the cells become malignant. Transformed cells are called transformant; transformants are cells whose characteristics are changed morphologically, even though they are in a normal culture condition. For example, transformed cells lose the contact inhibition control system.

In cells with high-grade malignancy, cells accumulate in multiple layers and rise to form a colony; generally this is called a focus. Transformation is classified (graded) into Types I, II, and III, covering the range from slight to serious malignancy. Type-II and III cells are defined as neoplastic transformant (Miyakoshi et al. 2000c). In culturing cells successively, it has been confirmed that a transformant appears naturally at an extremely low rate, even when no external treatment is given.

2.3.5 Summary

This chapter provides a brief, general summary of *in vitro* methods that should be helpful for engineers and for scientists from disciplines other than cell or molecular

biology. The organization here is parallel to that used in Chapter 5, where results of *in vitro* studies on magnetic field bioeffects are described.

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Part II

Extremely Low Frequency

3

Experimental Results: *In vivo*

Masamichi Kato

3.1 Behavioral Science Research

3.1.1 Experiments with rodents

Many neural systems are involved, anatomically and functionally, in learning and memory. Among them, central cholinergic systems have been studied extensively for their role in memory. In rodents, it is well documented that a decrease in cholinergic activity in the brain causes performance deficits in a radial arm-maze, which is used as a spatial memory test for rodents (Levine 1988).

Lai (1996) investigated effects of 750 μT, 60 Hz magnetic field exposure for 45 minutes immediately before each training session in a 12 arm radial-maze. Over a series of 10 consecutive sessions, the exposed rats made more errors than did the sham-exposed group. Pretreatment with the cholinergic agonist physostigmine blocked the adverse effect, suggesting that the spatial learning deficit was caused by the effects of the ELF magnetic field upon cholinergic systems. More recently, Lai et al. (1998) showed poorer learning of rats in a Morris water maze with 60 Hz magnetic fields of 0.75 mT or 1.0 mT applied before training sessions.

Sienkiewicz et al. (1998a) investigated the effects of exposure to a 50 Hz, vertical, sinusoidal magnetic field at 750 μT for 45 min on performance of C57BL/6J mice in an eight arm radial-maze. Experimental subjects were exposed immediately before daily testing sessions. Exposure reduced the rate of acquisition of the task, but it did not affect overall accuracy. These results support those of Lai (1996), suggesting that initial stages of learning are more susceptible to disruption by ELF magnetic field exposure.

Sienkiewicz et al. (1998b) performed another series of experiments with mice to study the effects of magnetic fields on spatial learning of mice in an eight-arm radial maze. In the first experiment, they investigated the relationship between changes in behavior and flux density, seeking a dose-response relation. The second experiment examined the effects of the introduction of a delay between exposure and testing.

The third experiment tested if repeated, short-term magnetic field exposure exerted any effect on the retention and performance of the task. Exposure above a threshold, of between 7.5 μT and 75 μT, increased the number of errors and reduced the rate of acquisition of the task without any effect on overall accuracy. However, the imposition of a 45-min delay between exposure at 750 μT and behavioral testing resulted in the elimination of any deficit. Similarly, exposure to fields between 7.5 and 750 μT for 45 min each day for 4 days after training had no amnesic effects on the retention and subsequent performance of the task. Overall, these results provide additional evidence that 50 Hz magnetic fields can cause subtle changes in the processing of spatial information in mice. Sienkiewicz et al. (1996) saw no effect of 15 to 45 min exposures when the exposure was applied during task performance, rather than previous to training sessions.

Collectively, these investigations raise the possibility of an interaction between intensity and duration of the exposure, and also the importance of timing of the exposure. This last point suggests that the interfering mechanisms might be chemical, rather than due to direct induced current or electric field.

Kavaliers et al. (1996) reported that exposure to 100 μT, 60 Hz magnetic field for 5 min during task acquisition in a Morris water-maze significantly improved performance of female mice, eliminating the sex difference in acquisition by deer mice. Males perform this task better than do females. Enhancement of enkephalin activity with an enkephalinase inhibitor significantly reduced task performance by male mice. Both naltrexone treatment and exposure to a 60 Hz magnetic field attenuated the enkephalin-mediated reduction of spatial performance. These findings indicate that brief exposure to 60 Hz magnetic fields can enhance water maze task acquisition by female deer mice and suggest that these facilitatory effects on spatial performance involve alterations in opioid activity (see 2. 2.1.1.4.) Although these results (enhancement) are opposite in direction to those of Lai et al. (degradation), both groups found behavioral effects following ELF magnetic field exposure.

3.1.2 Experiments with non-human primates

Rogers et al. completed an extensive series of behavioral studies designed to assess the effects of electric, or electric and magnetic field, exposure on the behavior of a nonhuman primate, the baboon (*Papio cynocephalus*). The exposure facility design and data on its function during experiments are provided in Rogers et al. (1995e). Because animals can detect electric fields, experiments involving exposure to a strong electric field can alter behavior, either through (1) an indirect effect mediated by perception of the field, especially if the exposure is aversive, or (2) by a direct effect on the function of the nervous system. Thus, Orr et al. (l995a) used behavioral methods to assess the electric field detection threshold for baboons, finding it averaged which is close to the human threshold value of 10 kV/m (Kato et al. 1986: 1989). The baboons pressed buttons to earn food rewards for correct responses indicating presence or absence of an electric field. Rogers et al. (1995a) demonstrated that electric field exposure, at field strengths up to 66 kV/m, was not aversive for baboons; the subjects would not respond to end electric exposure. Then the authors examined

the effects of exposure to vertical electric fields (30 or 60 kV/m, 60 Hz) on operant behavior (Rogers et al. 1995b). Baboons were trained to respond on either a fixed ratio 30 (FR30) schedule of reinforcement when a red lamp was on or to differential reinforcement of low rate 20 sec (DRL 20) schedule when a green light was on; the two tasks alternated for 15 min periods. The subjects were assigned randomly to field-exposed and sham-exposed groups and studied using a design including (1) pre-exposure, (2) exposure and (3) post-exposure periods, each 6 wks in duration. On the first day of exposure, the field-exposed baboons showed 'work stoppage' on both schedules. After the first day, operant behavior was normal, leading the authors to conclude that the work stoppage was merely the result of initial exposure to a novel stimulus.

Next, Orr et al. (1995b) exposed baboons to a vertical, 6 kV/m electric field (60 Hz) along with a horizontal 50 μT magnetic field (60 Hz). On the first day of exposure, operant responding was not affected, as suggested by the electric field "perception" hypothesis. In these experiments, the baboons were trained to perform a match-to-sample task; this more demanding task assessed several cognitive functions, including short-term memory. In the final operant experiment, baboons were exposed to 30 kV/m and 100 μT. No work stoppage occurred, suggesting that the magnetic field exposure blocked the normal response to exposure to a clearly perceptible electric field. In all of these match-to-sample experiments, no adverse effects on task performance were detected. The same research group also studied the social behavior of groups of eight, young-adult, male baboons. Rates of performance by subjects randomly assigned to field-exposed or sham-exposed groups were compared during 6-week pre-exposure, exposure, and post-exposure periods. Temporary increases of some social behaviors, (passive affinity, tension and stereotypy) occurred with initial exposure to 30 or 60 kV/m (Coelho et al. 1991, Easley et al. 1991). However, adding a 50 μT magnetic field to 6 kV/m or adding 100 μT to 30 kV/m annulled the increased social behavior (Coelho et al. 1995). The authors again suggested that magnetic fields can inhibit the excitatory effects caused by exposure to perceptive electric fields.

Although the authors do not specifically mention any specific part of nervous system involved in the brain, it is conceivable those systems relevant to the above mentioned behavioral changes are influenced by electric and magnetic fields, contradictory each other.

3.1.3 Experiments with humans

Preece et al. (1998) examined the effect of 50 Hz, 600 μT horizontal, linearly polarized magnetic field exposure on cognitive function of 16 subjects. A randomized, three-way, cross-over design was used, and a computerized battery of cognitive function tests was completed in the presence and absence of the ELF magnetic field. Analysis indicated temporary declines in the accuracy of attentional task, choice reaction time (RT) and a recall tests (numerical working memory sensitivity index). In addition, the delayed work recall accuracy showed a temporary deterioration.

Trimmel and Schweiger (1998) investigated the effects of exposure to a 50 Hz, 1000 μT magnetic field on visual attention, speed and accuracy of perception, and verbal memory of 66 human volunteers. The double-blind investigation used three exposure conditions: (1) magnetic field with auditory noise of 45 dB, (2) noise of 45 dB, and (3) a control condition without magnetic field or noise. Attention and perception performance were reduced in the magnetic field plus noise condition. There also was a 'trend' ($P < 0.1$) towards reduction in memory performance. Then the authors separated the subjects into a 'sensitive' group - those that claimed to react exceptionally to electromagnetic field exposure - and an 'insensitive' group, there were clear performance deficits in the subjects rating themselves as 'sensitive' to electromagnetic field. No effects were found in 'insensitive' group. Also, there were no inter-group differences in the noise condition. In this study, because the magnetic field was applied with noise, it is possible that the effects were due to interaction effects or accumulative effects of magnetic field and noise. Strictly speaking, without an exposure to magnetic field alone, it is impossible to attribute, without ambiguity, these results to exposure to ELF magnetic fields.

Podd et al. (2002) investigated both the direct and delayed effects of 50 Hz, 100 μT magnetic field on human performance. Eighty subjects completed a visual duration discrimination task: half were exposed to the ELF field, and the other half were sham exposed. The delayed effects of exposure also were examined in a recognition memory task that immediately followed completion of the discrimination task. No effect was observed on RT and accuracy in the visual discrimination task. However, the field had a delayed effect on memory, producing a decrement in recognition accuracy.

3.1.4 Summary

Although the behavioral changes observed generally are subtle, the above mentioned studies on rodents, baboons, and humans, do indicate that some cognitive functions are influenced acutely by ELF magnetic field exposure.

3.2 Central Nervous System

3.2.1 Neurophysiology and clinical neurology

Since magnetic stimulation technique was introduced to the study of brain function, technical improvements have been made. Recently the repetitive transcranial magnetic stimulation (rTMS) technique has been widely used in neurophysiology and clinical neurology as a tool not only for functional cortical mapping of the motor system (Ueno et al. 1988, 1990) but also for studying other brain functions. Although little is known about their mechanisms of action, the list of physiological effects obtained by rTMS has been accumulating (Petersen et al. 2003), and the method has become established in clinical practice (Daeuper et al. 2002). From the beginning

of work with this technique, muscular twitching has been observed and electromyogram (EMG) activity has been recorded from appropriate muscle(s) following human motor cortex stimulation; these effects have been reported by many laboratories from around the world.

TMS has provided the first real evidence that direct, monosynaptic connections from the motor cortex to spinal motoneurons exist in humans (Petersen et al. 2003). In particular, the tibialis anterior muscle appears to receive a significant a monosynaptic corticospinal drive, just as do muscles in the hand. The reason for this may be the importance of this muscle in controlling the foot trajectory in the swing phase of walking. TMS is powerful technique in the analysis of motor control in human subjects. Another positive effect has been observed by TMS applied over the visual cortex. Amassian et al. (1989: 1994) investigated the effect of stimulation of human occipital cortex on perception alphabetical characters. When the interval between visual stimulus and TMS was less than 40–60 msec, or more than 120–140 msec, letters were correctly reported. However, at intervals of 80–100 msec, either a blur or nothing was seen. This phenomenon was studied further by Kastner et al. (1998), who reported that "transient visual field defects" and phosphenes were induced in normal volunteers by TMS applied above the inion on the midline.

For several other areas of cerebral cortex, effects also have been observed when TMS was applied over a relevant cortical area in combination with performance of appropriate task(s), either physiological or psychological. Some of the recent clinical work is described below.

Pascual-Leone et al. (1994a) studied the effects of TMS of the motor cortex on simple RT in 10 patients with Parkinson's disease compared with 10 age-matched, normal controls. The subjects flexed their right elbow rapidly in response to a visual "go" signal, and RT was measured as the interval to the onset of biceps EMG activity. On random trials, TMS was applied to the left motor cortex at varying delays after the go signal. In trials without TMS, the RT was 15% longer in the patients. However, in the trials with subthreshold TMS, patients and controls did not differ in RT. Pascual-Leone et al. (1994b) further studied the effects of rTMS of the motor cortex on choice RT, movement time, and error rate in a serial RT task in six medicated patients with Parkinson's disease and 10 age-matched, normal controls. In normal subjects, subthreshold, 5-Hz TMS did not significantly change choice RT, but it did shorten slightly movement time and increase error rate. In the patients, TMS significantly shortened choice RT and movement time without affecting error rate. These effects did not impair procedural learning. Performance on a peg-board test was improved by rTMS in the same Parkinsonian patients, especially when they were "off" medication, but worsened in the normal subjects. The authors indicate that repetitive, subthreshold motor cortex stimulation can improve performance in patients with Parkinson's disease and could be useful therapeutically.

Lesion studies and neuroimaging investigations suggest that left prefrontal lobe dysfunction is pathophysiologically linked to depression (George et al. 1994). It has been demonstrated recently that rTMS can affect mood in both normal and depressed patients. Focal stimulation over the left prefrontal cortex induced a transient feeling

of sadness, while right prefrontal cortex stimulation caused a sense of happiness (Pascual-Leone et al. 1996a).

Pascual-Leone et al. (1996b) reported on the effects of focal rTMS on frequency of symptoms of depression in 17 patients with medication-resistant depression. TMS was applied with a figure-8-shaped coil that produced focused stimulation. Left dorsolateral prefrontal cortex was stimulated over 5 consecutive days; each day 20 trains, 10 sec in duration at 10 Hz, were given at an intensity of 90% of the patient's motor threshold. Sham rTMS and stimulation of different cortical areas were used as controls. The study was designed as a multiple cross-over, randomized, placebo-controlled trial. Left dorsolateral prefrontal cortex rTMS resulted in decreased score on both the administered Hamilton Depression Rating Scale (from 25.2 to 13.8) and the self-rated Beck Questionnaire (from 47.9 to 25.7). Eleven of the 17 patients showed pronounced improvement that lasted for about 2 weeks after 5 days of daily rTMS sessions. These findings indicate a possible role of the dorsolateral prefrontal cortex in depression.

Loo et al. (1999) investigated efficacy of rTMS for treating resistant major depression in a double-blind study. Eighteen medication-resistant subjects were randomly assigned to 2 weeks of real or sham rTMS. Stage of depression was estimated by using the Montgomery-Asberg Depression Rating Scale, the Hamilton Depression Scale, and the CORE Scale. TMS was administered with a figure-8-shaped coil (70-mm diameter in each loop) applied to the left dorsolateral prefrontal cortex. A 10 Hz signal with a strength of 110% of the motor threshold was applied, using 30 trains of 5 seconds each delivered 30 seconds apart. The coil was tangential to the scalp for real treatment and at 45° for sham treatment (which delivered "a much weaker stimulus" instead of zero value for the usual sham control). After 2 weeks, the medication-resistant patients showed improvement on all three depression scales. However, there were no differences between the groups receiving real and sham treatment. This investigation is a little hard to evaluate with respect to the effects of magnetic stimulation, because in the sham group, the weak stimulus applied might have been effective. Further, placebo effects are sometimes strong in dealing with depression.

A test of human motor cortex excitability was recently devised by Tokimura et al. (2000). The test is based on coupling TMS with peripheral nerve stimulation. EMG responses evoked in a finger muscle after TMS over the appropriate motor cortex can be suppressed by electrical stimulation of the median nerve, if the time between the conditioning stimulation of the nerve and application of test TMS over the motor cortex is 2 to 8 msec longer than the time needed by the peripheral afferent nerve input to reach the cortex. This effect, named short latency afferent inhibition of the motor cortex, is produced by interaction within the cerebral cortex. Because the effect is reduced or abolished by intravenous injection of the muscarinic antagonist scopolamine, it is thought this test could be a noninvasive way of testing cholinergic activity in the human cerebral cortex (Di Lazzaro et al. 2002). The pathogenesis of Alzheimer's disease appears to involve several different mechanisms, the most consistent of which is a deficit in cholinergic activity (Coyle et al. 1983). Di Lazzaro et al. (2002) used short latency afferent inhibition to assess cholinergic transmission

in the cerebral cortex of 15 patients with Alzheimer's disease and 12 age-matched healthy adults. The afferent inhibition was reduced to 86% in the patients, compared with reduction of 45% with the controls. The findings suggest that this method can be used as a non-invasive test of cholinergic pathway in Alzheimer's disease.

3.2.2 Neurotransmitters

3.2.2.1 Magnetic field exposure and transmitter release

Because classical transmitters are removed quickly from the synaptic cleft, as is mentioned in Chapter 2, no method is available to directly and instantly measure *in vivo* the amount of transmitter released from a nerve terminal. Therefore, only a very few studies, which have used *ex vivo* or *in vitro* approaches, have been reported possible effects of magnetic field exposure on transmitter release.

Dixey and Rein (1982) used PC12 cells, which were derived from a transplantable rat pheochromocytoma, to examine the effects of magnetic field exposure on transmitter release. PC12 cells synthesize, store, and release dopamine, noradrenalin and acetylcholine (ACh). Magnetic field exposure was pulsed at 500 Hz, using either 160 or 850 μT, for 15 minutes. During the exposure period, ^{3}H-NA release was elevated by 28%, as compared to the control value.

Rosen (1992) studied the effects of a static magnetic field on the frequency of miniature end-plate potentials (MEPPs) recorded from a rat phrenic nerve and diaphragm preparation. MEPPs, which are spontaneously occurring, small (about 0. 5 mV) depolarizing potentials recorded from the post-synaptic membrane of the end-plate region of skeletal muscle, are thought to reflect random quantum release of ACh from the synaptic vesicles of the nerve terminal. In the presence of a 120 mT field, statistically significant changes in MEPP were observed. There was a modest increase in frequency of MEPPs at temperatures at and below 34°C, and a prominent decrease in frequency of MEPPs at temperatures above 35°C. This temperature-dependent phenomenon was not seen in the absence of Ca^{2+} in the perfusate. The author interpreted these results as an example of the phase transition temperature where the diamagnetic anisotropy of the presynaptic membrane is sufficient to influence neurotransmitter release by altering the function of the transmembrane Ca^{2+} transfer mechanism. Although not mentioned, the author probably avoided ac magnetic field exposure due to technical difficulty of recording MEPPs under ac magnetic field. Strong induction of ac fields into the recording system is a common difficulty.

These two studies suggest that the transmitter release is affected by magnetic field exposure *in vitro* in the first study and *ex vivo* in the second study.

3.2.2.2 Magnetic field exposure and transmitter receptors

Several papers have been published dealing with the effects of magnetic field exposure on various types of receptors in brain. It is noted here that all the receptors to which any effects of magnetic field exposure have been recognized are classified

as metabotropic type (see Table 2.1). The one exception is reported involving an NMDA-class glutamate receptor (Kole et al. 1999).

Lai et al. (1993) found that Sprague-Dawley rats exposed to 60 Hz, 0.75 or 2.0 mT horizontal, magnetic field for 45 minutes had decreased cholinergic activity in the frontal cortex and hippocampus - in an apparent dose-dependent manner - as measured by sodium-dependent, high-affinity choline uptake (Fig. 3.1). The author further investigated whether these magnetic-field-induced decreases were mediated by endogenous opioids present inside the brain. The effect was blocked by treating the animals before magnetic field exposure with the centrally acting opiate antagonist naltrexone, whereas treatment with the peripheral opiate antagonist naloxone methiodide was ineffective.

In a subsequent experiment Lai and Carino (1998) studied the involvement of opiate receptor subtypes, finding that both μ- and δ-opiate receptors in the brain are involved in the magnetic field-induced decreases in cholinergic activity in the frontal cortex and hippocampus. The authors indicate that magnetic fields activate endogenous opioids in the brain, which in turn causes a decrease in cholinergic activity, although it is not known how magnetic field exposure affects the endogenous opioid system. It is well known that endogenous opioids modulate the activity of cholinergic systems in the brain. Lai and Carino (1999) investigated the effects of different exposure intensities and durations on cholinergic activity in the frontal cortex and hippocampus of the rat. When the rats were exposed for 60 min at 0.5, 1.0, 1.5, or 2.0 mT, a significant decrease was observed only after exposure to 2.0 mT. When the exposure was for 30, 45, 60 or 90 min at 1.0 mT, a decrease ($P < 0.01$) was found only after 90 min exposure. In a further experiment, when the exposure duration was extended to 3 hours at 0.05, 0.5, or 1.0 mT, a significant decrease ($P < 0.05$ or $P < 0.01$) was observed after exposure to all three magnetic flux density. These results indicate that the intensity and duration of exposure interact.

Several investigators have searched for biological data to support the clinical findings described in section 3.2.1., focusing on animal studies of neurotransmitters and receptors in various regions of the brain. Kole et al. (1999) reported that a single TMS of 120 A/μsec for 3 sec at 20 Hz caused specific and substantial increase of $5HT_{1A}$ (serotonin, 5HT) receptors in the frontal cortex (layers 5-6), cingulate cortex (layers 1-3), and anterior olfactory nucleus along with increased NMDA receptors in the hippocampal CA1, CA3, and dentate gyrus 24 hours after stimulation of rat brain. As no change in 5HT uptake was found in either the dorsal raphe region or the rest of the brain, the authors claim non-specific effects of the experimental procedure on 5HT uptake sites were absent.

Ben-Shachar et al. (1999) investigated the effects of rTMS on brain monoamine levels and their receptors of Sprague-Dawley rats. Daily treatments consisting of 52 stimuli were applied to the head at 15 Hz for 3.5 sec at 2 Hz for 10 days. Noradrenalin, dopamine, dihydroxy-phenylalanine, homovanillic acid, 5HT, and 5-hydroxyindolacetic acid in frontal cortex, striatum, hippocampus and midbrain showed no changes after the rTMS. β-adrenergic receptors were up-regulated in the frontal cortex, down-regulated in the striatum, and unchanged in the hippocampus. $5\text{-}HT_2$ receptors were down-regulated in the frontal cortex but were not changed in

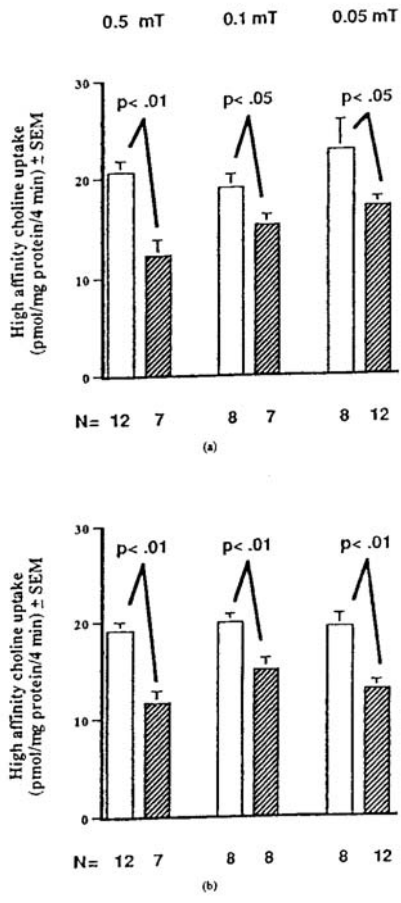

Fig. 3.1. High-affinity choline uptake in (a) the frontal cortex, and (b) the hippocampus of rats after 3 hrs of exposure to a 60 Hz magnetic field at 0.5, 0.1, or 0.05 mT. Shaded bars are for magnetic field-exposed rats, and open bars are for "bucking" mode controls (Lai and Carino 1999).

the other brain areas. No changes were demonstrated in benzodiazepine receptors in the frontal cortex and cerebellum. These findings demonstrate specific and selective alterations can be induced by rTMS.

Serotonergic neurotransmission is considered to be a neuromodulatory system interacting with other neurotransmitters. Various specific 5-HT receptors, at least 14, mediate serotonergic function. Among them is the 5-HT_{1B} receptor subtype located on serotonergic (autoreceptor) and non-serotonergic (heteroreceptor) terminals where it regulates the release of the corresponding neurotransmitter. Massot et al. (2000) carried out *in vitro* experiments to see if 5-HT_{1B} receptors were sensitive

to 50 Hz magnetic fields exposure. Rat and guinea pig brain cortices were exposed eight different magnetic field intensities, ranging from 0 to 2,500 μT. They found that magnetic flux densities above 400 μT specifically interacted with 5-HT_{1B} receptors (Fig. 3.2). At the molecular level, the effect of magnetic field essentially corresponds to a decrease of the affinity of the natural agonist (5-HT) for 5-HT_{1B} receptors. This change is reversible, because the effect disappeared when magnetic field exposure ceased. It also appears to be very specific to the 5-HT_{1B} receptors because no other receptor binding measures tested were affected by magnetic fields.

Kavaliers et al. (1998) studied the effects of exposure to a 60 Hz, horizontal, magnetic field of 141 μT for 30 min on male CF-1 mice. The investigators assessed whether field exposure affected nociceptive responses, which were determined using a 'hot plate' test. Individual mice were placed on a metal surface maintained at 50 ±0.5°C, and the latency of the foot-licking response was recorded. Compared with sham exposure, field exposure had no significant effect on the nociceptive response. However, exposure to the 60 Hz magnetic field significantly reduced the analgesic effects of the centrally produced neuroactive steroid, 3α-hydroxy-4-pregnen-20-one (3αHP). This inhibitory effect on analgesia involved alterations in the functioning of calcium channels, because the effects of 3αHP have been shown to involve, at the membrane receptor level, alterations in Ca^{2+} flux, the functioning of calcium channels, and Ca^{2+}-activated protein kinase C (e.g., Dhanvantrai and Wiebe 1994).

These results extend the prior findings of significant antagonistic effects of ELF magnetic fields on opioid-induced analgesia (Ossenkopp and Kavaliers 1987). The authors raise the possibility that ELF magnetic field might, in part, exert their actions through effects on diverse, neuroactive, steroid-modulated processes.

Zecca et al. (1998) performed an experiment to see if combined electric and magnetic field exposure for 8 months, which is about one-third of the rats' life span, caused any changes in dopamine, noradrenalin, 5-HT, μ-opioid receptors, and D-2 dopamine receptors in brains of Sprague-Dawley rats. Two combinations of 50 Hz fields were tested: 5 μT + 1 kV/m, and 100 μT + 5 kV/m. Frontal cortex, parietal cortex, striatum, hippocampus, hypothalamus, cerebellum and pineal gland were assayed. No differences were found between sham-exposed and field-exposed groups in the concentrations of noradrenalin, dopamine and its metabolite (DOPAC), 5HT and its metabolite (5-HIAA) and D-2 dopamine receptors. However, an increase of the μ-opioid receptor was found in the hippocampus and a decrease was observed in the frontal cortex. In the parietal cortex an increase occurred at the lower field strength, while a decrease was observed at the higher strength. No changes of μ-opioid receptor activity were observed in the other areas.

Calvo and Azanza (1999) performed experiments using single neurons of the subesophageal ganglia of a snail (*Helix aspersa*). Fields (50 Hz of 1–15 mT) were applied for 1 min at time intervals of 1 min. Neuronal action potentials were recorded by an intracellular microelectrode. With or without magnetic field exposure, test ganglia were bathed with Ringer solutions containing different concentrations of Ca^{2+}, K^+, caffeine, glutamate or ACh, and the neuronal responses were analyzed. The 50 Hz magnetic fields did not modify synaptic activity induced by glutamate or ACh. When calcium concentration in the bathing solution was decreased, the firing

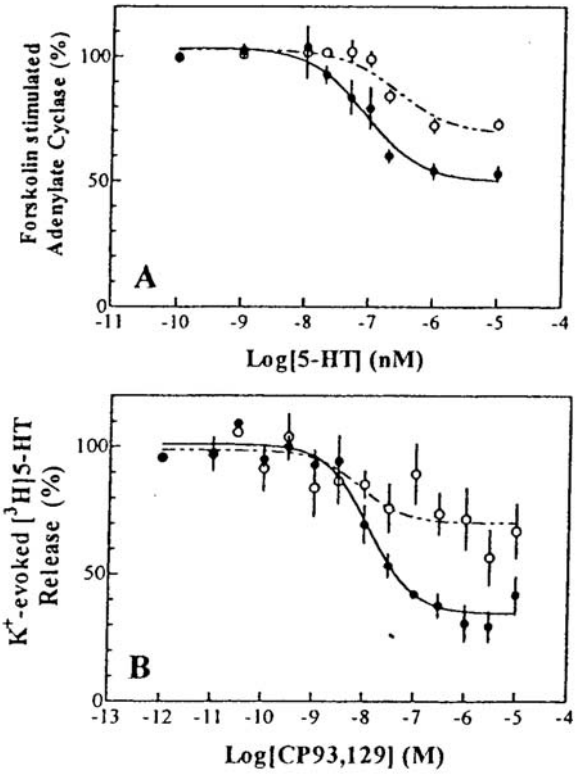

Fig. 3.2. Effects of 50 Hz magnetic fields on 5-HT_{1B} activity The dashed line in each portion is for the control group, and the solid line is for experimental (magnetic field exposure) group. (A) 5-HT_{1B}-dependent adenylate cyclase. Adenylate cyclase activity was measured in h5-HT_{1B} CHO-transfected cell membrane by determining [^{32}P]cAMP formation. Open circles are exposed, and filled circles are without exposure. (B) Effect of 50 Hz magnetic fields on the synaptosomal release of 5-HT. Rat brain synaptosomes were loaded with 20 mM [^{3}H]5-HT before 5 min of stimulation with K^+. (Massot et al. 2000)

frequency of the neurons decreased during magnetic field exposure. This suggests that the magnetic field effect on the neuron was mediated by a calcium-dependent metabolic process.

3.2.3 Pain

Pain threshold increases during night and decreases during daytime. Jeong et al. (2000) investigated, using the hot plate test, the effects of exposure of magnetic fields

(60 Hz, 2 mT, for 24 h) on pain thresholds in mice. Magnetic field exposure inhibited the nocturnal increase of pain thresholds; it also produced hyperalgesia at daytime. The increase of pain thresholds induced by melatonin given during the day was inhibited by exposure to magnetic fields or by the opioid antagonist naloxone. The magnetic field and naloxone synergically inhibited hypoalgesia produced by melatonin. The daytime hyperalgesia after magnetic field exposure was potentiated by the benzodiazepine agonist, diazepam, and inhibited by the benzodiazepine antagonist, flumazenil. These results suggest that exposure to 2 mT, 60 Hz magnetic fields inhibits the increase of pain threshold at night and produces hyperalgesia during the day, operating with the involvement of opioid and benzodiazepine systems.

Choi et al. (2003) also investigated whether exposure to 60 Hz, 1.5 mT magnetic field affects the normal diurnal rhythm of the pain threshold in mice. An increase of pain threshold on the hot plate test was observed during night compared to that during daytime. This rhythm was attenuated by both (1) constant exposure to light for 5 days, by decreasing the night time threshold to the daytime level, and (2) constant exposure to dark for 5 days, by increasing the daytime threshold to the level of night. Under dark exposure, the diurnal rhythm in pain threshold was restored when mice were exposed to the magnetic field for 12 h daily for 5 days. Exposure during night did not restore the rhythm. The authors mentioned that ELF magnetic field exposure might participate in the diurnal rhythm of pain threshold by acting on the system that is associated with the environmental light-dark cycle.

Kavaliers and colleagues also have noted that the exposure to ELF magnetic fields affects pain threshold and behavioral activity. Kavaliers and Ossenkopp (1986) reported that exposure to a 0.5 Hz rotating magnetic field (0.15 – 9 mT) for 60 min reduced the day-time analgesic effect of morphine in CF-1 mice. Intracerebroventricular injections of a calcium chelator, EGTA, blocked the effect, and administration of a calcium ionophore, A23187, potentiated the inhibitory action. These results suggest that exposure to magnetic field may alter morphine-induced responses in mice, in a manner comparable and consistent with effects on Ca ion and possibly other divalent ions. Kavaliers et al. (1998) further showed that a brief exposure of male mice to an ELF magnetic field (60 Hz, 141 μT peak, for 30 min) significantly reduced the analgesic effects arising from intracerebroventricular administration of the centrally produced allylic neuroactive steroid, 3α-hydroxy-4-pregnen-20-one (3αHII). They also showed that the dihydropyridine (DHP) calcium channel antagonists, dilitazem and nifedipine, block the inhibitory effects of the 60 Hz ELF on 3αHP-induced analgesia. These results suggest that exposure to a 60 Hz magnetic field affects the analgesic effects of neuroactive steroids such as 3αHP through alterations in calcium channel function. They claim that these effects were not caused by induced currents (Prato et al. 1995), because when the time-varying aspect of the magnetic field was kept constant but the other aspects were altered, the biological effect varied. These phenomena can be explained in a manner consistent with the predictions of Lednev's parametric resonance model (Lednev, 1991). Prato et al. (2000) investigated the effect of 30 or 60 Hz magnetic field exposure on avoidance behavior of snails. When the magnetic fields were set to parameters for the predictions of Lednev's parametric resonance model for the calcium ion, they observed either (1) reduced, (2) unaffected,

or (3) increased endogenous opioid mediated analgesia. This model is discussed in Chapter 8.

3.2.4 Electroencephalogram

Bell et al. (1992) examined the effects of magnetic field exposure on human electroencephalogram (EEG) activity. The head and chest of each 10 volunteers and 10 neurological patients were exposed in a series of 2 sec "on" and 5 sec "off" trials to either: 1) 78 μT static magnetic field; 2) 78 μT, 60 Hz magnetic field; or 3) static + 60 Hz fields simultaneously. EEG activity during the exposure period was compared with that in the preceding no-exposure period by obtaining the power spectrum of numerous EEG bands, each 0.5 Hz in width. The authors suggested that magnetic field exposure resulted in some changes in the central nervous system, because there were changes in the EEG associated with magnetic field exposure.

Lyskov et al.(1993) performed double-blind studies of the effects of continuous and regularly intermittent (1 sec "on" and 1 sec "off") exposure to ELF magnetic fields on EEG activity in 17 male and 17 female volunteers. The head was exposed for 1 h to a 45 Hz, 1.26 mT magnetic field. The authors report decreased activity (EEG power) in the lower frequency bands (δ and θ) and increased activity in the higher frequency bands (α and β).

EEG characteristically is quite sensitive to changes in internal and external situations, such as fatigue, sleep, body movement, emotional changes, visual stimuli, noise, thinking, level of consciousness, etc. It is not easy to control and equalize these factors between the control and experimental groups. Also, the multivariate data analysis required to examine many frequency bands is complex and difficult to interpret. For these reasons, the small set of EEG results, although intriguing, is not very informative.

3.2.5 Evoked potentials

3.2.5.1 Sensory-evoked potentials

Lyskov et al. (1993) reported no effect on the P_{200} potential of the auditory evoked potential (EP) of rats after regular, intermittent (on vs. off periods f 15 min) exposure to 45 Hz, 1.26 mT magnetic fields for 24 hours. Graham et al. (1999) found no effect on auditory brainstem EPs, visual EPs elicited by pattern reversal stimuli, or somatosensory EPs. The subjects, 18 men and 18 women, were field-exposed or sham-exposed for 45 min to either 60 Hz magnetic fields of 14.1 μT or 28.3 μT.

These two studies suggest that ELF magnetic field exposure has little influence on the peripheral and central nervous functions that are related to sensory evoked potentials.

3.2.5.2 Event-related potentials

Crasson et al. (1999) studied the influence of 50 Hz magnetic field exposure on human performance and psychophysiological parameters. The healthy adult male sub-

jects were asked to perform several kinds of psychological tests while event-related potentials were recorded from frontal (Fz), central (Cz), and parietal (Pz) regions of the scalp. A 100 μT magnetic field was applied to the head of the subjects for 30 min (continuous or regularly intermittent, with 15 s on/off cycles), and the results from sham-control and field-exposed sessions were compared. Psychological tasks used included dichotic listening task, auditory and visual "oddball" tasks (discrimination of a novel stimulus), memory tasks (Rey auditory verbal learning task and digit span tasks), etc. They found continuous exposure produced (1) a decrease of N1 amplitude of event-related potentials recorded from frontal region, (2) increased P2 latency at Fz and Cz, and (3) increased RT on the visual "oddball" paradigm.

Although the few differences observed were small and transitory, the results seemed to be specific to some aspects of cognitive function of the human brain. Initial research examining many endpoints and finding a few effects always is problematic. Replication is needed to see if the same or if different endpoints are affected in a second (identical or highly similar) experiment.

3.2.6 Perception of electric and magnetic fields.

In mammals, including the human, there exist many types of specifically developed sensory receptors - such as the rods and cones of the retina that signal light stimuli, the hair cells in the inner ear that sense sound stimuli, the Pacinian corpuscles in the limbs that detect vibration, the baroceptors in the aorta that are sensitive to blood pressure changes, and the receptors on the tongue that provide taste sensation and others. Besides the natural stimuli that elicit activity of each receptor type, electrical stimulation also can activate effectively all of these receptors. Some kinds of fish, e.g., eel, skate and shark, possess electroreceptors that are called ampullary lateral line organs. These electroreceptors sense disturbance of electric field surrounding the fish (Chichibu 1970, also see Fig 1.13). However, there is no evidence indicating the existence of specific receptors in mammalian body that are sensitive solely to electricity.

Mammals, including humans, can detect an alternating current electric field because of hair movement that stimulates the hair follicle receptors of the skin (Cabanes and Gary 1981), causing a kind of tactile sensation with a threshold around 10 kV/m (Kato et al. 1986: 1989). Hair movement might not be a sole mechanism for electricity perception since some people claim to feel 60 Hz electric field on palms of hand. However, to the best knowledge of the present author, there is no published paper on this aspect.

Sensitivity to the Earth's magnetic field is widespread among reptiles and birds. Both behavioral and neurophysiological evidence suggests that there might be two magnetoreception mechanisms present in some vertebrates, one system functioning as a source of directional compass information and the other providing a geographic position ("map") information based on geomagnetic cues (Phillips and Deutschlander 1997). However, there is no known specialized 'magnetoreceptor' whose location and structure have been confirmed anatomically and functionally (Phillips 2005).

Contrary to perception of electric field, no literature has been reported that human can detect magnetic field. For example, Schmitt and Tucker (1978) could not verify that human perceived magnetic field sensation with 0.7 – 1.5 mT, 60 Hz magnetic field exposure.

3.2.7 Kindling

Kindling is an augmented state of neural firing, a sort of epileptic reaction, that following repetitive electrical stimulation at a certain intervals with sufficient stimulus strength to produce after-discharges of the neurons at the site of stimulation. Frontal cortex, temporal cortex, amygdala, hippocampus, and septum usually are selected for stimulation. This phenomenon, first reported by Goddard et al. (1969), subsequently has been established as an animal model of epilepsy, based on detailed behavioral observations and EEG studies by Wada and colleagues (Wada and Sato 1974, Wada 1976).

Kindling, which is not based on morphological changes of the brain, is characterized by lasting enhanced functional changes of information transmission via synapses. This process has been confirmed to develop in all animal species so far studied, such as cat, crocodile, frog, monkey, mouse, rabbit, and rat. There is a tendency for kindling to be acquired faster in phylogenetically lower species and to require many repetitions of stimulation in higher species. The preferable frequency is 50 or 60 Hz, with either rectangular or sinusoidal waveforms. Stimulus strength to elicit after-discharges of the neurons typically is determined by raising strength with steps of 25 or 50 μA. The weakest stimulus to elicit the after-discharges is called the after discharge threshold, which varies depending on animal species and stimulation sites. Stimulus duration usually is for 1 sec, and the interval between successive stimulation tests is either 12 or 24 hours (Sato and Akiyama 1984).

Kindling acquisition is classified into five stages in most animal species four stages are recognized in rhesus monkey, and six stages are identifiable in cat and rabbit. Characteristic features of the stages are: Stage I, mouth and facial movement; Stage II, head nodding; Stage III, head turning, with extension of contralateral forelimb or unilateral convulsion; Stage IV, seizure generalization, without postural loss; Stage V, generalized seizure, with postural loss. Stimulation number to reach the stages differs depending on animal species. For example, in baboon about eight repetitions are required for Stage I (8 days when the stimulation interval is 24 hours), 22 repetitions for Stage IV, and 72 repetition for Stage V.

Ossenkopp and Cain (1988) reported that exposure of male Long-Evans rats to a 60 Hz, 100 μT magnetic field for 1 hr prior to each daily brain stimulation with 200 μA, which was well above after-discharge threshold for all the subjects, resulted in an increase in the mean number of after-discharges required to reach each of the five stages of the kindling process and in a significant reduction in after-discharge duration in each stage. These data suggest a weak retardation of kindling in the group exposed to a 60 Hz magnetic field.

Potschka et al. (1998) studied effects of 50 Hz magnetic fields on kindling acquisition and fully kindled seizures in female Wistar rats. In their chronic experi-

ments, rats with electrodes implanted in the basolateral amygdala were exposed to either a 100 μT magnetic-field or a sham-field condition before and after onset of daily electrical stimulation over an 8-week period of kindling development. The pre-kindling after-discharge threshold was increased by magnetic field exposure. Fully kindled rats given acute exposure (1 to 2 hours) to 50 Hz, 100 μT magnetic fields had no changes in after-discharge threshold or seizure parameters recorded at the after-discharge threshold. These data indicate that chronic exposure of rats to magnetic fields exerts weak inhibitory effects on some seizure parameters of the kindling model.

In summary, these studies suggest that ELF magnetic field exposure can interact with the process by which electrical stimulation of the brain produces kindling. However, the mechanisms involved are not clear. This could be a fertile area for future research.

Although the observed phenomenon is different, it is interesting to mention here that Rogers et al. (1995b) reported a suppressive effect of magnetic field exposure (60 Hz: with 50 μT and 30 kV/m or 100 μT with 60 kV/m) on electric-field-induced work stoppage in baboons performing two tasks: (1) a compound operant schedule consisting of fixed ratio 30 and differential reinforcement of low rate 20 components, and (2) DRL20 visual match-to-sample task.

3.3 Development and Regulation of the Cell Axis, Neurite Growth, and Nerve Regeneration

3.3.1 Regulation of the cell axis

During morphogenesis in the vertebrate embryo, a DC (0 Hz) voltage gradient of 0.5 to 1 V/mm exists across the wall of the early neural tube, and this gradient is required for normal cranial development (Shi and Borgens 1994, Borgens and Shi 1995). Controlling cell division is fundamental to normal development, and the regulation of the axes of cell division is considered to have major morphogenetic impact. Appropriate cell-cell contact directs the orientation of mitotic spindles (Goldstein 1995), whereas chemotaxis modulates cell migration (Parent et al. 1998). Naturally occurring electric fields also orient and direct cell migration (Zhao et al. 1999).

In adult myelinated nerve, Schwann cells divide along a mitotic spindle oriented parallel to the nerve. Mitosis is controlled, in part, by electrical activity of the nerve, because when it is inhibited by using tetrodotoxin, cell division is inhibited (Martin and Webster 1973). Action potential activity in myelinated nerves uses saltatory conduction between nodes; this involves extracellular current flow. Periodic propagation of nerve impulses will create pulsed, extracellular electric fields oriented parallel to the nerve, which could influence mitotic spindle orientation of Schwann cells.

Song et al. (2004) studied how electrical cues regulate the orientation and frequency of cell division and the rate of wound healing *in vivo* using the cornea of the rat as the model. The mammalian cornea establishes an internally positive transcorneal potential DC difference of +40 mV by pumping Na^+ and K^+ in and Cl^-

out. Corneal epithelial wounds were made through the whole epithelium. The authors found that: (1) the axis of cell division was oriented at the edge of a wound, (2) orientation declined with distance from the edge, (3) increasing the wound-induced electric field increased orientation and decreasing the electric field decreased it, and (4) healing was faster when the wound electric field was increased and slower when it was decreased, and (5) the proliferation of epithelial cells was regulated by the wound-induced electric field.

3.3.2 Neurite growth

When a DC voltage gradient is applied across a culture chamber (Jaffe and Poo 1979), neurites grow towards the negative electrode (cathode) and away from the positive electrode (anode). Borgens et al. (1981) reported that severed lamprey reticulospinal tract in the spinal cord regenerated toward the cathode in an *in vivo* study.

3.3.3 Nerve regeneration

Transected axons within the spinal cord of the guinea pig can regenerate in the presence of an extracellularly applied electric field (Borgens et al. 1986). Borgens (1999) further studied the effect of extracellularly applied electric field on regeneration of damaged spinal cord axons. The DC electric field (100 μV/mm) was imposed within a hollow silicon rubber tube implanted into the damaged spinal cord for 3 wks. A robust regeneration of axons into the tube was observed, providing evidence for not only the facilitated regeneration of adult mammalian central nervous axons but also for their guidance by an applied DC electric field.

Levi-Montalcini (1952) first discovered nerve growth factor (NGF) in mouse sarcoma cells. Extensive studies since then have revealed that NGF belongs to neurotrophin family within a wider concept of neurotrophic factors (NTFs). NTFs are a group of proteins involved in the process of differentiation and growth of neurons, maintenance of nerve function, synaptic plasticity, and nerve regeneration. Subsequently it has been discovered that NTFs have many other important functions in other cell systems, including immune function.

In the normal animal, NGF is present in low concentration in serum and is produced and released by target cells. Neurites elongate following concentration gradients of NGF and eventually reach the target cells. Once synapses are established between neurites and the target cells, NGF is transported retrograde from the target cells to the nucleus of the neuron (Heumann et al. 1984). This action of NGF, which is most conspicuous in sympathetic nerves, is maintained lifelong. In sensory nerves NGF appears to play an important role during periods of early developmental stages.

Axotomy triggers several cellular and intracellular processes that constitute the early events of regeneration. Sciatic nerve has been used extensively for study of the regeneration process. NTF-producing-cells are amply distributed within such tissues as target cells of the axon, nerve ganglia (aggregate of nerve cell bodies), Schwann cells of myelinated nerve fibers, and fibroblasts. When the nerve is damaged, production of NTFs - such as NGF, brain-derived neurotrophic factor (BDNF), and leukemia

inhibitory factor (LIF) – is facilitated within Schwann cells and fibroblasts in the distal side of the damaged nerve. Hence the concentration of NTFs is higher on the distal side. As a consequence sprouting of the axon from the proximal end of the damaged nerve is facilitated, and elongation to the distal end follows according to concentration gradients of the NTFs (Furukawa and Furukawa 2000).

Pulsed electromagnetic field (PEMF) exposure has been reported to promote peripheral nerve regeneration (Sisken et al. 1993). PEMF exposure also facilitates functional recovery, such as walking, over a period of 6 weeks (Walker et al. 1994). Longo et al. (1999) tested the hypothesis that PEMF alters levels of NGF activity in injured nerve and/or dorsal root ganglia neurons during the first stages of regeneration (6–72 hours). Sprague-Dawley rats with a transection of sciatic nerve at mid-thigh were exposed to 0.3 mT, with pulses 20 msec duration given at 2 Hz for 4 h/d for different time periods. PEMF caused a significant decrease in NGF activity in both proximal and distal segments and also in contralateral, non-operated nerves compared to sham-treated unoperated nerves. PEMF-treated proximal nerve segments demonstrated an 18% increase in NGF at 6 hr, followed by decreases of 30% at 24 hrs and 9% at 72 hr.

The findings of Longo et al. (1999) demonstrate that PEMF can affect NGF activity and levels and raise the possibility that PEMF might promote nerve regeneration by amplifying the early, post-injury decrease in NGF activity. This study alone does not create a cause-effect link between PEMF exposure and promotion of nerve regeneration, but it does suggest that NGFs should be further considered as important candidates for mechanisms by which PEMF might influence nerve regeneration and recovery processes.

A high-strength magnetic field (many T) can align the collagen gel. Dubey et al. (1999) used this phenomenon to develop an *in vitro* assay to study neurite elongation into the magnetically aligned collagen gel rods from chick embryo dorsal root ganglia explants placed onto one end of the rods. The depth of neurite elongation from the ganglia neurons into the rods was greater than that observed in controls and increased with an increase in magnetic field strength. DC fields of 0 (control) and 4.7 T to 9.4 T were used. The authors concluded that the use of magnetically aligned collagen gel rods in repairing transected nerves could lead to significant advances in both speed and the functional recovery of regeneration.

PEMF also has been shown to promote neurite outgrowth *in vitro* (Sisken et al. 1990). Macias et al. (2000) applied pulsed magnetic field to dorsal root (sensory) ganglia neurons of rat embryos *in vitro* in order to determine whether the induced current would direct and enhance neurite growth in the direction of the current. In the presence of NGF in the media,neurons exposed to the pulsed magnetic field exhibited asymmetrical growth parallel to the current direction with concomitant enhancement of neurite length. The pulsed magnetic field signals used here were a train of 22 rectangular voltage pulses of 20 μsec duration with 200 μsec between pulses. This pulse sequence was repeated at between 10 and 25 Hz. For the 20 μsec pulse, the induced electric field was 0.25 V/m.

3.4 Endocrine System

3.4.1 Field exposure and endocrine functions

The effects of electric field exposure on a variety of hormones have been investigated since the mid-1970s, when possible biological effects of electric fields first drew attention of researchers. Most of the early research did not yield any consistent positive results.

Wertheimer and Leeper (1979) reported an association between magnetic field exposure and childhood leukemia. At about the same time, Wilson et al. (1981) reported that night-time melatonin secretion was inhibited following exposure to 60 Hz electric fields. Although only a preliminary finding, the observation of an effect on melatonin provided a first plausible mechanism by which power-frequency electromagnetic field exposure might affect the development of cancer. Public concern and research interests rapidly shifted to possible bioeffects of magnetic fields, and studies of melatonin received considerable attention. The pineal gland and its product melatonin are part of the endocrine system. However, given both their importance in research relating to ELF bioeffects and the relatively large volume of work on melatonin is considered in its own section.

3.4.1.1 Melatonin

3.4.1.1.1 Effects of manipulation of geomagnetic field on melatonin

Semm et al. (1980) provided the first evidence that the cells in the pineal gland can respond to stimuli other than light. These researchers demonstrated a significant diminution of *in vivo* electrical activity of single pinealocytes of guinea pig following acute inversion of the vertical component of the Earth's geomagnetic field by means of a Helmholz coil. This finding suggested that pineal melatonin synthesis might be affected by magnetic fields.

Welker et al. (1983) demonstrated that rats exposed to a 15-min inversion of the horizontal component of the Earth's (DC) geomagnetic field during the nighttime hours in the presence of dim red light, had a significantly decreased pineal melatonin synthesis as compared to unexposed control animals. Olcese and Reuss (1986) reported that, in both albino and pigmented rats, melatonin synthesis was markedly inhibited following a single, 30-min exposure to a DC magnetic field stimulus consisting of a 50° rotation of the Earth's horizontal geomagnetic field.

3.4.1.1.2 Effects of 60 Hz electric fields on melatonin in rodents.

Wilson et al. (1981, 1986) reported that the normal nocturnal melatonin peak in male rats was greatly reduced, after 21 days of exposure to 60-Hz electric fields of between approximately 2 and 40 kV/m. Reiter et al. (1988) demonstrated melatonin reductions in rats exposed to 10, 65, or 130 kV/m electric fields *in utero* through weanling. These studies suggested an all-or-none, rather than a graded, dose-response effect.

Other investigators soon reported that extremely low frequency magnetic fields also could inhibit melatonin.

3.4.1.1.2.1 Assessment of melatonin concentration Based on experiments using rats, mice or hamsters, there have been many reports that ELF magnetic field exposure inhibits melatonin production. Kato et al. (1993: 1994abc: 1999) completed 21 experiments in which melatonin was assayed. To date, this is the largest set of melatonin and power-frequency field experiments completed by any single research group. Conducted between September of 1989 through February of 1996, 18 experiments involved simultaneous magnetic field and sham exposure. In addition, three complete 'negative control' experiments were conducted in which the exposure coils were not activated. These were spread out during the research program, providing 'historical control' data for comparison with data of other experiments. Albino Wistar-King rats were used for 19 exposure experiments, and pigmented Long-Evans rats were used for the remaining two experiments.

Among the 18 exposure experiments, Kato et al. performed five using a circularly polarized, 50 Hz field, with the experimental group at 1.4 μT_{rms}. The rotating plane was perpendicular to the horizontal component of the geomagnetic field. In each of these experiments, the sham-exposed control group was exposed to 0.02 μT_{rms}. The intent here was to establish across time the continuing efficacy of the critical 'positive control' experiment.

In the initial two experiments, samples were collected every 4 hr, meaning the sample sizes per time point – typically six or seven – were smaller that the later experiments in which samples were collected every 12 hours. In the later 19 experiments the sample size was about 24 per group per time point. In all experiments after the initial two, only daytime (at 12:00 h) and nighttime (at 24:00 h) samples were obtained. A 12:12 light-dark cycle was used; lights were turned on at 06:00 h and turned off at 18:00 h. Melatonin was assayed from both plasma and pineal gland.

Figure 3.3 presents data obtained from 7 μT and 350 μT_{rms} circularly polarized field exposed groups and a sham-exposed control group. For the field-exposed subjects, the plasma concentrations were reduced at nearly all timepoints Similar results were obtained from pineal gland melatonin concentration.

Figure 3.4 shows the combined control (no exposure) data and the results of three different exposure experiments. In general, any 50 Hz magnetic field intensity exceeding 1.4 μT_{rms} produced a suppression of melatonin in both pineal gland (shown) and plasma (not shown). Furthermore, the degree of melatonin suppression was the same for magnetic field intensities between 1.4 and 350 μT_{rms}. These results suggest a sharp, all-or-none (i.e., step-function) relationship, rather than a more gradual dose-response relationship, between magnetic field intensity and melatonin suppression.

To test if differences of exposure parameters were important, the effects of a 50 Hz, ellipsoidal as well as linearly polarized magnetic field exposure on plasma and pineal gland melatonin concentration were investigated, using the same species and a consistent protocol within Kato's laboratory (Kato et al. 1994abc, 1999). To assess the effect of orientation of the circularly polarized field, one experiment was completed in which the rotating plane was parallel to the horizontal component of the geomagnetic field (Kato et al. 1994b). The experimental group received 1.4 μT_{rms}, and the sham-control group received 0.02 μT_{rms}. To test the effects of different expo-

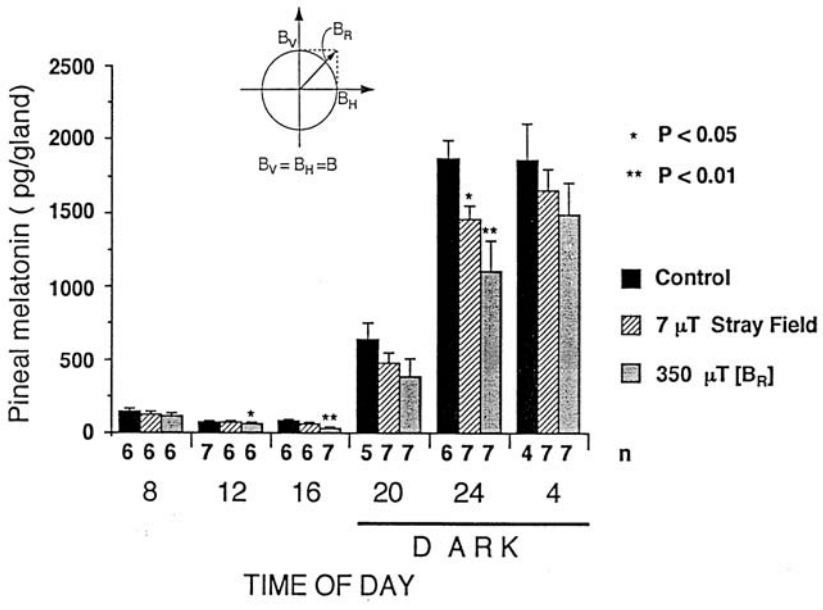

Fig. 3.3. Pineal melatonin concentrations of sham-exposed and magnetic-field-exposed rats differ considerably through the light:dark cycle. The period of darkness, 18:00–6:00 hours, is indicated. Means and standard errors (SEs) are shown, and number of samples (n) is indicated. Stars indicate time-points at which the difference from the control value is statistically significant (Kato et al. 1993.)

sure parameters, elliptical magnetic fields were exposed in four experiments (Kato and Shigemitsu 1997): in two exposure experiments an elliptical field, for which the ratio of major vs. minor axes was 2:1, was used; in the other two experiments, the ratio was 4:1. Thus, the comparison is between a relatively circularly 50 Hz magnetic field and a rather oval-shaped 50 Hz magnetic field. The intensities were at 1.4 and 7.0 μT_{rms}. For the ellipsoidal magnetic field exposure, when the ratio of major versus minor axes was 2:1, there was a reduction of melatonin at both 1.4 μT_{rms} and 7.0 μT_{rms} (Fig. 3.5). However, there was no effect on pineal function when the ratio was 4:1 at either 1.4_{rms} or 7.0 μT_{rms}. When the magnetic field was linearly polarized, horizontal fields at 1.0 and 5.0 μT did not induce melatonin suppression, and the vertical field at 1.0 μT did not produce the effect (Kato et al. 1994abc). On this point, Löscher et al. (1994) reported that serum melatonin was suppressed after 8–9 weeks of 50 Hz vertical magnetic field exposure, at strength of 0.3–1.0 μT, in Sprague-Dawley rats that had been pretreated with DMBA. Different lengths of exposure period or species difference might account for the different outcomes.

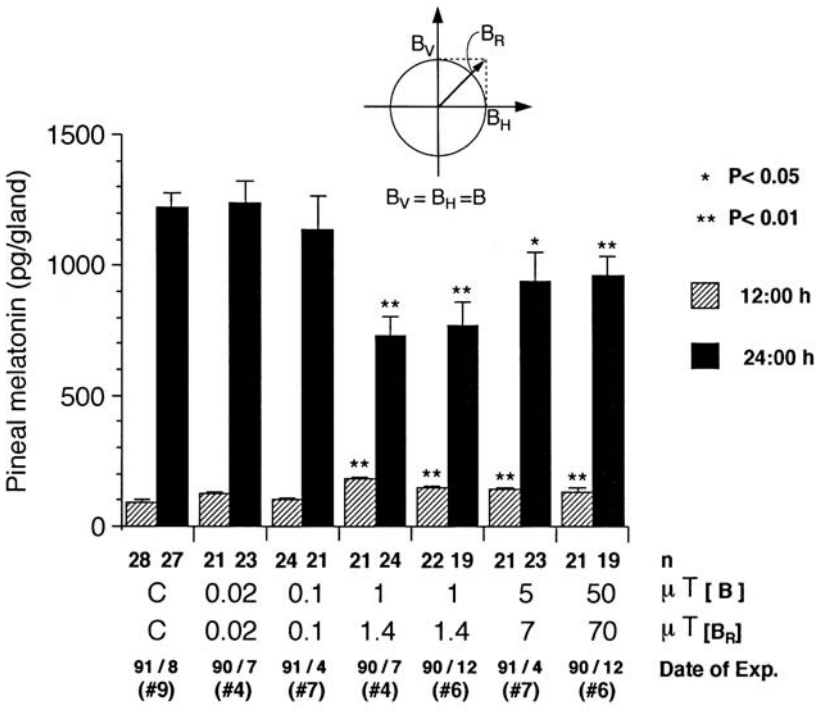

Fig. 3.4. Melatonin concentrations in the pineal gland at 12:00 (light on) and 24:00 (light off) hours at different flux densities of a 50 Hz, circularly polarized magnetic field. The experiment was repeated four different times during two calendar years. Significant decreases of melatonin concentration always occurred with magnetic fields stronger than 1.4 μT (Kato et al. 1993).

Also to test the effects of polarization of the magnetic fields, a vertical field of 1.0 μT and horizontal fields of 5.0 μT and 1.0 μT were used. The sham-exposed groups received 0.1 or 0.02 μT, respectively.

Some authors, such as Yellon (1994), have noted that positive results in the initial melatonin suppression experiments have not been substantiated as more experiments were completed. In contrast to the six-week experiments of Kato et al., Yellon used single 15-minute exposures. As possible explanations, variables such as (1) age or species of subjects; (2) assay methodology; 3) exposure parameters, such as duration, field intensity, presence of transients, and vector alignment; and (4) photoperiod or season, have been offered (Rogers et al. 1993). Because of such concerns about repeatability, Kato et al. (1993) conducted replicate experiments. They confirmed the effect in four out of the five experiments, showing good reproducibility of the effect.

In summary, Kato et al. have established convincingly that nocturnal melatonin concentration is decreased at the end of a 6-week exposure period. They also have

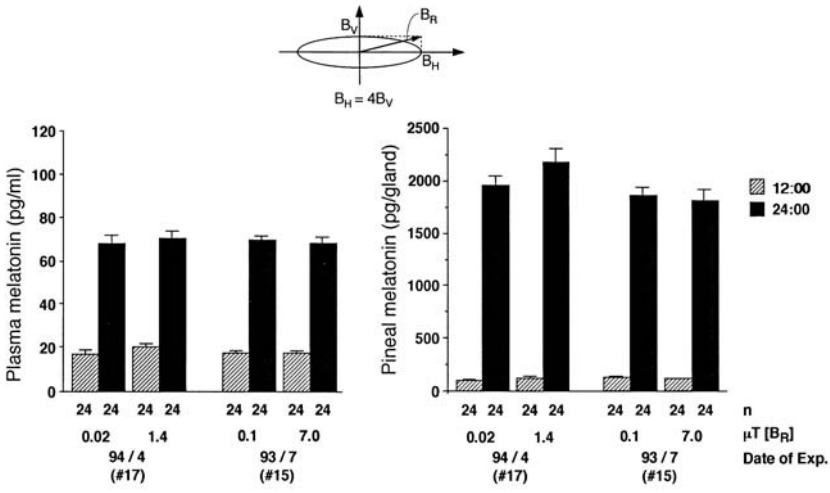

Fig. 3.5. An elliptically polarized magnetic field, with an axis ratio of 4:1, does not produce melatonin suppression. Plasma (left) and pineal gland (right) melatonin contents are shown. There are no statistically significant differences (Kato and Shigemitsu 1997).

shown that melatonin has returned to its normal concentration at both 1 and 4 wks after cessation of exposure, indicating the magnetic field-induced suppression effect is both short-lived and fully reversible (Kato et al. 1994a). Lynch et al (1984) compared thresholds of light intensity required to suppress melatonin contents of both pineal gland and plasma for albino Sprague-Dawley and pigmented Long-Evans rats, anticipating the 'the albino's inability to attenuate light impinging on its retina would increase its sensitivity to the photic suppression of pineal melatonin content and of circulating melatonin levels'. They found, however, that the pigmented Long-Evans strain is 5-fold more sensitive to light; threshold intensities for melatonin suppression were 0.022 μW/cm^2 in the Long-Evans versus 0.110 μW/cm^2 in the Sprague-Dawley strain.

These results prompted Kato et al. (1994c) to compare the effects of ELF magnetic field exposure on pineal function of pigmented and albino rats. Pigmented Long-Evans rats were exposed to a circularly polarized, 50 Hz magnetic field for 6 wks to contrast effects with those observed in the albino Wistar-King strain. Magnetic field exposure at 1.4 μT significantly reduced melatonin contents of both plasma and pineal gland, indicating the 50 Hz, circularly polarized magnetic field is just as potent in pigmented rats as it is with an albino strain.

Taking into account both these results with magnetic fields and the results of Lynch et al. (1984) with light stimulation, it would be reasonable to assume that some common, or similar, mechanism exists between photo- and magneto-sensitivity. However, it also is possible that pigmented and nonpigmented rat strains differ in

other important respects besides pigmentation that complicate elucidation of the photoreceptor and magnetoreceptor inter-relationship.

As noted above, Yellon (1994) observed exposure to a 100 μT, 60 Hz magnetic field for 15 minutes beginning 2 hours before lights off decreased and/or delayed the night-time rise in the melatonin rhythm of adult Djungarian hamsters.

Selmaoui and Touitou (1995) investigated the effects of duration and intensity of magnetic field exposure on pineal function of male Wistar male rats. The magnetic fields used were 50 Hz, sinusoidal, horizontal magnetic fields at 1, 10, or 100 μT. Exposure durations were either short-term (12 hours) or long-term (30 days). Serum melatonin concentration and pineal N-acetyltransferase (NAT) and hydroxyindole-0-methyl transferase HIOMT activities were measured. Short-term exposure depressed NAT activity and melatonin concentration at the highest intensity of 100 μT. Long-term exposure depressed nocturnal peak serum melatonin concentration and NAT activity at 10 and 100 μT. These results suggest that effects depend on both the duration and intensity of magnetic fields, and the sensitivity threshold varies with the duration. Overall the results suggest a cumulative effect of magnetic fields on pineal function.

Löscher et al. (1998) conducted experiments to see the effect of exposure of female Sprague-Dawley rats to 100 μT, 50 Hz magnetic fields for periods of 1 day or 1, 2, 4, 8 or 13 wks. Inconsistent changes in nocturnal level of melatonin were observed. In one experiment after 2 weeks of exposure, a decrease in serum melatonin was observed at 6 hours after onset of darkness. In all the other experiments, however, no change was observed. They have no plausible explanation for the discrepancy, though several variables such as sex, age, season, and exposure parameters were pointed out for consideration.

Besides Löscher et al., several other researchers have mentioned that they could not confirm the melatonin reduction in repeated exposure experiments, even though they found decreased melatonin concentrations by magnetic field exposure in their earlier experiments. Using Djungarian hamsters, whose neuroendocrine response is very sensitive to photoperiod, Yellon (1996), Truong and Yellon (1997), and Wilson et al. (1999) carried out experiments to see whether magnetic field exposure resulted in changes of melatonin production in either short-light (SL) or long-light (LL) conditions. In the Yellon (1996) and Truong and Yellon (1997) studies, the animals were kept in LL (16L:8D) or SL (10L:14D) conditions for 3 or 6 weeks before the exposure experiments. Magnetic field exposure with 60 Hz at 0.1 mT was for 15 minutes, occurring 2 hours before dark. In both long and short photoperiod conditions, no changes in melatonin concentration of pineal gland and blood were observed. Once again, it must be noted that these are acute experiments involving a single, brief (15 min) magnetic field exposure followed rapidly (2 h later) by the melatonin measurement. This is very different from the 6-wk exposures reported originally by Wilson et al. and studied extensively by Kato et al.

In the Wilson et al. (1999) experiments, Djungarian hamsters were maintained in either SL (8L:16D) or LL (16L:8D) photoperiods. Acute exposure (15 minutes) of both SL and LL animals to a horizontal 60 Hz, 0.1mT magnetic field resulted in a significant decrease in pineal melatonin content, whereas at 50 μT no effect

was observed. In SL animals an increase in noradrenalin was observed in the medial basal hypothalamus, including the suprachiasmatic nucleus, after acute exposure. Repeated magnetic field exposure of SL animals to a combination of steady-state and on/off 60 Hz magnetic fields at 0.1 mT for 1 h/d for 16 days was associated with a reduction in melatonin concentrations, while continuous exposure to 3 h/d for 42 days resulted in no change. These data indicate that both one-time and repeated exposure to 0.1 mT, 60 Hz magnetic field can give rise to neuroendocrine responses in Djungarian hamsters.

Selmaoui and Touitou (1999) studied the effects of exposure to 50 Hz, horizontal magnetic fields at 100 μT for one week (18 h/d) to either aged (23 months) or young (9 weeks) Wistar rats. Serum melatonin concentration decreased by 28% and pineal NAT activity decreased by 52% in the young rats. However, no effect was observed in the aged rats, suggesting that old rats are insensitive to the magnetic field.

Collectively, the melatonin literature is the most convincing data set indicating that exposure to ELF magnetic fields can have an important physiological effect. Although many negative outcomes have been reported, the balance of the evidence clearly suggests there is a robust and reliable effect. The mass of experimental data is now large enough that some reasonable hypotheses have emerged about the details of the mechanism(s) involved and the reasons why different experiments using different conditions report differing results. However, the existent data are not sufficient to provide definitive answers. (The problem is like degrees of freedom in statistics or solving unknowns with simultaneous equations. Alas, the number of questions exceeds the df, or the number of unknowns exceeds the number of available equations.) Therefore, additional work in this area is highly desirable. In the *in vivo* ELF magnetic fields bioeffects world, melatonin appears to the best available system for untangling the mechanism(s) of action of ELF magnetic field bioeffects.

3.4.1.1.2.2 Morphological studies Morphological data concerning magnetic field exposure effects on the pineal gland have been reported by several authors. Synaptic ribbons of pinealocytes show a circadian rhythm in which the number of ribbons is high in the night and low during the daytime. Martinez-Soriano et al. (1992) studied the effects of 50 Hz, 5.2 mT, pulsed magnetic field exposure on the diurnal rhythms of both synaptic ribbons and serum melatonin levels in Wistar rats. The rats were exposed to magnetic fields for 30 m/d (during 9:00–13:00). Synaptic ribbons were examined by electron microscopy at 1, 3, 7, 15 days of exposure, and serum melatonin concentration was measured at days 3 and 15 of exposure. Synaptic ribbon numbers were decreased at days 15 and 21, in association with decreased melatonin levels at day 15.

Matsushima et al. (1993) studied the effects of exposure to a 50 Hz, circularly polarized magnetic field for 6 weeks at 7 μT_{rms} on pineal gland volume and pinealocyte size in Wistar-King rats. The exposure caused a slight but significant ($P < 0.05$) increase in pineal volume. Furthermore, the size of pinealocytes in the distal and proximal, but not in the middle, regions were affected by magnetic field exposure. Hence a power-frequency magnetic field might exert an effect on mechanisms controlling day-night rhythms of pinealocyte size in the rat.

3.4.1.1.3 Experiments with farm animals.

Lee et al. (1993: 1997) penned female Suffolk lambs directly under a 500 kV, 60 Hz transmission line where the mean electric field was 6 kV/m and the mean magnetic field was 4 μT. Serum melatonin level were measured at predetermined intervals over the period from April until the following February. The control group was penned 229 meters from the line, where the electric and magnetic fields were at ambient levels. In three repeated experiments, they could not find any group differences.

Burchard et al. (1998a) exposed dairy cows to 10 kV/m and 30 μT for 28 days, comparing nocturnal melatonin concentrations among pre-exposed, exposed, and post-exposed periods each 28 days in duration. They found no group differences.

3.4.1.1.4 Experiments with non-human primates

Rogers et al. (1995c) obtained data from a pilot experiment with three baboons (*Papio cynocephalus*), each implanted with an indwelling venous cannula to allow repeated, automated blood sampling, showing a profound reduction in nocturnal serum melatonin concentration of baboons. In this experiment, the exposure schedule was irregular, in terms of times of field onset/offset and in duration and scheduling of the daily exposure periods, which could include more than one "field on" period per day. On some days, exposure occurred in both day-time and night-time. Field strength (60 Hz) combinations of either 6 kV/m and 50 μT or 30 kV/m and 100 μT were used. Furthermore, in this pilot experiment (and only in this experiment) transients were allowed to accompany field onset and offset. Rogers et al. (1995c) suggested that animals frequently adapt to constant environmental conditions, so exposure conditions deliberately made variable Also, transients might be the relevant mechanism, so the fields were turned on and off in an electrically 'noisy' manner. In this pilot experiment, which was done first, all variables were set according to hunches about what might be most effective. In the following main experiments, a conventional experimental design emphasizing rigid control was used, and no effect was found. When contrasted with the next set of experiments (Rogers et al. 1995d), this report suggested that different exposure paradigms could produce very different results.

Rogers et al. (1995d) performed an ambitious set of experiments employing non-human primates (*Papio cynocephalus*) to study the effects of either 6 kV/m and 50 μT or 30 kV/m and 100 μT, 60 Hz electric and magnetic field exposure on serum melatonin concentration. Each baboon was implanted with an indwelling venous cannula to allow repeated, automated blood sampling. Three baboons were field-exposed only during the light phase (7:00–22:50) for 6 weeks, and three baboons were sham-exposed. Timing of field onsets and offsets was strictly controlled. Because the transformers were turned on and off slowly, there were no transients associated with field onset and offset. During the exposure period, the daily pattern of melatonin secretion was determined using blood samples collected every 2 hours around the clock. Diurnal samplings completed during 6-wk pre-exposure, exposure, and post-exposure periods provided within-subject data for each animal. The investigators found no differences between the exposed and sham-exposed groups during any of the three periods.

3.4.1.1.5 Experiments with humans

When an experimenter can obtain data directly from humans, the problem of extrapolating from animal model data to humans is avoided. There are two categories of studies, experimental and observational, of whether ELF magnetic field exposure affects melatonin production of human subjects. Each of these approaches has its own advantages and disadvantages. In experimental studies, the researchers can determine the experimental conditions, such as parameters of the fields - e.g., strength, wave form (sine wave, pulse or others), frequency, duration, etc. - and can recruit experimental subjects according to the concerns of the researchers. Lighting schedule, darkness, room temperature, etc. also can be controlled. Another important advantage is that the experimenter can obtain data directly from humans, thereby avoiding efforts to extrapolate animal data to humans. Ethically and practically, the limits of experimental studies on human subjects are that they are restricted to acute exposures lasting for only a few days. Experiments with humans cannot be planned if adverse effects are predicatable, again from ethical point of view.

Observational studies have the advantage of providing a way to assess potential effects of chronic, long-term exposure in the real world. Therefore, the obtained results could provide, for example, reference data for epidemiology studies. The limits are that exposure is not determined by the researchers, and characteristics of the exposure - e.g., waveform, strength, variations, etc. - might not be fully controlled. Hence the responsible parameters of the fields might be left unresolved.

As of fall of 2005, there are at least 12 studies in human populations examining whether exposure to power frequency magnetic fields reduces or otherwise disrupts the nocturnal production of pineal melatonin.

3.4.1.1.5.1 Laboratory experiments with healthy humans Graham and colleagues in the USA, Selmaoui and colleagues in France, Warman and colleagues in the United Kingdom and Wood and colleagues in Australia have studied patterns of melatonin secretion of healthy human subjects exposed to magnetic fields in specifically built magnetic field exposure laboratories.

A series of controlled laboratory-based study on human subjects were first carried out by Graham et al. (1996a). Thirty-three male human volunteers of 18–35 years were either sham-exposed or field-exposed during the night to circularly polarized magnetic fields of either 1 or 20 μT. In their initial two series, a regular, intermittent exposure paradigm was adopted that provided 1 h off/1 h on (with the field cycling on and off every 15 second throughout each of the 1 h on periods) from 23:00 to 7:00. Overall the *a priori* hypothesis of a group difference did not occur. However, *a post hoc* analysis suggested a significant decrease of melatonin concentration occurred only in the subjects with pre-existing low melatonin concentrations. This hypothesized effect did not occur in the second experiment. Overall, the intermittent exposure was not effective in altering nocturnal melatonin release patterns in human subjects. In the third experiment (Graham et al. 1996b) used continuous magnetic field exposure at the same strength for 8 hours (23:00–7:00). No overall effects on melatonin concentrations were found. Graham et al. (2001) studied if exposure to 60 Hz magnetic fields at an intensity well within the upper range of occupational

exposure resulted in suppression of melatonin or 6-OHMS. The 24 male volunteers were exposed either to continuous or regularly alternating (1-hr field-on and 1 hr field-off), circularly polarized, 60 Hz sinusoidal magnetic fields at 127 μT. Exposure was from 23:00 to 07:00. For either type of exposure, no differences were observed between field-exposed and sham-exposed control groups. These results indicate that the nocturnal secretion of melatonin is not affected in humans by acute field exposure at intensity over 600 times higher than that typically encountered in the home.

Graham et al. (2000) carried out laboratory experiments in which 30 young healthy men were exposed to 60 Hz, 28.3 μT magnetic fields for four consecutive nights. The magnetic field intensity was picked to match a typical occupational field intensity. The measurements were evaluated using a randomized, double-blind test protocol. No differential effects on concentrations of melatonin or 6-OHMS compared to equivalent sham-control conditions were detected. Repeated nightly exposure to the magnetic field was associated with reduced consistency, contrasting to the consistent intra-individual measurements in the sham control group. Morning 6-OHMS measures obtained after exposure on night 4 differed ($P < 0.01$) from measures obtained after the second and third exposure nights. While overall results indicate there is no difference between the exposed and sham control groups, some suggestion of a cumulative effect of magnetic field exposure is presented. Remember that Kato et al. showed their effects in rats after 6 wks of exposure, and Wilson et al. (1981, 1986) reported effects appeared in the third week of exposure. The possibility remains that a longer period of controlled exposure might result in differences with human subjects.

Selmaoui et al.(1996) performed two experiments to investigate the effects of a 9-hour exposure to a 50 Hz horizontal magnetic field at 10 μT. Exposure was continuous in the first study and intermittent in the second study, which was carried out 1 month later. None of the exposures affected serum melatonin or urinary 6-OHMS of the 32 young men (20–30 years old).

Warman et al. (2003) conducted experiments on 19 male subjects (18–35 years)to see if 2-hour exposure to 200 – 300 μT, 50 Hz circularly polarized magnetic field had any effect on melatonin production. Exposure was timed to occur before or during the nightly melatonin rise. The data indicated no significant suppression, alteration of peak levels, or change in timing of the nighttime melatonin rise.

Wood and colleagues investigated melatonin production by focusing on the 'phase' of the circadian rhythm. Previously Armstrong's group (Trinder et al. 1996, Luke et al. 1996) identified a window of time for sensitivity to light illumination for inhibition of melatonin secretion onset in humans. Wood et al. (1998) studied whether any such window exists for sensitivity to ELF magnetic field exposure. Subjects were 30 males of 18–49 years. Experiments were carried out on three successive Friday night/Saturday morning periods. The first night was a 'screening night' to establish the basic melatonin rhythm of each participant. The second night was for exposure to magnetic fields, and the third night was for the sham-exposure. Exposure durations were between 1.5 to 4.0 hours in duration and occurred either: (1) before the rise in melatonin, (2) during the rising limb, and (3) after the peak level of melatonin rhythm had been attained. The magnetic field strength was 20 μT with

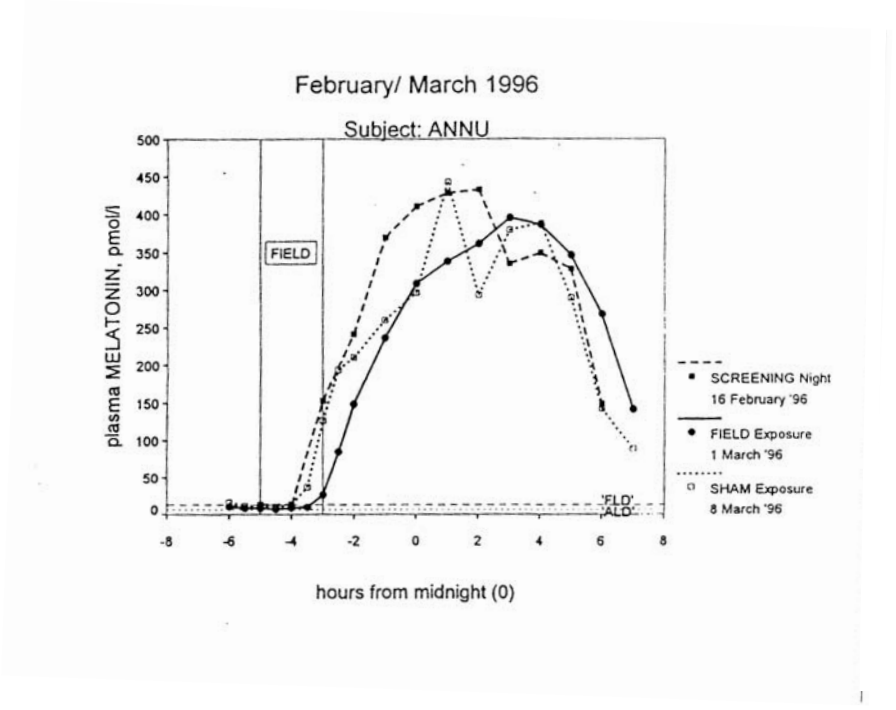

Fig. 3.6. Melatonin profiles from an individual human subject. On a screening night, time of onset of rise in melatonin (Mel On) for this subject was determined to be 21:00 hr (−3 hr from midnight). Exposure to 20 μT magnetic fields and sham-exposure is between 19:00–21:00 hr on the subsequent two experimental nights (Wood et al. 1998).

50 Hz circularly polarized fields, either sinusoidal or square-wave. A regular intermittent exposure was used with field on for 15 sec and then off for 15 sec. When the magnetic field exposure preceded onset of rise, a significant delay in melatonin onset time of 27 minutes (P = 0.021) was observed with both sinusoidal and square-wave conditions (Fig. 3.6). Magnetic fields generated by square-wave current produced more marked reductions in the maximum melatonin level compared to sinusoidal wave form. When the fields were exposed during the rising period or during the time of expected peak level, there was no change in melatonin.

3.4.1.1.5.2 Laboratory measurement on patients The above three groups investigated the effects of magnetic field exposure on healthy subjects under controlled experimental conditions in which the exposure time was relatively short. Polish researchers have studied ELF magnetic fields and melatonin using patients. Karasek et al. (1998) measured diurnal rhythms of blood melatonin concentrations in 12 male patients with low back pain syndrome. The authors compared the diurnal melatonin rhythm profiles of one day before the exposure (base line) and one day after exposure to 2.8 mT, 40 Hz, square-wave, bipolar-pulsed magnetic fields for 3 weeks (20 min/d,

5 d/wk). By placing a coil, the magnetic field exposure was localized to the lower-back region. Karasek et al. (1998) reported depression in nocturnal melatonin level after ELF magnetic field exposure. Whole body exposure was not used; thus the head, which includes pineal gland and the eyes, two organs hypothetically susceptible to magnetic field exposure, might not have been exposed 'directly' to magnetic field. For this reason, this study provides a challenge concerning the possible mechanisms of magnetic field exposure upon the pineal gland. In a subsequent study Karasek et al. (2000) examined whether or not whole body exposure to magnetic fields of differing parameters exerted the same effect. The study was carried out in seven men (mean 37 years, range 32–42) suffering from low back pain. These patients were exposed to a pulsing magnetic field (induction = 25 – 80 μT; frequency = 200 Hz, modulated, automatically programmed; complex saw-wave-like impulse shape; bipolar) for 3 weeks (5 d/w, twice a day at 08:00 and 13:00, for 8 min each), applied to the whole body in patients lying in a horizontal position. Diurnal serum melatonin profiles were estimated 1 day before exposure (baseline) plus 1 day and 1 month after the last exposure. No changes in melatonin concentrations were observed. The authors attribute the different results between the initial positive and the second negative study to differences in field strength, frequency, and impulse shapes.

3.4.1.1.5.3 Occupational exposure studies with humans Four groups of researchers have investigated the effects of ELF magnetic field exposure on melatonin secretion of workers under the occupational conditions.

Pflüger and Minder (1996) examined the effects of 16.7 Hz electromagnetic field exposure on melatonin excretion under regular working conditions of 66 engine drivers and 42 control workers (train attendants and station managers) of the Swiss Federal Railways by measuring urinary 6-OHMS. The study compared 6-OHMS levels between exposed and control groups; within-subject comparisons also were made in the two groups during non-working periods and on days following the return to work. The exposure averaged 20 μT in the most exposed workers and around 1 μT in the least exposed. Evening 6-OHMS concentration (or daytime level) of railway engineers was less than that of the control group. Among engine drivers, evening 6-OHMS values were lowered by a factor of 0.81 (95% CI;0.73 – 0.90) during workdays compared to leisure days. At day 1 P = 0.01 (127 drivers vs 90 controls), and at week P = 0.03 (88 drivers vs 50 control) Furthermore, during subsequent leisure periods, evening values recovered significantly. No significant difference between work and non-work days was observed in the control group.

Burch et al. (1998: 1999a) studied whether nocturnal excretion of urinary 6-OHMS was influenced in electric utility workers. Using personal dosimeters, exposure was measured at 15-second intervals over three consecutive 24-hour periods, and exposure metrics based on magnetic field intensity and temporal stability was calculated for periods of work. A rate-of-change metric (RCM) was used to estimate intermittency, and the standardized RCM (RCMS = RCM/standard deviation) was used to evaluate temporal stability. Low RCMS values correspond to relatively small differences between successive measurements, indicating that magnetic field exposures were stable over time. When each of the two magnetic field metrics was analyzed separately, no associations between 6-OHMS excretion and magnetic field

intensity or intermittence were found. When RCMS was analyzed as a continuous variable, low RCMS exposures were associated with lower nocturnal 6-OHMS concentration ($P < 0.01$). This result provided evidence that temporally stable magnetic field exposures are associated with reduced nocturnal 6-OHMS excretion. This finding might be regarded as contradictory to a result reported with non-human primates; exposure to rapidly switched and irregularly intermittent electromagnetic fields for a few days produced a profound suppression of melatonin (Rogers et al. 1995c).

Burch et al. (1999b) further studied the relationships of geomagnetic disturbance and 60 Hz magnetic field exposure on 6-OHMS excretion in an occupational population, electric utility workers. They found that a greater reduction of 6-OHMS was observed when increased geomagnetic activity was combined with elevated 60 Hz magnetic fields ($P < 0.02$).

Magnetic fields in close proximity to an energized 3-phase conductor are either circularly or elliptically polarized, whereas those associated with single-phase conductors are linearly polarized (Deno 1976, Yasui 1994). In laboratory experiments both circularly polarized magnetic fields and linearly polarized fields have been studied with either animals (Kato et al. 1993) or humans (Graham et al. 1996; Wood et al. 1998). Elliptically polarized fields also were studied with animals (Kato et al. 1999). A linearly polarized magnetic field simply reverses its direction over time. With a circularly polarized magnetic field, the direction of the field moves in a circle, and there are smaller peaks and valleys in the induced current (see Chapters 6 and 7). Therefore experimental subjects get more field exposure from a circularly polarized field. The existence of elliptically polarized field in substations, under power lines, and in residential area has been reported (Dietrich et al. 1992, Yasui 1994).

Burch et al. (2000) measured environmental ELF magnetic fields in relation to urinary 6-OHMS excretion of electric utility workers over three consecutive workdays. There was a magnetic-field-dependent reduction in 6-OHMS levels among men working more than 2 hours per day in substations and 3-phase environments, but there was no effect among those working 2 hours or less. No change was observed among men working in single-phase environments. These results suggest that circular or elliptical magnetic field polarization is associated with melatonin suppression in humans. Another point is that length of exposure is critical for the suppression.

Davis et al. (2001) investigated whether magnetic field exposure was associated with lower nocturnal urinary concentration of 6-OHMS in 203 women aged 20–74 years with no history of breast cancer. Each woman was interviewed and provided data on the following for 72-hour periods at two different seasons (repeated approximately 3 or 6 months later) of the year: (1) magnetic field and ambient light measured every 30 seconds in her bedroom, (2) personal magnetic field (measured with a dosimeter worn on the body) measured at 30-second intervals, and (3) complete nighttime urine collections made on three consecutive nights. Lower nocturnal urinary 6-OHMS concentration was associated with more hours of daylight, older age, higher body mass index, current alcohol consumption, and current use of medications - classified as beta blockers, calcium channel blockers, or psychotropics. After "adjustment" for these factors, higher bedroom magnetic field level during night (> 0.2 μT) was associated with lower urinary concentration of 6-OHMS during the same

night. Those persons with lower baseline melatonin levels were more susceptible to the effects of magnetic fields. This is in accordance with the results obtained in an initial, but unreplicable laboratory experiment by Graham et al. (1996a). Furthermore, the strongest magnetic field effects were observed during the summer months, when melatonin levels were lowest.

Contrary to the above reports, three papers have recently appeared which observed no effects of magnetic field exposure.

Juutilainen et al. (2000) performed a study to determine whether daytime occupational exposure to extremely low frequency magnetic fields suppresses melatonin production. Subjects were female workers in a garment factory exposed to the magnetic fields emitted by sewing machines. Eye-level magnetic field intensity was used to classify the workers into high (> 1 μT, 10 workers) or low (0.3–1.0 μT, 22 workers) exposure groups. A third group of eight factory workers was exposed to average magnetic field levels (3.2 μT, for 83% of the day) from other sources. A reference group consisted of 15 employees of a government organization and six university staff members. Melatonin production was assayed by determining 6-OHMS in urine. Urine samples were collected on Friday and Monday morning for 3 consecutive weeks.The average 6-OHMS excretion on Friday was lower among the factory workers than among the reference group. The ratio of Friday morning to Monday morning 6-OHMS was calculated to test the hypothesis that melatonin production is suppressed after 4 days of daytime occupational magnetic field exposure, with significant recovery occurring during the weekend. The ratio was close to 1.0, suggesting that there was no increase in melatonin production over the weekend.

Levallois et al. (2001) studied the effect of residential exposure to electric and magnetic fields from high-power lines on female urinary 6-OHMS in the Quebec City, Canada. A sample of 221 women (45.5 ± 11.2 years) living near a 735-kV line was compared with 195 women (45.8 ± 12.4 years) living away from any power lines. Mean 24-hour magnetic field intensity was greater (0.33 μT) in the group living near the lines than in the control group (0.13 μT). Personal exposure to magnetic fields was assessed by means of an EMDEX Lite meter. Participants provided morning urine samples on 2 consecutive days. After adjustment for other factors associated with low melatonin secretion, such as medication use or light exposure, nighttime concentration of 6-OHMS was similar in the two groups.

In search of any cumulative effect from chronic occupational exposure, Touitou et al. (2003) examined the circadian rhythm of melatonin in 15 men (32–46; mean 38.0 ± 0.9 yr) exposed to a 50 Hz magnetic field for 1–20 yr in the workplace. The estimated weekly geometric mean of individual exposures ranged from 0.1 to 2.6 μT. Control subjects were 15 men(35−47; mean 39.4±1.2 yr) who were exposed to 0.004 to 0.092 μT. Blood samples were taken hourly from 20:00 to 08:00, and nighttime urine was also collected and analyzed. This work revealed that subjects exposed on a daily basis to ELF magnetic fields over a long period did not differ from control subjects without appreciable exposure in their plasma melatonin or their urinary 6-sulfatoxy-melatonin concentrations.

3.4.1.1.6 Mechanism of magnetic field effects on pineal gland.

It long has been known that ELF and transient magnetic fields of moderate densities generate visual phenomena, known as magnetophosphenes (d'Arsonval, 1896). Lövsund et al. (1979: 1980) reported that magnetophosphenes are generated in the retina. The threshold values varied with the frequency of the magnetic field and the luminance level of the background light, with the maximum sensitivity being at 20 Hz and about 8 mT. Lövsund et al. (1981) studied retinal ganglion cells that responded to both light and magnetic field; the authors assumed the eddy current gave rise to the effects produced by the magnetic field. However, they recorded action potentials of optic tract from multifiber samples, not from single fiber specimens. Therefore, one cannot say if a single retinal photoreceptor responded to both light and magnetic field stimulation, or if receptors exist that responded specifically to magnetic stimulation. Still these results suggest that the retinal photoreceptors and/or synaptic connections within the retina are likely sources of magnetic field reception.

Olcese and colleagues (Olcese and Reuss 1986, Stehle et al. 1988, Olcese 1990, Schneider and Semm 1992) have emphasized the role of the retina in responding to magnetic fields. Olcese et al. (1985) reported that acutely blinded rats, following section of optic nerve, showed no inhibition of pineal melatonin synthesis produced by magnetic stimulation as compared with intact subjects. Reuss and Olcese (1986) reported that pineal responsiveness to a magnetic field stimulus was absent in intact rats housed in total darkness. From these results, the authors hypothesized that retinal photoreceptors might be capable of responding to a magnetic field.

Complete blindness generally results in the loss of synchronization of circadian rhythms to the 24-hour day and in recurrent insomnia in humans. Czeisler et al. (1995) studied melatonin secretion in 11 completely blind patients; the cause of blindness differed among patients. All the subjects reported that they had no conscious perception of light. Additionally, their behavior was consistent with that of a blind person. Ten out of the 11 subjects showed a diurnal melatonin pattern; in one patient, plasma melatonin was undetectable. In three out of the remaining 10 subjects, plasma melatonin concentration markedly decreased in response to exposure to bright light (10,000 lux). These results suggest that the photoreceptive system mediating the melatonin circadian rhythm differs from the photoreceptive system that mediates the conscious perception of light.

Following these observations, big progress was reported in both human and animal studies. Lucas and Foster (1999) and Lucas et al. (1999) attempted to define the photoreceptors responsible for inhibition of pineal melatonin production. They used C3H mice with a mutation affecting retinal disruption of rod phototransduction (rd) with a transgenic ablation of cones (rd/rd cl mice). Monochromatic green light of wavelength 509 nanometer at 2.6×10^2 μW/cm^2 induced complete suppression of melatonin production in the rd/rd cl mice as well as wild-type mice. Bilateral enucleation abolished the response even to 6 μW/cm^2, 509 nm light. The data indicate that mammals have additional ocular photoreceptors that are involved in the regulation of pineal melatonin production.

The cellular localization of these novel, non-image-forming photoreceptors was studied by Sekaran et al. (2003) by monitoring the effects of light stimulation (470 nm) on intracellular Ca^{2+} via FURA-2 imaging. The authors identified approximately 2.7% of light-sensitive neurons in the ganglion layer. A small population of widely dispersed retinal ganglion cells projecting to the suprachiasmatic nucleus in the hypothalamus is intrinsically photosensitive and expresses the opsin-like protein melanopsin.

Lucas et al. (2003) reported that in mice with the melanopsin gene ablated, retinal ganglion cells retrograde-labeled from suprachiasmatic nucleus were no longer intrinsically photosensitive. Melanopsin is a novel opsin synthesized in a small subset of retinal ganglion cells. Ganglion cells expressing melanopsin are capable of depolarizing in response to light in the absence of rod or cone input and are thus intrinsically light sensitive. Melanopsin ganglion cells convey information regarding general levels of environmental illumination to the suprachiasmatic nucleus.

Belenky et al. (2003) reported that melanopsin ganglion neurons in the mouse receive bipolar and amacrine terminals, suggesting that rod and/or cone signals might be capable of modifying the intrinsic light response in these retinal ganglion neurons.

Thapan et al. (2001) analyzed the novel non-rod, non-cone photoreceptor system in human by drawing an action spectrum for melatonin suppression. The observed spectrum shows short-wavelength (424 nm and 472 nm) peak sensitivity that is very different from the known spectral sensitivity of the scotopic (505 nm) and photopic (555 nm) response curves shifted approximately 50 and 100 nm to the left of the peak scotopic and photopic responses, respectively. The human retina contains one class of rod (496 nm) and three classes of cone photoreceptors (S-cone, 419 nm; M-cone, 531 nm; L-cone, 558 nm).

Retinal ganglion cells, which are output neurons of the retina, generally are divided into three classes (Type I, II and III cells) on the basis of morphological and functional characteristics. Type I neurons project to the lateral geniculate nucleus, which constitutes the pathway to the visual cortex where conscious perception of light takes place and images of the outside world are generated. Moore et al. (1995) investigated the retinohypothalamic tract originates from retinal ganglion cells and projects to the suprachiasmatic nucleus, site of the circadian clock, by using transneuronal transport. They identified a subpopulation of Type III cells that gives rise to the retinohypothalamic projection in the rat, suggesting a specific set of photoreceptors is involved in circadian entrainment.

Raybourn (1983) reported that DC magnetic fields at 5–10 mT suppress the b-wave of an electroretinogram, providing evidence that either photoreceptors or synaptic processes in the retina are involved in magneto-reception. Based on behavioral experiments conducted with newts, Phillips and Borland (1992) suggested that magnetic compass orientation is affected by the wavelength of light, again indicating that photoreceptors, be they traditional image-forming receptors or newly found non-image-forming receptors, are responsible for detecting magnetic field information. The literature cited above provides experimental data supporting "retina hypothesis" of the mechanisms of "magnetosensitivity".

The other hypothesis postulates that the electric (eddy) current induced by magnetic field exposure causes biological responses of central nervous structures such as pineal gland and/or suprachiasmatic nuclei (Lerchl et al. 1991). Brendel et al. (2000) performed a series of experiments in which direct effects of weak magnetic fields were studied in isolated pineal glands from Djungarian hamsters. During morning hours, pineal glands were removed and placed individually into glass chambers perfused with oxygenated buffer. Experiments lasted for 8 hr. Magnetic fields at 86 μT, at either 16.7 Hz, which is used by the railway system, or 50 Hz, were generated by Helmholz coils. Maximum melatonin production was achieved after 5–7 hr. In all experiments, maximum melatonin concentrations at both 16.7 Hz and 50 Hz were lower in the exposed groups compared with the sham-exposed controls. These results show that the pineal gland itself responds to magnetic field exposure with a significant decrease in melatonin production.

At this time, neither the 'retina hypothesis' nor the 'direct effect hypothesis' provides convincing data in an *in vivo* experimental set-up. The induced current mechanism is discussed in Chapter 7.

3.4.1.2 Effects of electric and magnetic field exposure on sex hormones

Melatonin has an antigonadotropic action (Chapter 2); it inhibits the release of leutinizing hormone (LH) and follicule stimulating hormone (FSH) from the pituitary gland and reduces the hypothalamic leutinizing hormone releasing hormone (LHRH) level. Although an antigonadotropic action of melatonin has been demonstrated in several species, it is much less pronounced in humans, where the existence and importance of such an effect remains controversial (Touitou 1989).

Kato et al. (1994d) measured the testosterone concentration of male rats whose melatonin level was significantly reduced by about 20% following 6 weeks of exposure to a circularly polarized magnetic field of 50 Hz, at 50 μT. No significant effect on testosterone secretion was observed in three repeated experiments. These results indicate that ELF magnetic field exposure does not influence at least one aspect of the hypothalamic-pituitary-gonadal axis, even when melatonin is significantly reduced.

Picazo et al. (1995) studied the effects of 50 Hz, linearly polarized vertical magnetic field exposure for two generations on testicular morphology and serum testosterone levels. First, female and male OF1 mice were maintained from 6 weeks of age, and were mated under continuous exposure to magnetic fields of 15 μT. Male offspring then were exposed under the same experimental conditions. When necropsied at the age of 10 weeks, size and weight of the testes concentrations of testosterone in the blood all were increased relative to controls. The authors attribute the difference from the negative results of Kato et al. (1994d) to the different exposure periods.

Because melatonin is an antigonadotropic hormone, and if nocturnal melatonin is decreased by electric and magnetic field exposure, then gonadotropic hormones could be increased. Thus, Burchard et al. (1998b) designed the experiment to evaluate the effects of electric and magnetic field exposure on blood progesterone concentrations during estrous cycles in non-pregnant, lactating dairy cows. A vertical electric field of 10 kV/m and a uniform horizontal magnetic field of 30 μT, both

at 60 Hz, were used. The within-subject experimental design included three estrous cycles of 24 to 27 days. During the first and third estrous cycles, the electric and magnetic fields were off to serve as control periods; field exposure occurred during the second estrous cycle. No differences between field-exposed and control periods were detected in plasma progesterone concentration and area under the progesterone curve. In this experiment, melatonin apparently was not assayed.

Implantation and embryonic development take place only in a specific hormonal environment. Melatonin inhibits LH release and thus controls ovarian function and hormone secretion. The receptivity of the uterus to embryo implantation is under hormonal control (Weitlauf, 1994). Estrogen (mainly 17β-estradiol) and progesterone play key roles in implantation.

Huuskonen et al. (2000) studied the effects of 50 Hz horizontal magnetic field exposure on embryo implantation, serum 17β-estradiol, progesterone, testosterone, and melatonin concentrations, estrogen receptors, and progesterone receptors in the uterus during pre-implantation and implantation periods of Wistar rats. Magnetic flux densities were either 13 or 130 μT. Exposure occurred continuously during the period from approximately 8 hours after ovulation until 176 h after ovulation. Magnetic field exposure did not influence the mean total number of implantations. Although the differences were not statistically significant, the mean nocturnal serum melatonin concentrations were decreased by 34% and 38% at 13 and 130 μT, respectively, at 70 h after ovulation. Serum estradiol and progesterone concentrations were not changed.

Selmaoui et al. (1997) studied whether acute (9-hour) exposure to a 50 Hz, 10 μT, horizontal magnetic field had any effect on the hormones of the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-adrenal axes. Thirty-two men were either exposed to continuous or intermittent (one hour "off" and one hour "on", with the field switched "on" and "off" every 15 seconds) from 23:00 to 8:00. They measured circadian profiles of cortisol, 17-hydroxycorticosteroids in urine LH, FSH, thyroid stimulating hormone, free thyroxine, free triiodothyroxine, triiodothyroxine, thyronine, and thyroxine binding globulin. When the profiles of the field-exposed group and sham-control groups were compared, no differences were found.

Taken together, the results of the above five papers suggest that exposures shorter than 6 weeks are ineffective in causing changes in gonadal hormones. However, one study implies that exposure for two generations is long enough to bring about the effects. However, given the many differences among experiments, it is not clear if exposure duration plays a critically important role.

3.5 Bone Repair

3.5.1 Clinical studies

The pioneering studies by Yasuda (1954) and Fukada and Yasuda (1957) showed that substantial electric fields are endogenous to the skeleton. They measured piezoelectricity of bone, finding that the magnitude of the piezoelectric constant depends on the angle between the applied pressure and the axis of the bone. The piezoelectric

effect appears only when a shearing force is applied to the collagen fibers, making them slip past each other. The maximum value of the piezoelectric constant induced in bone is about one-tenth of the piezoelectric constant of quartz crystal. The origin of piezoelectricity in bone was ascribed to the piezoelectric effect of the crystallin micelle of collagen molecules, not to the soft tissues.

Based on the Yasuda and Fukada and Yasuda papers, application of electric fields to promotion of fracture healing was started. In the early trials, electrodes were implanted surgically to provide exogenous electrical stimulation. However, the surgical procedure, implantation and removal, produced several unfavorable by-products, such as electrochemical changes surrounding the electrodes and damage to the implanting regions. For these reasons Bassett et al. (1974) introduced the use of inductively coupled electromagnetic fields for research; later they began to apply this method to the treatment of non-union (or un-united) tibial fractures (Bassett et al. 1981). Since then many reports have claimed high success rates, although these claims have not based on reliable, double-blind testing methods.

Using a double-blind trial for the first time in clinical studies of the effects of magnetic fields and bone growth, Barker et al. (1984) reported in their preliminary study on pulsed magnetic field therapy for tibial non-union that no difference was found between the active treatment group (9 patients) and the control group (7 patients). Sharrard (1990) conducted a randomized, double-blind trial on a larger number of patients with delayed unions of tibial fractures from 1981 through 1987. A total of 45 tibial shaft fractures with delayed union, which had been conservatively treated for more than 16 weeks but less than 32 weeks, were enrolled. The fractures were selected for their lability to delayed union by the presence of moderate or severe displacement, angulation or comunition or a compound lesion with moderate or severe injury to skin and soft tissues. Plaster immobilization for 12 weeks was used for all subjects. Active electromagnetic stimulation units were used with 20 patients, and dummy (sham) units were used in 25 patients; assignment was random Neither the patients nor the surgeon knew whether the unit was active or dummy, because the unit were indistinguishable in external appearance and use. The units consisted of copper wire coils in two formed plastic bodies. The coils were 35 cm long; the therapeutically effective window was 20 cm long and 11 cm wide. In the active devices, pulses of current were passed through the coils to produce corresponding pulses of magnetic flux through the fracture region and pulses of electric induction in the tissues. The form of the signal used for the electromagnetic stimulation was quasi-rectangular biphasic pulses of 200 μsec duration followed by sharp reverse in form for 25 μsec. A burst consisted of 20 individual pulses, and the bursts were repeated at 15 Hz. Although no clear description of this point is given in the text, the value of dB/dt appears to be around 7.5 Tesla/sec, as judged from Figure 1 of Sharrard (1990). These values might correspond to electric fields to those observed during development or the mechanical deformation of bone structure (i.e., 0.1–1 V/m). A radiologist and an orthopedic surgeon assessed radiographs blindly and independently. The radiologist's assessment of the stimulated group showed radiological union in five fractures, progress to union in five, and no progress to union in 10. In the control group, there was union in one fracture, progress towards union in one, and no progress in 23. Us-

ing Fisher's exact test, the results were highly significant in favor of the stimulated group (P = 0.002). The orthopedic surgeon's assessments were not identical; the results being significantly in favor of the stimulated group.

Various kinds of magnetic fields, beside the ones mentioned above, are effective for healing nonunion bone fracture in humans. The most reliable material for promotion of bone regeneration is a fresh autogeneous graft harvested from the patient's own bone, such as ribs, iliac crest, or anterior tibia. However, harvesting of autogeneous grafts is accompanied with disadvantages, including prolonged operating time, blood loss, or infection of the wound etc. Therefore, there is a considerable interest in the development of alternatives to the bone grafts. Demineralized bone matrix (DBM) can induce bone formation. However, DBM implants require a prolonged time for bone formation, compared with that of an autogeneous bone graft, and produce insufficient amount of bone.

3.5.2 Animal studies

Takano-Yamamoto et al. (1992) investigated if magnetic field exposure could promote regeneration of bone mediated by DBM. A 2-mm, non-healing bony defect in the premaxilla of male Wistar rats was either treated with 7 mg DBM or was left as a non-grafted control. Solenoid coils generated a magnetic field (150–180 μT) with a frequency of 100 Hz, this signal was given as a 10 msec burst with 100 μsec-wide, quasi-rectangular pulses, repeating at 15 Hz. The DBM graft plus magnetic field group produced more bone, with almost complete osseous bridging in the defect sites, than did the group treated with DBM only on day 35. Alkaline phosphatase activity, an osteoblastic marker, was determined as an index of bone formation, and ^{45}Ca incorporation represents re-mineralization of bone. Alkaline phosphatase activity increased significantly from day 7 in the DBM graft plus magnetic field group and from day 10 in the DBM-graft group, both measures reaching their maxima on day 14, compared with those of the DBM-graft sham group, with a greater than two-fold rise in alkaline phosphatase activity and a three-fold rise in the amount of ^{45}Ca incorporation by the DBM graft plus magnetic group. These findings indicate that the pulsed ELF magnetic field had an enhancing effect on the bone-inductive properties of the DBM, mediated by stimulation of the osteoblast differentiation that was induced by DBM.

Margonato et al. (1995) investigated possible effects of magnetic field exposure on structure and function of tissues (e.g., bone marrow) that are characterized by high cell turnover rates. Male Sprague-Dawley rats 12 weeks of age began exposure for 22 h/d to 50 Hz, horizontal fields at 5 μT for 32 weeks, meaning they were exposed for about 70% of their life span. The exposure did not cause any changes in growth rates and bone marrow. This study shows that the normal bone did not respond to exposure to ELF magnetic fields.

Landry et al. (1997) aimed to determine whether a 100 μT, 60 Hz magnetic field affected periosteal proliferation and differentiation in either the normal rat tibia or in the tibia 1 to 14 days after a surgically induced defect. Six groups of 30 young, male Fischer rats were used: three groups had no induced bone damage, and three groups

had injury to bone. Three groups of "bone-control" rats were studied: sham exposed, and magnetic field exposed for either 24 or 72 hours. Three experimental groups were studied following surgical induction of unilateral defect in the anteromedial region of the tibia: sham-exposed, and magnetic field exposed for either 24 or 72 hours. Histological analyses with a computerized morphometric system were processed on six different occasions: days 1, 2, 3, 5, 7 and 14 following start of magnetic field or sham exposure. Proliferation and differentiation were unaffected by magnetic field exposure in normal (no bone injury) rats. In the bone-damaged rats, exposure for 24 or 72 hours had no effect on proliferation, but it did produce an increase in osteoblasts 72 hours after the injury. The authors conclude that magnetic field exposure can affect "bone repair", and they suggest that the primary effect of magnetic field exposure was to promote differentiation but not proliferation.

In their early experiments on proliferative activity of cells of a transformed bone cell line, McLeod et al. (1993) showed that a 1.8 mT, 30 Hz magnetic field exposure resulted in a significant enhancement in activity of alkaline phosphatase, an enzyme serving a critical role in matrix mineralization. McLeod and Collazo (2000) carried out experiments intended to determine if the cells or tissues responded directly to magnetic flux or to the electric fields induced by magnetic field. They exposed MC-3T3-E1 osteoblast-like cells to a 30 Hz, 1.8 mT magnetic field for 4, 8, 16, or 64 hours. *In situ* assays of alkaline phosphatase activity demonstrated a progressive inhibition of enzyme activity, the pattern of which mapped to the intensity of the induced electric fields. They interpreted these experimental results to indicate that cells respond to the induced electric field. They interpreted the different experimental results reported by various investigators might be due to differences in the types of cells used in the experiments.

Aaron and Ciombor (1996) reported an increase in bone and cartilage differentiation with a pulsed electromagnetic field used clinically for bone healing. RNA (mRNA) for the extracellular matrix molecules, proteoglycan and type II collagen have been shown to be upregulated by this PEMF. Aaron et al. (1999) further studied the ability of 60 Hz magnetic fields *in vivo* to accelerate cell differentiation in endochondral ossification. The acceleration of chondrogenic differentiation is associated with increased expression of transforming growth factor β_1 (TGFβ_1) mRNA and protein. DBM was implanted subcutaneously along the thoracic musculature in young, male Sprague-Dawley rats. The rats bearing DBM implants were exposed to 60 Hz magnetic fields of various strengths and daily exposure durations. Rats were placed in the exposure facility on the day after DBM implantation and were killed on day 8 of ossicle development, the time of maximal chondrogenesis. Significant increases in chondrogenesis were observed at 0.07 mT and 0.1 mT, with the largest response occurring with 0.1 mT and 7h/d exposure. Stimulation of chondrogenic differentiation was confirmed by increased expression of mRNA for the extracellular matrix proteins, proteoglycan (+57%) and type II collagen (+56%). These data demonstrate that transcription of mRNA for tissue growth factor (TGFβ_1),as well as the accumulation of active TGFβ_1 protein, are up-regulated by 60 Hz magnetic fields coincident with accelerated chondrogenic differentiation, suggesting these magnetic field effects might be mediated by TGFβ_1. Because TGFβ_1 has multiple regulatory

roles in many tissues, the ability of magnetic field to regulate TGFβ_1 suggests that TGFβ_1 may be general effector of magnetic field activity and might account for the wide variety of effects observed with magnetic field stimulation.

3.6 Immune Responses

3.6.1 Lymphocyte proliferation

In general, lymphocytes respond to foreign substances by proliferation and functional activation. Cossarizza et al. (1989) assessed the effect of a pulsed magnetic field (50 Hz, 2.5 mT) on the proliferation of human lymphocytes from young and aged subjects. Exposure to an ELF-pulsed magnetic field caused an increase of cell proliferation in lymphocytes stimulated by phytohaemagglutinin (PHA); this occurred in lymphocytes from both young and old subjects. Interestingly, lymphocytes from older individuals (mean age 86 years) had reduced proliferative responses when stimulated by PHA, as compared to lymphocytes from young adult subjects (mean age 24 years).

Murthy et al. (1995) investigated the effects of combined 60 Hz electric and magnetic field exposure on immune responses of baboon (*Papio cynocephalus*). The experimental design used pre-exposure, exposure, and post-exposure periods (each 6 wks in duration) with field-exposed and sham-exposed groups. In the fifth week of the combined 6 kV/m vertical electric field and horizontal magnetic field of 50 μT for 12h/d exposure, reductions were observed in $CD3^+$ and $CD4^+$ counts, IL-2 receptor expression (Fig. 3.7), and proliferative responses to pokeweed mitogen (PWM). By the fifth week of the post-exposure period, these immune parameters had returned to the baseline levels.

Tremblay et al. (1996) asked if 60 Hz magnetic field exposure would affect immune responses in a dose-dependent, time-dependent fashion. Fischer F344/N rats were exposed for 6 weeks to 60 Hz, linearly polarized, sinusoidal continuous-wave magnetic fields at sham, 2, 20, 200 and 2000 μT. Magnetic field exposure induced decreases in the number of $CD4^+$, $CD5^+$, and $CD8^+$ T cells, and linear regression analysis revealed a negative dose-response relationship for these three cell populations. Evaluation of splenic NK cell activity demonstrated 50% increase at the 2000 μT intensity, and the linear regression indicated a positive dose-response relationship between splenic NK cell activity and magnetic field intensity. These results suggest that an *in vivo* exposure of rats for 6 weeks to 60 Hz magnetic fields can induce significant immunological perturbations on effector cells of both natural and adaptive immunity in a dose-dependent manner.

Contrary to the above mentioned reports, Thun-Battersby et al. (1999) could not find any effects on leukocyte proliferation following *in vivo* exposure of female Sprague-Dawley rats to a linearly polarized, 50 Hz magnetic field at a flux density of 100 μT for periods of 3 or 14 days or 13 weeks. T-lymphocytes ($CD4^+$, $CD8^+$, $CD4^-$ and $CD8^-$), NK cells, B lymphocytes, macrophages were assessed, along with granulocytes in blood, spleen and mesenteric lymph nodes. Apparently this study did not use mitogen-stimulating agents, such Con A or PWM.

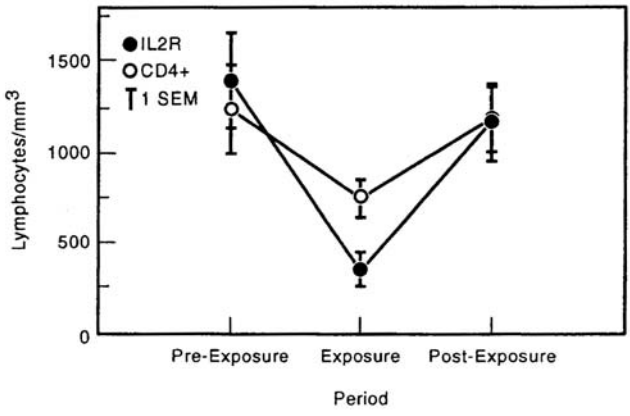

Fig. 3.7. Effects of 6 kV/m and 50 μT exposure on $CD4^+$ and interleukin 2 receptors. Open circles are for sham-exposed subjects (n = 6), and solid circles are for field-exposed subjects (n = 6). $CD4^+$ and interleukin 2 receptor (IL2R) T cells were enumerated from peripheral blood collected from baboons in week 5 of pre-exposure, exposure, and post-exposure periods, each 6 weeks in duration (Murthy et al. 1995).

Korneva et al. (1999) reported the results of three sets of experiments on the effects of 22 μT, sinusoidal, 50 Hz magnetic field applied for 1 h on 5 successive days on the level of host defense and on spleen colony formation in CBA male mice. The first set of experiments assessed effects on the ability of the bone marrow to produce precursor cells, as measured by exogenous and endogenous spleen colony-forming units (CFUs), and on the cellularity of the thymus. A 36% increase in the thymus weight and ratio of thymus to kidney weight were measured 1 h after the last of the repeated magnetic field exposures. There were no differences 24 or 96 hours after cessation of magnetic field exposure. In the second set of experiments, the mice were given a sub-lethal dose of X-ray (6 Gy) followed 2 h later by the first magnetic field exposure. The magnetic field exposure was repeated for 5 days as in the first experiment. The number of colonies per spleen showed a 96% increase with magnetic field exposure. In the third set of experiments, bone marrow was taken from mice that had been exposed to 22 μT fields and injected into mice which had been exposed to a lethal dose of X-ray (9.2 Gy). The mean number of CFUs per femur in the recipient mice was shown to be reduced at day 1 (54%) and day 4 (33%). The authors interpret the reduction of CFUs per femur as being due to the results of enhanced precursor cell migration, not to depression of proliferative activity.

Overall, Korneva et al. (1999) showed a stimulating effect of repeated magnetic field exposure on the level of host defense, by modulating activity in both mature immunity cells and precursor cells.

3.6.2 T lymphocyte activity

Mevissen et al. (1998) investigated the effects of 50 Hz, 100 μT magnetic field exposure, given to young-adult rats for 2, 4, 8, or 13 weeks, on the immune surveillance system. Following the different exposure periods, splenic lymphocytes were cultured and the proliferative responses to the T-cell selective mitogen concavalin A (Con A) and to the B-cell-selective mitogen PWM were determined. A triphasic alteration in T-cell function during the 13-week exposure period was observed: splenic lymphocyte blastogenesis was markedly enhanced after 2 weeks; a less marked increase was apparent after 4 weeks, no difference from sham control was observed after 8 weeks; and then a decrease was apparent following 13 weeks. In contrast to T cells, the mitogenic responsiveness of B cells and IL-1 production of PWM-stimulated cells were not altered during magnetic field exposure.

3.6.3 Natural killer cell activity

House et al. (1996) studied the effects of linearly polarized, 60 Hz magnetic field exposure at 0, 2, 200 or 1000 μT for either 28 or 90 days on the host immune responses in young adult B6C3F1 mice. Of many immune functions - such as antibody-forming cell responses, delayed-type hypersensitivity response, and natural killer (NK) cell activity - only NK cell activity differed in exposed animals. Mice exposed to 1000 μT for 28 days showed an increase ($P < 0.05$); NK cell activity was decreased ($P < 0.05$) after 90 days of exposure at 0.2 and 1 mT continuous and intermittent exposure.

Because NK cell activity in mice decreases with age (e.g., Albright and Albright 1998), House and McCormick (2000) further studied the effect of the above mentioned magnetic field exposure on mature (> 1 year old) mice, finding that continuous exposure at 1 mT for 90 days resulted in depression (18%) of NK cell activity. Exposure to lower flux densities (2 or 200 μT) had no effect.

The effects on NK cell activity on young adults somewhat resembles the triphasic effects of magnetic field exposure on T-cell function reported by Mevissen et al. (1998). In both studies an increase of the function was observed following 2–4 weeks exposure , but a decrease followed 13 weeks of exposure.

3.6.4 Cytokines

In the "normal" or "resting" condition (i.e., without mitogen stimulated activity) Pessina and Aldinucci (1998), Pessina et al. (1999), and Hefeneider et al. (2001) have reported no change in such cytokines as IL-1,IL-2, IL-6, and TNFα. However, Jonai et al. (1996) reported production of IL-1β was significantly increased by exposure at 1 and 3 mT and IFN-γ was decreased at 10 mT. All field-exposed cells (1, 3, 10 and 30 mT) showed decreases in TNF-α production. When the cells were stimulated with such substances as PHA before the magnetic field exposure, Pessina and Aldinucci (1998) found that the levels of IL-1β, IL-2, IL-6, and TNFα were higher.

Haussler et al. (1999) performed experiments to evaluate possible effects of exposure of rats to a 50 Hz, linearly polarized, 100 μT magnetic field on the *ex vivo*

production of interleukins by mitogen-stimulated splenic lymphocytes. No significant effect was observed on IL-1 and IL-2 production, contrary to the results obtained by Pessina and Aldinucci.

As mentioned above, experimental outcomes from immunological works vary considerably. Experimental conditions also vary greatly with respect to such potentially important variables as magnetic field parameters (e.g., sinusoidal vs. pulsed, duration, intensity, etc.), use of *in vitro* vs. *in vivo* or *ex vivo* conditions, animal species, ages, gender, season, etc. It seems reasonable to assume differences in these factors might contribute to differences in experimental results.

3.7 References

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4

Experimental Results: *In vitro*

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It is important to understand the mechanism of the effects of ELF magnetic fields on living organisms. Results of many previous *in vitro* studies have offered suggestions as to the mechanism of action. In general, results of *in vitro* studies conducted at extremely low magnetic flux densities (micro tesla), which are close to those in the living environment, have shown both positive and negative effects, thereby not leading to a consistent conclusion. However, studies with very intense magnetic fields (e.g. > 10 mT) more consistently tend to indicate occurrence of positive effects.

To really understand the mechanism of action, and so be able to make informed statements about the meaning of effects, it is important to find a cell system that responds reliably and robustly to ELF magnetic fields. The identification and scientific validation of such a system would be very important: it would allow (1) investigation of a dose-response relationship in the response of cells to ELF magnetic field exposure, and (2) evaluation of the effects of ELF magnetic fields on a biological system so that the mechanism of action could be pursued.

This chapter describes the response of cells to ELF magnetic fields at the molecular and cellular levels, including the results in studies of exposure to extremely strong magnetic fields (up to 400 mT).

4.1 Genotoxic Effects

The influence of ELF magnetic fields on genotoxic effects, including mutation, is particularly important, because genotoxicity is considered to be a critical factor for the multistage process of signal transduction, gene expression, cell proliferation, and carcinogenesis. The literature is complex and varied. Many published papers report that positive effects do occur in cell systems as a result of exposure to ELF magnetic fields, but many other studies do not. Unfortunately, there is such diversity in both independent and dependent variables that it is hard to reach firm conclusions based.

4.1.1 Chromosomal aberrations

Many researchers have determined the frequency of chromosomal aberrations and sister chromatid exchange (SCE) in response to ELF magnetic field exposure. Usually these studies were conducted using human peripheral blood lymphocytes. Typically, exposure to ELF magnetic fields (30 μT – 7.5 mT) did not cause an increase in the frequency of chromosomal aberrations or SCE (e.g., Antonopoulos et al. 1995, Cohen et al. 1986ab, Rosenthal and Obe 1989, Paile et al. 1995, Garcia-Sagredo et al. 1990). In this chapter a difference or a change typically is mentioned only when it is statistically significant. In the few instances where a result that is not statistically significant is mentioned, the exception is noted.

In a study using Chinese hamster V-79 cells, 24 h exposure to 25 μsec pulses of a magnetic field (0.18 – 2.5 mT) did not result in an increase in SCEs (Takahashi et al. 1987). However, in a study using human blood lymphocytes from three males, continuous exposure (72 h) to 10 msec pulses of a magnetic field (50 Hz, 1.05 mT) caused a significant increase in chromosomal aberration and SCE frequency (Khalil and Qassem 1991).

An increase in SCE frequency also was found in a study of 42 h exposure of mouse m5S cells to a highly intense (400 mT, 50 Hz) ELF magnetic field (Yaguchi et al. 1999). On the other hand, in a study also using m5S cells exposure to magnetic fields equal to or less than 50 mT did not cause an increase in SCE frequency.

Yaguchi et al. (2000) studied chromosome aberrations in m5S cells after treatment with ELF magnetic field (5 – 400 mT), mitomycin C, or X-rays. In a flux-density-dependent manner ELF magnetic field exposure enhanced the formation of spontaneous, antibiotic-induced, and X-ray-induced chromosomal aberrations (Table 4.1.). Increases in the frequency of chromosomal aberrations were observed in cells exposed to 400 mT, compared with unexposed controls. The aberrations induced by the ELF magnetic fields mostly were chromatid-type, not chromosomal-type. The cells exposed to 400 mT ELF magnetic field exhibited a three-fold higher level of chromatid-type aberrations than did the unexposed cells.

Continuous and intermittent (On/off every 15 sec) exposure of human amniotic cells to a 30 μT magnetic field over a 72 h period produced chromosomal aberrations, such as gaps; the frequency of aberrations was approximately doubled in field-exposed cells (Nordenson et al. 1994). In this report, however, an increased frequency of chromosomal aberration was not observed when continuous exposure to a 300 μT magnetic field was applied to the cells.

When mouse m5S cells were given concomitant treatment of 50 or 400 mT ELF magnetic field with either X-rays or mitomycin C, the frequency of chromatid-type aberrations was enhanced by field exposure (Yaguchi et al. 2000). Also, in a study using peripheral human blood lymphocytes, combined ELF magnetic field exposure (1.4 mT, 60 Hz) and ionizing radiation (3 Gy) produced a synergistic effect on the frequency of tetraploid chromosome appearance, compared to the effect produced by the ionizing radiation alone (Hintenlang 1993).

In summary, based on the available literature, there is little possibility that exposure to an ELF magnetic field of less than several mT will cause SCE and chromoso-

Table 4.1. Chromosomal Aberration in m5S Cells after Treatment with ELF Magnetic fields, Mitomycin C, or X-rays

Treatment[a]			Chromosomal Aberrations[b]								Aberrations / 100 cells		% Aberrant cells	
			Chromatid-type				Chromosome-type							
Pre-treatment	MF (mT)	No. of cells Analysed	ctg	csg	ctb	cte	Frag	Min	Dic	Ring	Total	Excl[c]	Total	Excl[c]
–	–	200	9	0	4	1	0	1	0	0	7.5	3	7	3
–	5	200	11	2	6	4	2	3	0	0	14	7.5	13[*d]	6.5
–	50	200	17	3	6	2	3	3	0	1	17.5	7.5	16[**d]	7.5[*d]
–	400	200	22	7	11	4	5	1	3	0	26.5	12	20[***d]	10[**d]
MMC	–	200	21	3	14	35	2	3	0	1	39.5	27.5	38	24.5
MMC	5	200	20	6	21	37	2	1	3	0	45	32	33.5	23
MMC	50	200	25	4	25	55	7	0	4	0	60	45.5	41.5	32.5
MMC	400	200	88	5	51	67	9	2	1	1	112	65.5	66.5[***e]	48.5[***e]
X-rays	–	100	5	2	5	4	26	22	21	5	90	83	64	61
X-rays	5	100	16	3	5	7	36	38	24	6	135	116	71	63
X-rays	50	100	15	2	12	12	26	30	37	3	137	120	74	68
X-rays	400	100	25	3	12	8	26	29	43	8	154	126	76	74[*f]

[a] Cells were pretreated or untreated with either MMC (1 μM for 1 h) or X-rays (3 Gy) and then exposed or unexposed to ELF magnetic field (400 mT) for 40 h.

[b] ctg = chromatid gaps, csg = isochromatid gaps, cte = chromatid exchanges. Isochromatid breaks (csb) were included in acentric fragments (Frag). Min = minute chromosomes, Dic = dicentrics.

[c] Excluding gaps.

[d] $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$; statistically different from the unexposed controls (Chi square test).

[e] $^{***}p < 0.0001$; statistically significant, compared to cells treated with MMC alone (Chi square test)

[f] $^{*}p < 0.05$; statistically significant, compared to cells treated with X-rays alone (Chi square test)

MMC = mitomycin C

mal aberrations in cells. However, stronger (> 50 mT) ELF magnetic fields do appear to cause effects, especially when combined with chemical or physical (X-rays) mutagens.

4.1.2 DNA strand breaks

Many chemicals can produce toxicity by damaging the chemical bonds of DNA, resulting in breakage. If only one strand is broken, the intact strand can readily serve as a template for repair. If both strands are broken, repairs can be made, but the possibility of error, which remains very low, is greater. Given the importance of DNA, cells have highly efficient DNA repair mechanisms. However, no repair process is 100% effective.

4.1.2.1 Induction of strand breakage

It generally is considered that the energy of an ELF magnetic field is insufficient by itself to physically cause breakage of DNA strands. In contrast, ionizing radiation, which is far more energetic, can cause breakage of DNA strands. Many experiment have been conducted to assess the possibility that exposure to ELF magnetic fields can break strands of DNA.

In most of the reported experiments, no DNA single-strand breakage was observed when the cells were exposed acutely to ELF magnetic fields (0.2 μT to 5 mT), e.g., Reese et al. (1988), Fiorani et al. (1992), Fairbairn and O'Neill (1994) and Cantoni et al. (1996).

In contrast, Lai and Singh (1997) reported on a study using *in vivo* exposure followed by *in vitro* assay. Rat brains were exposed to magnetic fields (0.1 – 0.5 mT) for 2 h; cerebral cells were removed for assay 4 h after exposure. The results of a comet assay indicated an increase in the frequency of single-strand breaks. This proactive finding stimulated additional research on this unanticipated possibility.

In a different study using the comet assay with human brain tumor (MO54) cells, there was no significant increase in DNA strand breaks when the cells were exposed to ELF magnetic fields (5 – 400 mT) for 30 min (Miyakoshi et al. 2000a). Also, in a study examining the effects of ELF magnetic field and oxidative stress, in which cells were treated with H_2O_2 and subsequently exposed to a magnetic field at 50 Hz (3 msec pulses) and 5 mT, the results of the comet assay revealed that there was no influence on DNA single-strand breakage (Fairbairn and O'Neill 1994). Similarly, exposure to 0.2 – 100 μT magnetic fields (50 Hz) had no influence on DNA single-strand breaks in cells treated with methylmethan sulfonate, chromate, ultraviolet light, or H_2O_2 (Cantoni et al. 1995: 1996).

Collectively, the recent data continue to indicate that, by itself ELF, magnetic field exposure does not cause single-strand breakage of DNA.

Some researchers have examined the related question of whether ELF magnetic field exposure can modify the repair of DNA damage induced by another source, such as ionizing radiation. Given that chemicals and background radiation normally result in a high rate of DNA damage and repair, it would be important if ELF magnetic fields affected the important normal cellular processes of DNA repair.

Following 50 μT - 2.5 mT magnetic field exposures, there was no inhibition of repair – which was determined using indexes of rejoining activity and unscheduled DNA synthesis activity – of DNA single-strand breaks (Cossarizza et al. 1989a, Frazier et al. 1990). However, when cells were exposed to 50 or 400 mT magnetic fields for 30 min after X-ray irradiation at 4°C, the results of an alkaline comet assay showed only a slight increase in the number of DNA strand breaks (Miyakoshi et al. 2000a).

In summary, the limited set of results available do not strongly support the idea that ELF magnetic field exposure can alter DNA repair initiated by ionizing radiation, even at field strengths of 50 mT or greater.

4.1.3 Micronucleus formation

The effects of ELF electric or magnetic fields have been Examined using micronuclei formation as the dependent variable. Micronucleus formation occurs when the chromosome, in part or in its entirety, is released from the nucleus. This is another form of DNA damage.

For example, Scarfi et al. (1993) examined genotoxic effects of 50 Hz (AC sinusoidal) electric fields of 0.5, 2, 5, and 10 kV/m using human lymphocytes. At all intensities tested, no differences in the frequency of micronucleui formation were found between exposed and control (unexposed) cultures. When the mutagen mitomycin C was added to the cultures, the micronucleus frequency was increased significantly, but no difference was found between field-exposed and unexposed cultures.

Many reports have shown no positive effect of 30 μT - 2.5 mT ELF magnetic field exposure on formation of micronuclei. (Saalman et al. 1991, Scarfi et al. 1991: 1994: 1999, Lagroye and Pongy 1997).

In contrast, a positive effect was reported by Simko et al. (1998b): an increase of the frequency of micronucleus formation was observed in human squamous cell carcinoma (SCL II) cells after 48 h and 72 h of continuous exposure to 50 Hz magnetic field (0.8 and 1.0 mT). However, in the same study, when using a non-transformed human amniotic fluid cell line, there was no increase in micronuclei by ELF magnetic field exposure. Another report by the same group (Simko et al. 1998a) indicated an increased frequency of micronucleus formation by amniotic fluid cell line cells with 24, 48, or 72 h of exposures to horizontal magnetic fields (50 Hz, 1 mT).

A related positive result was observed by Lagroye and Pongy (1997). To assess the modifying effect of ELF magnetic field exposure on induction of micronucleus formation by γ-rays, 24 h magnetic field exposure (50 Hz, 100 μT) was given following 6 Gy γ-ray irradiation. Magnetic field exposure caused an increase in the number of binucleate cells with micronuclei present, compared to the frequency produced by γ-ray irradiation alone.

The limited data base on the effects of ELF electric or magnetic field exposure on micronucleus formation is mixed. Both positive and negative effects have been reported from a small number of experiments conducted using a diverse array of field conditions. To obtain a clear picture, further research is required. It would be especially helpful if replicate studies were conducted using a limited set of very similar exposure conditions.

4.1.4 Mutation

The Ames assay using *Salmonella typhimurium* revealed that there was no effect of magnetic field exposure (0.3 mT; 60, 600, and 6000 Hz; 48 h) on mutation frequency (Morandi et al. 1996). In studies using mammalian cells, exposure of the Chinese hamster ovary (CHO) cells to ELF magnetic fields (1 μT; 50 Hz; 7 days) did not cause a significant increase in the mutation frequency of the gene encoding the hypoxanthine-guanine phosphoribosyl transferase (HPRT) enzyme (Nafziger et al. 1993). However, exposure of human melanoma (MeWo) cells to a highly intense

magnetic field (400 mT, 50 Hz) resulted in an increase in the number of HPRT gene mutations (Miyakoshi et al. 1996b: 1997). When the cells were exposed to the ELF magnetic field (400 mT, 50 Hz) in an annular culture plate (diameter: 15 cm), the frequency of mutation of the HPRT gene increased from the center of the plate toward the edge (Miyakoshi et al. 1996b). This suggests it is the induced electric current, rather than the applied magnetic field that is producing the effect. Furthermore, the induced mutation increased with the current density produced by the ELF magnetic field (Miyakoshi et al. 1996b).

Under conditions of inhibited DNA synthesis, no mutation induction was observed (Miyakoshi et al. 1996b). However, there were increased mutations during the S phase (DNA synthesizing phase) of the cell cycle (Miyakoshi et al. 1997). These results suggest that there is a good correlation between DNA synthesis and increased mutations caused by exposure to a highly intense ELF magnetic field. However, it appears that weak (i.e., environmentally relevant) magnetic fields have no effect on mutation frequency.

Also, in a study of the effect of ELF electric field exposure, in which an annular plate was used, the somatic mutation frequency in *Drosophila melanogaster* larvae was increased as a function of induced current (Koana et al. 2001). In a study using CHO cells, a 10-h ELF electric field exposure (10 V/m, 60 Hz) increased, by about two-fold, the HPRT gene mutation frequency, compared to sham-exposed cells (Ding et al. 2001).

Saos-LP-12 cells, which express the normal p53 gene due to the Lac switch system, were isolated from the human osteosarcoma Saos-2 cells, and then the p53 gene was deleted. These cells were subsequently exposed to an intense ELF magnetic field (400 mT, 50 Hz). The increased mutation frequency induced by the ELF magnetic field was inhibited by the induction of normal p53 expression (Miyakoshi et al. 1998a). These results suggest that the normal p53 protein is intimately involved in the repair processes associated with mutations caused by exposure to ELF magnetic fields.

In a study of 5 mT ELF magnetic field exposure, continuous 6-week exposure did not significantly increase the mutation frequency in CHO-K1 cells (Miyakoshi et al. 1999). Considering the magnetic intensity used in studies on the positive effects of such exposure, measured as an increase in mutation frequency, there is little possibility that the mutations are caused in the everyday environment, which entails exposure to ELF magnetic fields at micro tesla levels. The concomitant exposure to an ELF magnetic field (3 mT, 60 Hz) and the chemicals menadione (which induces the formation of free radicals), or methylnitrosourea (an alkylating agent that damages chemical bonds in DNA), did not influence the mutation rate of rat embryo fibroblast cells, which were induced using *E-coli lacI* gene target (R2λLIZ) (Suri et al. 1996).

With ionizing radiation-induced mutation, two research groups have revealed that HPRT gene mutation frequency in CHO-K1 cells was increased by 0.7 and 5 mT ELF magnetic field exposures following irradiation (Miyakoshi et al. 1999, Walleczek et al. 1999). Following X-ray irradiation (4 Gy), human glioma MO54-SY4 cells, whose NF-κB gene was inactivated by induction of mutant Iκ-Bα, an inhibitor

of phosphorylation, were cultivated for 8 days under the magnetic field (5 mT, 60 Hz). The frequency of the HPRT gene mutation was increased approximately 4-fold relative to that caused by X-rays alone (Ding et al. 2000). These results suggest the possibility that the ELF magnetic field might modify the cellular actions of ionizing radiation and some chemical agents.

In summary, there is little evidence that weak ELF magnetic fields directly cause mutations in quiescent cells. However, there is evidence that ELF magnetic fields can affect mutation-related processes when (1) the cells are in an activated state and/or (2) other mutagentic agents are administered along with the magnetic field exposure. Effects are reported more commonly with mT fields than with μT fields.

4.2 Cellular Proliferation

If a signal increases cell proliferation, i.e., growth, it increases the risk of carcinogenesis. Non-dividing cells are at little risk, but dividing cells are vulnerable to various forms of DNA damage and/or faulty DNA repair. An uncontrolled growth signal is not good for cells. If ELF magnetic fields stimulated cellular proliferation, it would have bad implications with for carcinogenicity. Conversely, a signal that leads to a reduction of cellular proliferation, or to cell death, has bad implications for toxicity. Therefore, many researchers have examined these possibilities using a variety of similar or inter-related approaches.

4.2.1 Cell proliferation and survival

In most reports to date, there is little possibility that the ELF magnetic field exposure induces cell killing, irrespective of the magnetic intensity. For example, in studies of changes in cell proliferation conducted using CHO cells, in comparison to growth curves, there was no significant effect of the ELF magnetic field exposure at either 220 μT or 5 mT (Livingston et al. 1991, Miyakoshi et al. 1996a).

However, positive effects have been reported. A single exposure for 1 h to a 2 mT, 50 Hz magnetic field caused quasi-oscillatory changes in SV40-3T3 mouse fibroblast cells (Schimmelpfeng and Dertinger, 1997). Also, a 30 min exposure (50 Hz, 80 μT) resulted in an increase in cell proliferation rate in human AMA cells (Kwee and Raskmark 1995). In this report, however, effects of ELF magnetic field exposures were not observed at more or less than 80 μT or with exposure times longer or shorter than 30 minutes.

Human skin cells from normal subjects and ataxia telangiectasia patients were exposed to an ELF magnetic field (400 mT, 50 Hz) for 2 h following X-ray irritation. Field exposure had no effect on the survival of either cell line (Miyakoshi et al. 1994).

In a study of ELF magnetic field exposure (45 mT, 1 h) using human myelogenous HL60 cells, an increased number of apoptotic cells was observed (Narita et al. 1997). In that study, however, no apoptosis was observed in the human peripheral lymphocytes that were exposed to the ELF magnetic field.

Effects of rapidly changing magnetic gradient fields were examined on fetal human lung fibroblasts. The cells were exposed for 2 to 24 h to trapezoid-shaped waveforms of 500 and 75 Hz base frequency and an amplitude of 2 mT. A specially designed incubation system was placed inside the magnet (Rodegerdts et al. 2000). Controls were sham-exposed simultaneously in an identical incubation chamber and treated exactly the same way as exposed cells, except that the incubation chamber was placed outside the magnet room. Cell cycle analysis was carried out until 24 h after exposure to detect alterations in cell division. In addition, cell proliferation was monitored for 3 wks after exposure. There were no differences in proliferation and cell cycle distribution of exposed and unexposed cells.

The conventional wisdom is that ELF magnetic fields do not promote cell death. However, there are experiments which indicate that this viewpoint is not correct. Once again, further research will be required to sort out the tangle of unanswered questions produced by the use of diverse exposure regimens and cell assessment strategies.

4.2.2 Melatonin and cell proliferation

It long has been recognized that melatonin is oncostatic, i.e., it inhibits the growth of cells. It would have important implications if it was established the ELF magnetic fields inhibited an inhibitor, producing a net increase in cellular growth.

Liburdy et al. (1993) reported effects of ELF magnetic field exposures on cell proliferation of human breast cancer MCF-7 cells. It is known that melatonin, the primary neurohormone of the pineal gland, inhibits the proliferation of MCF-7 cells when cells are treated with it at physiological concentrations. When cells were exposed to a sinusoidal field (1.2 μT, 60 Hz) with concomitant melatonin treatment, the cell proliferation-inhibiting effect of melatonin was reduced. In the same series of studies, it was demonstrated that exposure to ELF magnetic fields reduced the ability of tamoxifen to inhibit cell proliferation (Harland and Liburdy 1997). Tamoxifen is drug clinically used for the treatment of breast cancer because it inhibits cell proliferation. However, when using a full-wave rectified 60 Hz field of 1.2 μT, these effects were not observed.

The inhibition of the effect of melatonin by the ELF magnetic field exposure was interpreted as follows: the "melatonin effect" is inhibited because a power-frequency magnetic field reduces the ability of melatonin to scavenge free radicals. This proactive study has received considerable attention.

In a study using MCF-7 cells (which had been obtained from the Japanese Cell Bank), the effect of ELF magnetic field exposure on the oncostatic action of melatonin was not verified (Tachiiri et al. 1999). It was hypothesized that these findings differed from those of Liburdy et al. (1993) because of possible differences in the property of the MCF-7 cell lines used. The MCF-7 cell line used by Liburdy *et al.* was very sensitive to melatonin and surely had an estrogen receptor; however, the MCF-7 cell line used by Tachiiri et al. (1999) had a weak response to melatonin and possibly had no estrogen receptor. In medical practice, whether a patient's breast

cancer cells are positive or negative for estrogen receptors has a considerable impact on choice of chemotheraputic drug.

The results of Liburdy et al. (1993) provoked considerable interest, and various groups tried to replicate and/or extend the work. As has happened many times in ELF bioelectromagnetics, the "hot" new phenomenon gradually faded away, primarily because addition positive studies were not published. Also, interest moved on to other topics before all of the hard work needed to fully understand the situation could be completed.

4.2.3 DNA synthesis

The results of studies using DNA synthesis as an index of cellular growth can be broadly classified into three categories: (1) stimulation, (2) inhibition, and (3) no effect. Two examples of each kind of outcome are summarized briefly below.

For example, when human fibroblast cells were exposed to various ELF magnetic fields (2.3 – 560 μT) of different frequencies (15 – 4000 Hz), DNA synthesis was promoted (Liboff et al. 1984). Exposures to pulsing magnetic fields of 0.18 to 2.5 mT also stimulated DNA synthesis (Takahashi et al. 1986).

On the other hand, ELF magnetic field exposures (230 – 650 μT, 1 – 200 Hz) inhibited DNA synthesis in the human lymphocytes, the division of which had been induced by phytohaemagglutinin (Conti et al. 1983, Mooney et al. 1986).

Finally, in a study using normal human fibroblasts (HL-19), 30 h exposure of to ELF magnetic fields (20 μT – 20 mT, 50 Hz) had no effect on DNA synthesis (Cridland et al. 1996).

4.3 Gene Expression

Because of its fundamental biological importance, including implications for carcinogenesis, gene expression is one of the fields that has been examined and discussed with considerable animation. It is a fundamental question when one considers the molecular aspects of ELF magnetic fields. Although a variety of cellular systems could be used to examine this issue, a handful of dependent variables have received the most attention: they are *c-myc*, *c-fos*, and *c-jun*. All of these genes are proto-oncogenes. Oncogenes are genes that, when they become activated, transform normal cells into cancer cells. Proto-oncogenes are genes that are readily converted to oncogenes.

4.3.1 *c-myc*

Goodman and Henderson (1991) completed a study using human leukemia- derived HL60 cells; the transcription (mRNA level) of *c-myc*, an oncogene, was enhanced by a 20 min exposure to an ELF magnetic field (200 μT – 2.3 mT, 15 – 150 Hz). The maximum expression occurred at 45 Hz. It is known that the *c-myc* gene is intimately

involved in cell proliferation, and its transcription activity is transiently enhanced by the stimulation such as growth factors.

The same research group (Lin et al. 1994) used chloramphenicol transferase expression as a marker of *c-myc* activation following ELF magnetic field exposure. A construct consisting of the upstream of the *c-myc* gene and expressing chloramphenicol transferase was transfected into human HeLa cells. Chloramphenicol transferase expression was enhanced following a 20 min ELF magnetic field exposure (8 μT, 60 Hz). These results indicate that a specific region of the *c-myc* promoter is responsive to the ELF magnetic field.

However, several other research groups have failed to reproduce the effect of enhanced *c-myc* expression by ELF magnetic field exposure (Lacy-Hulbert et al. 1995, Saffer and Thurston 1995, Balcer-Kubiczek et al. 1996, Miyakoshi et al. 1996a).

In a study using human HL-60 cells, ELF magnetic field exposure (60 Hz, 1 mT, 75 min) caused no change in transcription rate of *c-myc* or β-actin but enhanced 45S-RNA transcription (Greene et al. 1993). Phillips et al. (1992) used human lymphoblastoid cells (CEM-CM3), and the transcription activity of protein kinase C (PKC) was investigated. PKC is involved with several oncogenes (*c-fos*, *c-jun*, *c-myc*) and with signal transduction. It was reported that the transcription activity was increased by exposure to an ELF magnetic field exposure (60 Hz and 100 μT).

4.3.2 *c-fos* and *c-jun*

Many researchers have examined the effect of ELF magnetic fields on the expression of other oncogenes, such as *c-fos* and *c-jun*.

In a study using CEM-CM3 T-lymphoblastoid cells, exposure of cells to the ELF magnetic field (60 Hz, 100 μT, 30 min) up-regulated *c-fos* transcription (Phillips et al. 1992). In this study, it also was reported that ELF magnetic fields caused an increase in *c-jun*.

In another study, a chloramphenicol transferase gene construct, containing the upstream regulatory region of the *c-fos* gene (from base pairs −700 to +42), was transfected into HeLa cells. The cells subsequently were exposed to an ELF magnetic field (60 Hz, 6 μT, 20 min): there was an approximate 1.2-fold increase in chloramphenicol transferase expression after 60 minutes (Rao and Henderson 1996).

Another group has reported similar positive findings. Primary rat-derived RTE cells, which were exposed to an ELF magnetic field (50 Hz, 100 μT, 30 min), had approximately a three-fold enhancement of *c-jun* protein expression (Lagroye and Poncy 1998). In the same study, however, *c-fos* expression was decreased to approximately 70% of control levels following 5-h ELF magnetic field exposure.

Finally, in a study using HL60 cells, 10 to 40 minute ELF magnetic field exposures (60 Hz; 5.7 or 570 μT) caused no change in steady-state mRNA expression for *c-fos* (Balcer-Kubczek et al. 1996).

The existence of at least four positive reports from two research groups suggests that ELF magnetic field exposure *in vitro* can affect gene expression. However, the existence of at least one negative report also suggests that all of the conditions

involved in this effect are not understood. Given the importance of the question, additional research is highly desirable. It should be noted that the relationship of these *in vitro* observations to carcinogenicity *in vivo* remains to be determined.

4.3.3 Heat shock protein

The effects of ELF magnetic fields also have been assessed from the perspective of the induction of cellular stress. An increase in amount of heat shock protein (hsp) is considered to be part of a cell's generalized response to physical stress, such as elevated temperature Because it has been speculated that ELF magnetic field exposure is an environmental stressor for cells, investigators have examined hsp production following magnetic field exposure.

It had been reported previously that ELF magnetic fields alter the expression of hsp. The Goodman research group, which had demonstrated increased *c-myc* transcription of HL-60 cells was produced by exposure to ELF magnetic fields, exposed HL-60 cells to a 60 Hz, 8 μT signal for 20 min. They found that transcription of hsp70 was increased by about 1.8-fold (Goodman et al. 1994).

Furthermore, the same research group (Lin et al. 1998b) observed that yeast (*Saccharomyces cerevisiae*) cells showed increased transcription of the heat shock gene SSA1 under the same conditions. Furthermore, using HL60 cells and the CAT assay, they confirmed that the increased expression of hsp70 induced by ELF magnetic field exposure was caused by the binding of *c-myc* protein to sites within the hsp promoter region. The ELF magnetic field apparently increased the number of binding sites.

These results suggest that the enhancing effects of an ELF magnetic field on the transcription of hsp70 might be caused by protein-DNA binding within the hsp promoter region (Lin et al. 1998a).

However, other researchers have reported that exposure to ELF a magnetic field alone had no effect on hsp expression. In a study using C3H mouse mammary carcinoma derived 34i cells, ELF magnetic field exposure (50 Hz, 1.5 and 3 mT) had no effect on expression of hsp70 and hsp90 (Kang et al. 1998). Also in a study using HL60RG cells, 2 h or 20 h exposures to an ELF magnetic field (50 Hz, 5 mT) had no influence on expression of hsp70 protein (Miyakoshi et al. 2000c). In this study, however, hsp70 expression was inhibited by the concomitant application of 50 mT ELF magnetic field exposure and mild heat (40°C or 42°C) treatment.

4.3.4 Neuron-derived orphan receptor-1

The gene encoding the neuron-derived orphan receptor 1 (NOR-1), which belongs to the superfamily of nuclear receptors, responds with great sensitivity to various stimuli. Therefore, the effects of ELF magnetic fields on the ability of NOR-1 to act as a transcription factor have been investigated.

Miyakoshi et al. (1998b) demonstrated (Fig. 4.1.) that exposure to a 400 mT magnetic field caused a transient increase in the expression of the NOR-1 gene in CHO cells (maximum: about 6 h). However, in a similar study using 5mT, the same research group found that the NOR-1 gene was not affected by exposure to an ELF

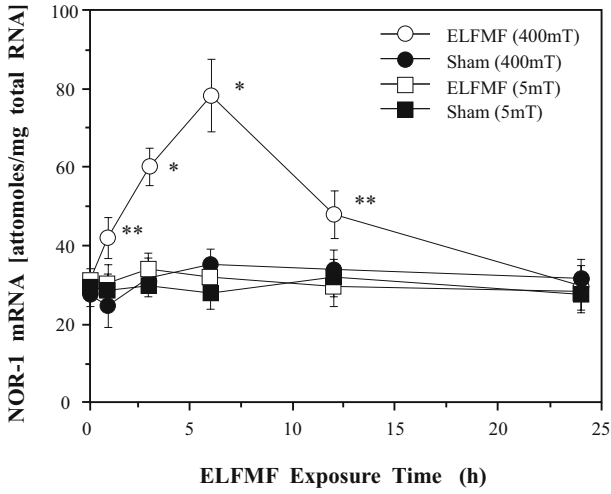

Fig. 4.1. Effect of exposure to 50 Hz magnetic fields of 5 mT or 400 mT on time course of NOR-1 mRNA levels. CHO-K1 cells were sham-exposed (open circles) or exposed to 400 mT (closed circles); other sets of cells were exposed to 5 mT (closed squares) or were sham exposed (open squares). Four plates were used for each experiment. The experiment was repeated twice (n = 8) for 5 mT and sham exposures; the experiment was repeated three times (n = 12) for 400 mT and associated sham exposures. Error bars represent S.E.M. $^{*}p < 0.01$ and $^{**}p < 0.05$), show the statistical significance of differences between 400 mT and its associated sham-exposed group.

magnetic field (Miyakoshi et al. 1998b). This suggests that there might be a field intensity threshold, which might be equal to or greater than 5 mT, for ELF magnetic field induction of NOR-1 gene expression.

4.4 Signal Transduction

Cells receive external stimuli as a signal that activates intra-nuclear genes through cytoplasmic messengers, resulting in induced gene expression and consequently increased production of proteins. This sequence of processes is called signal transduction; it is a fundamental mechanism regulating all types of cells.

The signaling pathway activating β-galactosidase in PC12-VG cells derived from pheochromocytoma cells of lacZ gene-introduced into rats serves as an example (Fig. 4.2.). Forskolin, using cAMP or protein kinase A as a messenger, is a signal that activates the lacZ gene that had been introduced into the cell nucleus. This induces transcription and produces mRNA. Other signals, such as 12-O-tetradecanoylphorbol-13-acetate (TPA) and increased free intracellular Ca^{2+} elicit a similar process, using PKC as a messenger. Then, β-galactosidase is produced as a protein product via translation. The following two sections provide explanations as to how ELF magnetic fields could have an effect on signal transduction of cells.

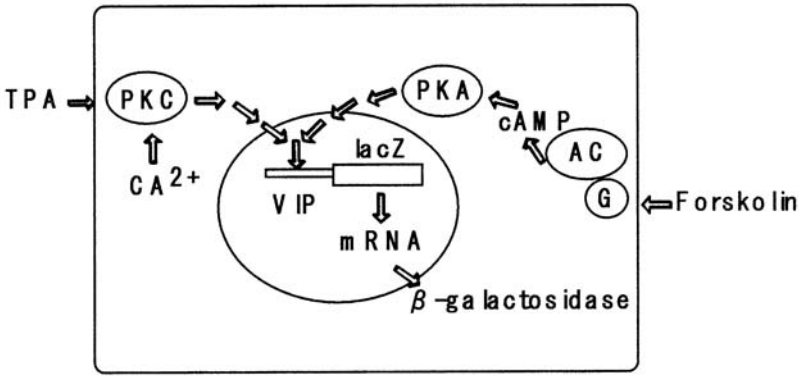

Fig. 4.2. An example of signal transduction. (See text in detail. Miyakoshi, unpublished)

Signal transduction conventionally is discussed from the perspective of molecular biology. To a molecular biologist of the late 20th century, it is an extremely obvious and important question to ask, does ELF magnetic field exposure affect signal transduction in cells? Three of the most intensively studied signal transduction pathways involve Ca^{2+}, protein kinase C (PKC), and ornithine decarboxylase (ODC), and molecular biologists interested in ELF magnetic fields have assessed each of these. In addition, two other important aspects of cellular communication, gap junctions and interleukin 2 have been used to experimentally address ELF magnetic field bioeffects.

4.4.1 Calcium ion

Many researchers are intimately involved in studying the role of Ca^{2+}, because this ion is essential to the regulation of many signal transduction pathways. As has been demonstrated repeatedly in this chapter, contemporary knowledge from molecular biology has been applied to the study of ELF magnetic field bioeffects.

In a study using the mononuclear blood cells from healthy adult volunteers, cells were stimulated by phytohaemagglutinin and exposed to a squared waveform field (3 Hz, 6 mT). Ca^{2+} uptake in the exposed cells was lower than in cells treated with phytohaemagglutinin alone (Conti et al. 1985).

On the other hand, a 60 min exposure of rat thymic lymphocytes, maintained at 37°C, to an induced electric field of 1.0 mV/cm produced an average 2.7-fold increase in concanavalin A-dependent Ca^{2+}-uptake compared to non-exposed, isothermal control (Walleczek and Liburdy 1990).

Lindstrom et al. (1993) indicate that intracellular Ca^{2+} oscillations were induced in a human T-cell line (Jurkat) by exposure to an ELF magnetic field (50 Hz, 0.1 mT). In another study by the same researchers, Jurkat cells responded to an applied magnetic field by displaying intracellular Ca^{2+} oscillations; the frequency range for

5 to 100 Hz was examined: the amplitude of the responses was greatest at 50 Hz (Lindstrom et al. 1995). Besides showing a frequency dependence, the response also showed a dependence on field intensity. No effect was observed at 0.04 m; the effect was maximal at 0.15 mT, and no further increase occurred at 0.3 mT.

On the other hand, another research group that attempted to replicate this experiment reported that the ELF magnetic fields (60 Hz, 0.15 mT) had no effect on intracellular calcium signaling in Jurkat E6-1 cells (Lyle et al. 1997).

Together, these results imply that exposure to an ELF magnetic field alone without stimulation of proliferation, i.e., without stimulation by concanavalin A or phytohaemagglutinin, had no effect on Ca^{2+}.

4.4.2 Protein kinase C

Exposure to an electric field (60 Hz, 100 to 1000 mV/cm, 1 h) significantly decreased the activity of cytosolic PKC in HL60 cells (Holian et al. 1996). However, no concomitant rise in particulate PKC activity was observed. Here the electric field apparently promoted down-regulation of cytosolic PKC activity.

ELF magnetic fields (60 Hz, 100 μT) also activate protein tyrosine kinases and PKC in human pre-B leukemia cells (Uckun et al. 1995).

Using PC12-VG cells produced by transfection of a β-galactosidase expression plasmid into rat pheochromocytoma PC12 cells, the effect of the ELF magnetic field exposure (50 Hz, 400 mT) on signal transduction was investigated (Ohtsu et al. 1995). Exposure enhanced β-galactosidase expression in PC12-VG cells stimulated by forskolin (2 μM). The enhanced β-galactosidase expression produced by this magnetic field exposure was almost completely inhibited by concomitant treatment with calphostin C, a PKC inhibitor.

Also in the effect of ELF magnetic field exposure on NOR-1 gene expression (Miyakoshi et al. 1998b) described above, the gene expression-inducing effect of 400 mT exposure was inhibited by treatment with calphostin C, or dantrolen (a Ca^{2+} influx inhibitor). These results suggest the likely possibility that the ELF magnetic field exposure has an effect on the signal transduction system involving PKC.

4.5 Ornithine Decarboxylase

Ornithine decarboxylase (ODC) activity is controlled by a signal transduction pathway involving the cytomembrane, and ODC is known as a rate-limiting enzyme in the pathway synthesizing putrescine from ornithine. (This is one of the polyamine synthesis pathways.)

In a study using human lymphoblastoid cells (CEM), mouse myeloma cells (P3) and rat hepatoma cells (Reuber H35), Byus et al. (1987) found that exposure to an ELF electric field (60 Hz, 0.1 to 10 mV/cm) caused a several-fold increase in ODC activity. The change was transient.

Also in a study using mouse L929 cells, it was shown that the exposure to an ELF magnetic field (60 Hz, 1 to 100 μT) produced an approximate two-fold increase

in ODC activity compared to control levels (Litovitz et al. 1991). However, in experiments designed to replicate this result using the same cell type, there was no significant change in ODC activity produced by the ELF magnetic fields (Azadniv et al. 1995, Cress et al. 1999).

4.5.1 Interleukin-2

The effects of the exposure of mitogen-stimulated human lymphocytes, from aged subjects, to low-frequency pulsed magnetic fields were studied by (Cossarizza et al. 1989b), who measured the production of IL-2 and the expression of IL-2 receptor. Microtiter plates were placed between a pair of Helmholtz coils powered by a pulse generator. The pulse duration was about 2 msec, and the repetition rate was 50 Hz. The intensity of the magnetic field was 2.5 mT, and the average time-variation of the magnetic field was on the order of 1 T/sec. The maximum induced electric field was estimated to be 0.02 mV/cm. Cultures were exposed for 18 h for determination of IL-2 receptor-positive cells and percentage of T-activated lymphocytes; exposures of 24 or 48 h were used to assess IL-2 production.

Compared to controls, cultures exposed to ELF pulsed magnetic fields had increased [^{3}H]thymidine incorporation, i.e., the cultures were actively synthesizing DNA. Exposed cells also had lower amounts of IL-2 in their supernatants, higher percentages of IL-2 receptor-positive cells, and increased numbers of T-activated lymphocytes (Cossarizza et al. 1989b).

4.5.2 Gap junctions

Clone 9 cells from rat liver first were treated with chloral hydrate to partially inhibit intracellular communication via gap junctions (Benane et al. 1996). After 24 h of exposure to the chemical, the cells were exposed to ELF magnetic fields (7.9 – 49.9 μT, 45 Hz) for 30 min. Magnetic field exposure significantly depressed intracellular communication via gap junctions GJIC.

Li et al. (1999) exposed Chinese hamster lung cells to 12 TPA, alone or in combination with a 50 Hz ELF. Treatment with TPA for the last hour of a 24 h ELF magnetic field exposure to 0.2, 0.4, or 0.8 mT significantly reduced intracellular communication via gap junctions, compared to TPA treatment alone.

As part of a larger experiment (Griffin et al. 2000) used a similar protocol, except that rat liver clone 9 cells were used. Here exposure to an ELF magnetic field had no effect on intracellular communication via gap junctions.

The paper by Griffin et al.(2000) included two additional complexities: two cells from two different sources were compared, and a very specific magnetic field exposure regime was used. First, some clone 9 rat liver cells were obtained from another investigator, and others were purchased from the American Type Culture Collection (Rockville, MD). All cells then were cultured under the same conditions. The cells were treated with chloral hydrate for 24 h prior to ELF magnetic field exposure (Griffin et al. 2000).

Second, the exposure conditions were determined in accord with predictions of the ion parametric resonance model. Exposure experiments were undertaken using a 45 Hz magnetic field with flux density of 23.8 μT (r.m.s.) in parallel with a 36.6 μT static magnetic field for 40 to 45 min.

Exposures experiments were carried out either blinded or unblinded. In blinded experiments, cells from the American Type Culture Collection and cells which had originally been grown in the other investigator's laboratory were used.

There was no statistically significant effect of magnetic field exposure on intracellular communication via gap junctions in both of the blinded experiments using either cell line (Griffin et al. 2000).

4.6 Cell Transformation

West et al. (1996) used anchorage-independent cell growth as the dependent variable. Mouse epidermal JB6 cells (clone 41) were exposed to 60 Hz magnetic fields of 1, 10 or 100 μT for 8 or 14 days. Colony forming efficiency was increased in the field-exposed cells.

On the other hand, in a study using co-cultured C3H/10T1/2 cells of mouse fibroblasts and mutant daughter10e cells, 28 day exposure to an ELF magnetic field (60 Hz, 100 μT, 1 h epochs give four times per day) caused no increase in transformation frequency, which was determined using focus formation (Cain et al. 1993). In the same culture system, however, concomitant treatment with ELF magnetic field exposure and TPA caused a significantly increased focus formation (by an average of 150%) compared to the TPA-alone treatment groups.

On the other hand, there are some reports suggesting no effect of ELF magnetic fields on transformation. In a soft-agar assay, 60 Hz magnetic fields of 0.01, 0.1, 1.0 or 1.1 mT flux density did not induce anchorage-independent growth of mouse epidermal JB6 cells (Saffer et al. 1997). In addition, these magnetic fields did not enhance TPA-induced transformation: there was no increase in the maximum number of transformed colonies and no shift in the dose-response curve. Continuous exposure for 24 hr at 200 μT (60 Hz) also produced negative results in two transformation systems (Syrian hamster embryo cells and CH310T1/2 , clone 8) with or without post-exposure treatment with TPA (Balcer-Kubiczek et al. 1996).

CH310T1/2 clone 8 cells were exposed to high-intensity magnetic fields (5 to 400 mT) to investigate changes in transformation frequency, using focus formation as the dependent variable. No significant increase in frequency of focus formation was produced by field exposures (Miyakoshi et al. 2000b). However, in this experimental system, the transformation frequency for pre-treatment with X-rays (3 Gy) followed by ELF magnetic field was decreasing compared with X-rays alone (Fig. 4.3.).

No increase in transformant frequency was observed following exposure to ELF magnetic field (5–400 mT), either with or without TPA treatment. The transformation frequency was increased by X-irradiation and further increased by TPA. However, when cells were exposed to ELF magnetic field after X-irradiation, the transformation frequency was decreased, in a manner inversely proportional to the increase in

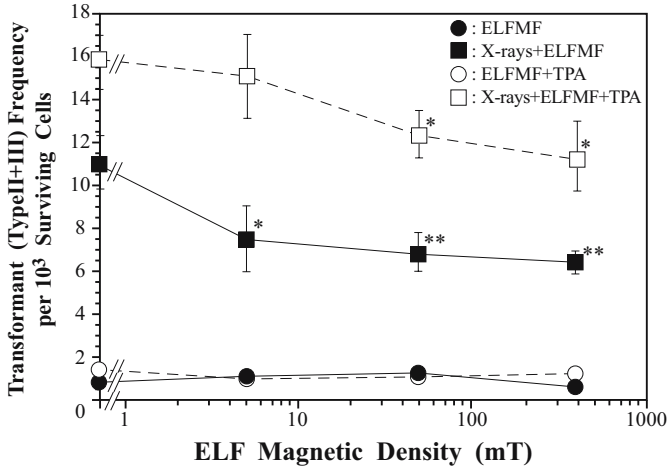

Fig. 4.3. Transformant (Type II + III) frequency of C3H10T1/2 (clone 8) cells exposed to X-rays (3 Gy) and/or ELFMF at 5, 50, and 400 mT for 24 h with or without TPA (0.3 ng/ml) treatment. Error bars represent the standard error. Asterisks, $^{*}P < 0.05$ and $^{**}P < 0.01$, show the statistical significance as compared to sham-exposure.

magnetic flux density. This suggests that high intensity ELF magnetic fields suppressed X-ray-induced transformation. In addition, long-term exposure for 6 weeks at 5 mT (60 Hz) suppressed both spontaneous and X-ray-induced transformation significantly (Miyakoshi et al. 2000b).

4.7 Summary

4.7.1 Summary of findings

Scientists interested in how exposure to ELF magnetic field might affect cells have used a variety of method and endpoint from contemporary molecular biology. In addition, a wide variety of exposure conditions have been used.

Many studies have examined genotoxicity, assessing SCE formation, production and repair of DNA strand micronucleus formation, and direct mutagenic action. Some papers report positive effects from ELF magnetic field exposure, and others do not.

To look at cell growth, studies have examined cell proliferation, DNA synthesis, and the role of melatonin in suppressing cellular proliferation. Some papers report positive effects from ELF magnetic field exposure, and others do not.

Another group of studies has looked at gene expression; *c-myc*, *c-jun*, *c-fos* and hsp have been used as makers, along with other related endpoints, such as NOR-1. Some papers report positive effects from ELF magnetic field exposure, and others do not.

Effects of ELF magnetic fields on signal transduction have been assessed, focusing on Ca^{2+} and PKC. Some papers report positive effects from ELF magnetic field exposure, and others do not.

Other experiments have investigated inter-cellular communication using ODC, Il-2, and gap junctions. Some papers report positive effects from ELF magnetic field exposure, and others do not.

Cell transformation has been assed: some papers report positive effects from ELF magnetic field exposure, and others do not.

4.7.2 Commentary

So, is the glass half full or half empty? If driven by a sense of awe and curiosity, it is remarkable the ELF magnetic fields can affect cellular processes of fundamental importance. If requiring incontrovertible proof before accepting a scientific concept, the existing literature does not prove that ELF magnetic fields can affect cellular processes of fundamental importance.

Given the lack of basic understanding and the paucity of the data base for any particular circumstance, it is very difficult to reach conclusions. To progress further, the survey stage needs to end. Instead of examining diverse exposure situations with any convenient technique from molecular biology, people need to focus on some specific problems and tease them apart until either (1) understanding of the mechanisms is achieved or (2) the apparent phenomenon unravels into scientific meaninglessness.

Weak (environmentally relevant) ELF magnetic fields don't appear to have much of an effect, so safety is not an issue. However, strong ELF magnetic fields can do many things to cellular function. These should be studied as a matter of basic science at the interface of biology and physics, not as a matter of hazards research. Moving away from weak fields will allow use of biologically effective doses without being limited by concern for environmental relevance. With a sufficient dose, i.e., where effect size is large relative to noise, it should be possible to establish robust, reliable bioeffects. Then research can begin to address the intriguing question of mechanism of action. Discoveries made while doing this will advance basic science and almost certainly will have unanticipated, but important, applications throughout biology and medicine.

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5

Dosimetry Related to ELF Electromagnetic Field Exposure Experiments

Tsukasa Shigemitsu

In this chapter the discussion considers two primary areas, (1) the engineering needed to produce effective exposure systems, and (2) assessment of the coupling between electromagnetic fields and biological systems. First instrumentation and exposure systems will be discussed. Next, control of the environment of the exposure experiment will be covered. Finally, the coupling between the electromagnetic fields and biological organisms will be considered.

5.1 ELF EMF Coupling and Dosimetry with Biological Systems

As described in Chapter 3, numerous experiments have been conducted to clarify the possibility of biological effects of extremely low frequency (ELF) electromagnetic fields from naturally originating or from artificial sources on biological systems. A relationship between the ELF electromagnetic fields of approximately 10 Hz that accompany lightning discharges and human behavior and physiology has been recognized. Thus, effects on biological systems of electromagnetic fields at 1–15 Hz have become known (König et al. 1981). However, these effects are weak.

Since the first publication of a report, which came from the former Soviet Union, concerning the health effects of electric utility employees (Asanova and Rakov 1966), there have been numerous experiments relating to biological effects of power frequency (50 or 60 Hz) electric and/or magnetic fields on animals and cells. In addition, many epidemiological studies and some experiments with human volunteers have been completed.

There are several steps requiring completion to evaluate the health effects of electromagnetic field exposures on humans (Fig. 5.1). The first step is to demonstrate biological effects on animals; as part of this, it is necessary to determine the "dose" in the *in vivo* experiments. Then the results are extrapolated to estimate the health effects expected in humans. However, there are major problems in the extrapolation of the results from animals to humans. Issues to be considered include differences in size – as well as anatomy and physiology – between animals and humans. One of the important problems is to estimate the "dose" in humans that would be equivalent to

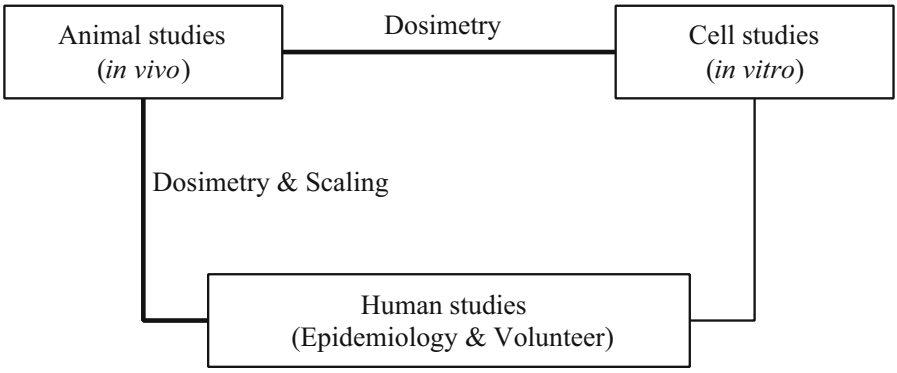

Fig. 5.1. The relationship between the three principal components required in EMF research conducted to assess safety for humans.

the "dose" used in an animal experiment. Quite similar issues are involved in doing *in vitro* experiments to clarify the mechanisms of action for ELF electromagnetic fields.

It is very important to know the exposure (dosimetry) in animals and to predict (scaling) the equivalent exposures in humans. Thus, methods for dosimetry and scaling must be employed. The proper dosimetry and scaling are required for extrapolation of data, which typically are obtained with small laboratory animals, to humans. As described below, size is an important attribute affecting how the energy in an ELF electomagnetic field is coupled, or connected, into an organism.

In order to conduct ELF electromagnetic field exposure experiments with animals or cells that will provide useful information with respect to humans, it is necessary to understand the exposure conditions and to estimate the dose. Therefore, particular attention must be paid to (1) instrumentation issues, (2) development of electric and magnetic field exposure systems, (3) coupling, (4) and scaling.

In the following section, instrumentation problems are treated in the context of macroscopic dosimetry and coupling issues are treated in the context of microscopic dosimetry. The material on macroscopic dosimetry is divided into three parts: (1) electric field exposure systems for *in vivo* study, (2) magnetic field exposure systems for *in vivo* study, and (3) *in vitro* exposure systems. The issue of coupling between electric field and magnetic field into biological system is treated separately in a subsequent section.

5.2 Macroscopic Dosimetry

There are a number of problems that often are not addressed adequately in ELF electromagnetic field research. For example, when animals in cages are placed in exposure systems for exposure experiments, contamination of non-conductive cages with conducting materials, such as urine or feces (plus saliva, food residues and skin

oils) from the animals, can result in distortion of the electric field. The field can be also distorted by the metallic portions, making it difficult to maintain accurate, stable electric field strength during exposures. Also, if appropriate measurements are made, changes with time or space are observed during both *in vivo* or *in vitro* experiments.

However, as the study of electromagnetic field effects became an interdisciplinary research area with researchers from both biology and engineering became involved, exposure facilities for experiments were improved, and the properties of experimental systems, dosimetry, etc. became defined from the perspective of electrical engineering. This allowed collection of experimental data with a much higher degree of confidence as to the validity of the results. In the ELF region, the electric and magnetic field exposure systems can be considered separately.

5.2.1 Electric field *in vivo* exposure systems

In designing a laboratory exposure system, careful attention must be given to many details. To generate the electric field for *in vivo* experiments, parallel plate electrodes usually are used. This configuration provides a uniform electric field distribution between the parallel plate electrodes, except in the distorted areas around the edges. Although the theoretical formulation for parallel plates assumes an infinite plate size, real-world plate sizes are small and distortion at the edges can be appreciable, even when relatively large diameter, rounded edges are used.

The nominal electric field strength (i.e., the unperturbed electric field) can be obtained by dividing the applied voltage by the distance between plates. For example, if the plate spacing is 0.5 m and the applied voltage is 10 kV, the apparent field strength is 20 kV/m. With high voltages applied to parallel plates, guard rings typically are installed to prevent corona discharges from occurring at the edges of the electrodes and supporting structures. Although corona discharge is a secondary phenomenon, it must be eliminated by a careful approach.

The parallel plate configuration can generate a uniform electric field, allowing performance of experiments with animals. One problem is the spacing of the plates. In order to maintain uniform electric field inside the space between the two plates, spacing between plates must be approximately three times greater than the height of animals, such as rats when standing erect (Bridges and Preache 1981).

In the ELF region, animals are assumed to act as conductors, and the electric field is distorted by the presence of the animals as compared to the situation without animals. Even if a homogeneous electric field is desired and is produced by well-engineered equipment, the field inevitably will be non-homogeneous with test animals present. Experiments typically are described and compared in terms of the unperturbed electric field strength. The electric field is enhanced on the surface of an animal (or human), because of charge accumulation along the electric flux lines (perturbed electric fields). Thus, an applied ELF electric field is perturbed by the presence of an organism. Furthermore, the external electric field is attenuated greatly inside the body. Therefore, when a subject is exposed, the surface and induced electric fields are very different from the unperturbed electric fields. The surface electric

fields are enhanced compared to the unperturbed electric field, and the induced electric fields inside the body are attenuated strongly.

Furthermore, the surface and induced electric fields which animals and humans experience are different, even when they are exposed to same unperturbed electric field intensity. Simply because of the way differing geometries affect the field: body interaction, the situation for an erect human with a height of 1.7 m in a 3 kV/m field beneath a transmission line will be very different from that of a horizontal rat with a height of 4 cm in a laboratory-generated field of 3 kV/m. Electric field enhancement on the top of the human's head will be much greater than the electric field enhancement on the back of a rat. This is the scaling problem, which means that in order to compare the biological response of different species, it is necessary to understand the process of how size and shape affect interactions with electric fields.

In addition to these problems, the uniform electric field in an exposure system is disturbed by many factors. One factor is the presence of animals in cages. The mutual shielding of animals, the top and walls of the animal cages, wooden chips used for bedding, drinking bottles, test apparatus (such as response levers), etc. all can cause perturbation and shielding effects, which can substantially distort the originally uniform electric field. The electric field strengths inside an animal cage are attenuated strongly by the shielding effects of metallic, or even plastic, cage walls.

The electric field inside cages has been found to be highly dependent on the surface conductivity of the plastic cages that commonly are used (Baum et al. 1991, Shigemitsu et al.1981). As the surface conditions of cages become contaminated by animal occupancy, with resultant gradual accumulation of urine and excrement, the walls become conductive. Thus, the electric fields inside cages can become highly attenuated, compared to the electric field originally present in the cages, because of shielding effects. Therefore, although the electric field outside the cage can be made uniform in space and constant with time, the field inside the cage is temporally and spatially unstable. It is very difficult to maintain constant electric field conditions during long-term exposure experiments. Frequent provision of clean cages can mitigate this problem, but frequent animal handling, and/or the need to turn the fields off to change cages, can produce their own biological problems. Thus, development of improved cages for the study of the biological effects of electric fields is necessary.

Another possible side effect (even artifact) is shock discharge from water bottles. When the animals drink water from water bottles in high electric fields, they can experience electric micro-shocks while drinking. This effect must be kept to a minimum. To minimize or avoid this problem, typically a resistance to ground is placed in the "circuit" formed by (1) sipper tube and water, (2) animal in contact with cage floor, and (3) electrical ground. If the shock discharge is painful, it can prevent animals from drinking. Thus, the adverse effects of dehydration could inadvertently be attributed to electric field exposure per se. Conversely, if the system is well designed, current flow at drinking will be imperceptible to the subjects, meaning their normal drinking behavior will not be altered.

An electric field exposure system for small animal such as rats or mouse is shown in Figure 5.2 (Shigemitsu et al. 1981). This exposure system was developed by the Central Research Institute of Electric Power Industry (CRIEPI) in Japan. In this ar-

rangement, the animal housing space, which consists of a metal cage part with lower field-generating electrode, is grounded. From the electrical point of view, concentric sphere geometry would provide good uniformity in the electric field distribution. The lower part of the outer grounded electrode is a roughly half-spherical area for animal housing. A ring-shaped electrode, made of brass plate with a guard ring to prevent corona, is placed inside the grounded electrode. Brass plates are supported by overhead insulators. The food container and water bottle are placed on the lower part of the grounded electrode.

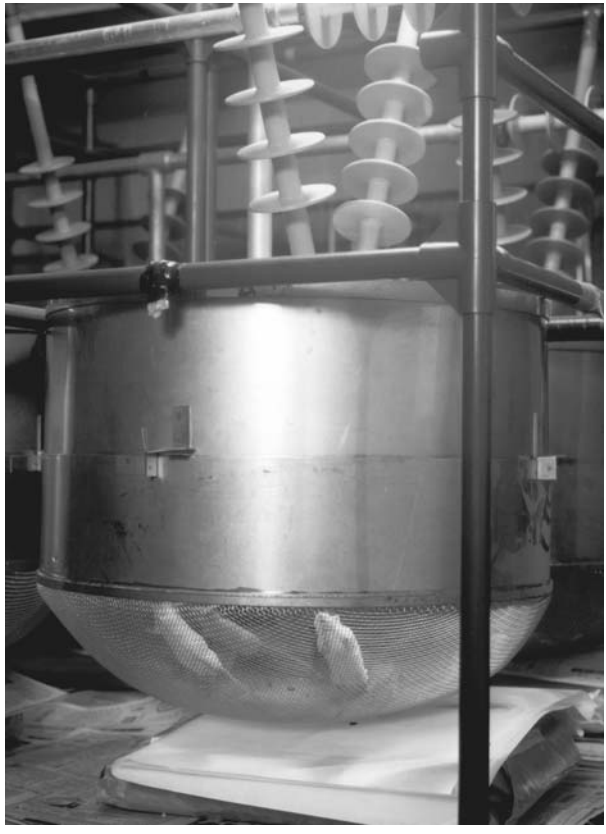

Fig. 5.2. An improved electric field exposure system for experiments with small animals, such as mice and rats.

5.2.2 Magnetic field *in vivo* exposure systems

In general, Helmholtz coils are used for magnetic field exposure experiments. Two-paired Helmholtz coils can be used to generate uniform magnetic fields in the space

Table 5.1. Comparison of the Characteristics of Various Systems Designs for Magnetic Field Exposure Experiments.

Coil Type	Coil shape	No. of Coils	Coil length of Diameter	Coil spacing	AT ratios
Helmholtz	Circular	2	d, d	−0.25d, +0.25d	1/1
Merritt-1	Square	3	d, d, d	−0.4106d, 0, +0.4106d	39/20/39
Merritt-2	Square	4	d, d, d, d	−0.5055d, −0.1281d, +0.1281d, +0.5055d	26/11/11/26
Rubens	Square	5	d, d, d, d, d	−0.5d, −0.25d, 0, +0.25d, +0.5d	19/4/10/4/19
Tetracoil	Circular	4	0.672d, d, d, 0.672d	−0.399d, −0.149d, +0.149d, +0.399d	73/107/107/73

between them. This gives a uniform magnetic field in an area perpendicular to and around the center of the axis between the two coils. In theory, in the case of two coils of radius r with separation distance of $2a$, coils satisfying the condition $r = 2a$ are known as Helmholtz coils. The field is uniform in a small volume the near the center of this configuration.

Because of the difficulty in establishing uniformity of the magnetic fields over a large volume within a limited experimental space, the use of two-paired circular Helmholtz coils is not practical. Thus, a variety of alternative exposure coil systems have been designed (Table 5.1). From the basic concepts, various systems consisting of different coil shapes, numbers of coils, and different sizes of coils have been proposed by various researchers.

For animal exposure experiments, use of square coils with four windings – the arrangement formulated by Merritt et al. (1983) – best satisfies the requirements for magnetic field uniformity for a relatively large volume in a limited space. Therefore, this coil system is a very suitable exposure system for animal experiments with magnetic fields (see, Figure. 5.3, Shigemitsu et al. 1993).

If there are requirements for both horizontal and vertical magnetic fields, two sets of this coil system can be installed to provide vertical or horizontal orientation, respectively. For example, vertical magnetic field can be obtained in the condition which the coils are oriented above and below of the objects are being exposed. Horizontal magnetic field is given by the coils that are oriented on the left and right sides of the targets. Two pairs of orthogonally oriented coils can produce the rotating magnetic fields.

Circularly polarized magnetic field can be obtained using of two sets of orthogonally placed coils. The vertical and horizontal components of circularly polarized magnetic field are equal in magnitude, frequency and 90° phase difference between two components. The principal artifacts in the development of magnetic field exposure systems are heating, vibration, and noise.

When designing *in vivo* exposure experiments for either electric or magnetic fields, there are a number of engineering, environmental, and biological parameters that must be considered and controlled (Table 5.2).

5.2.3 *In vitro* exposure systems

Magnetic field exposure systems for *in vitro* exposure of cells in culture dishes can be similar to those used for *in vivo* systems (Figure. 5.4). This exposure facility can

Table 5.2. Key Parameters in *In Vivo* ELF Electric and Magnetic Field Exposure Experiments.

Experimental variables
• Exposure system (electrode configuration, material)
• Frequency, wave shape (sinusoidal or pulsed, intermittent, transient, harmonics, *etc*)
• Strength of electric and magnetic fields in exposure and sham-exposure systems
• Degree of spatial uniformity
• Orientation and polarization (linear, vertical, horizontal, circular, elliptical fields, *etc*)
• Stray electric and magnetic fields and their reduction
• Power supply specification
• Exposure duration and schedule (acute, sub-chronic or chronic, circadian rhythm, time of day of exposure)
• Time between exposure and sampling
Subject variables
• Animals (species, strain, sex, size, age, weight)
• Animal housing density per cage
• Animal care and procedures (researchers – animal interaction)
• Number of subjects
• Sham (and control) animal
• Sampling (time of day, double-blind)
Environmental variables
• Temperature, relative humidity, air quality, lighting during exposure experiment
• Background electric and magnetic fields (AC field, sources, temporal and spatial variation)
• Corona discharge, ozone, audible noise, air ions
• Spark discharges, micro-shocks (current between the animals and water-bottle, feeder)
• Heating and vibration of coil in magnetic exposure experiment
• Noise and its level from generating coils
• DC magnetic field (geomagnetic fields, strength, direction)
• Material of animal cage and its location
• Material of water system and its location
• Material of feeding system and its location

be developed for wide application to cell exposure to ELF magnetic fields (Yamazaki et al. 2000). This exposure system consists of Merritt's 4-sqaure coils with magnetic field intensity of 10 mT at 50 Hz with spatial field uniformity less than ±3% in a 400 mm cube. Rotating and linearly polarized magnetic fields can be generated in this system with concentric compensation coils to reduce stray magnetic field.

ELF electric field exposure methods for cells or tissues can be broadly divided into two method categories: The first involves the use of electrodes to cause the flow of a uniform electric current to produce a uniform electric field. Misakian et al. (1993) presented an example of this method for generating a uniform electric field (Fig. 5.5). The second method involves the use of a time-varying magnetic field to produce a uniform electric current flow. For *in vitro* experiments with cell cultures, parallel flat plate electrodes can be use to generate electric fields for exposures.

For all *in vitro* experiments, the cells or tissues are placed in a media (e.g. saline solution) so they can live and grow. In the case of electric field exposure experiments, the electric field (E) and electric current density (J) have the following relationship $E = \sigma J$, where E is V/m, J is A/m^2 (μA/m^2 or mA/m^2), and ρ is resistivity ($\Omega \cdot m$). This equation is based on Ohm's law. Conductivity (σ) and resisitivity (ρ) have the relationship $\sigma = 1/\rho$. In other words, when the resistance of the media is constant,

Fig. 5.3. The newly developed magnetic field exposure system for *in vivo* experiments.

it is possible to achieve a uniform electric current flow distribution and electric field distribution.

The electrochemical reaction occurs at the electrodes used as a source for flowing electric current. This effect cannot be ignored. It must be taken to avoid electrode polarization and electrochemical reactions. Metal ions from the electrodes will be deposited into the solution, and these metals can damage or kill the cells. Thus, agar (most commonly) or other media are used between the electrodes and the solution bathing the cells.

The uniformity of the electric field and the spatial distribution of the electric current density are determined by the configuration and size of the electrode (Durney and Christensen 1999). When designing exposure apparatus for *in vitro* exposure experiment, it is necessary to consider list of parameters (Table 5.3). It is important that biologist and engineer collaborate to handle all aspects of the situation. Often

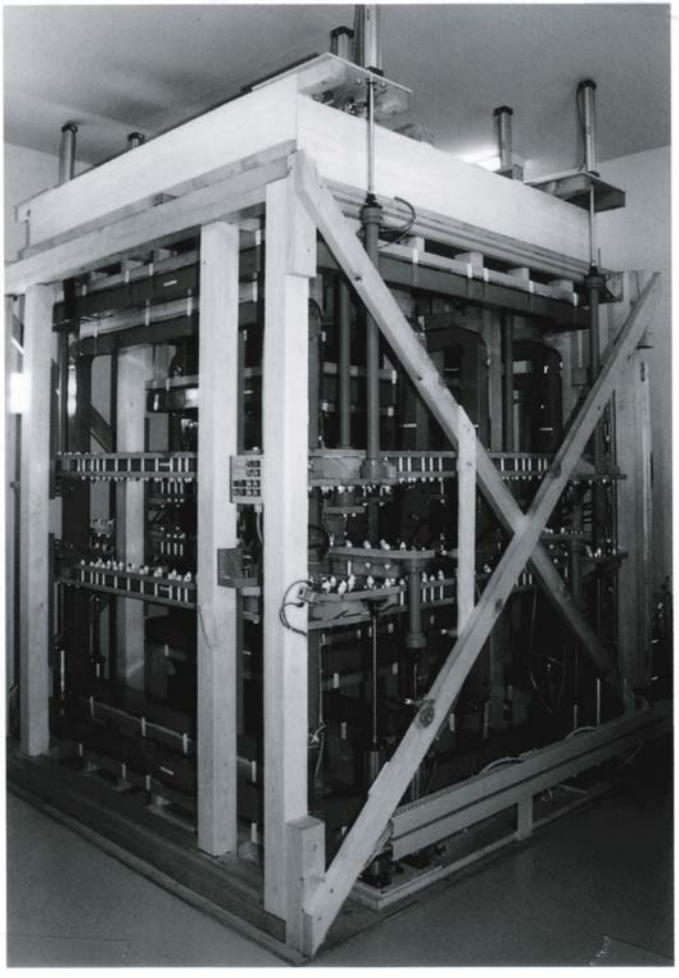

Fig. 5.4. The newly developed magnetic field exposure system for *in vitro* experiments.

compromises must be made. The biologist must decide issues such as cell line and number and specify the conditions (e.g., CO_2, O_2, temperature, and humidity) required to allow the cells to grow properly. The biologist must consult with the engineer on issues such as dimensions of cell culture dishes and culture units, because the biological customs and requirements must be considered in light of the realities of design of exposure systems. The engineer must listen to the biologist's description of what is required to perform a biologically valid experiment: such issues cannot be compromised for expediency, or "bad science" will result.

The second method involves the use of a time-varying magnetic field to induce electric field and electric current. Usually a circular Petri dish is used for magnetic field exposure experiments. When a Petri dish is filled with a medium with conduc-

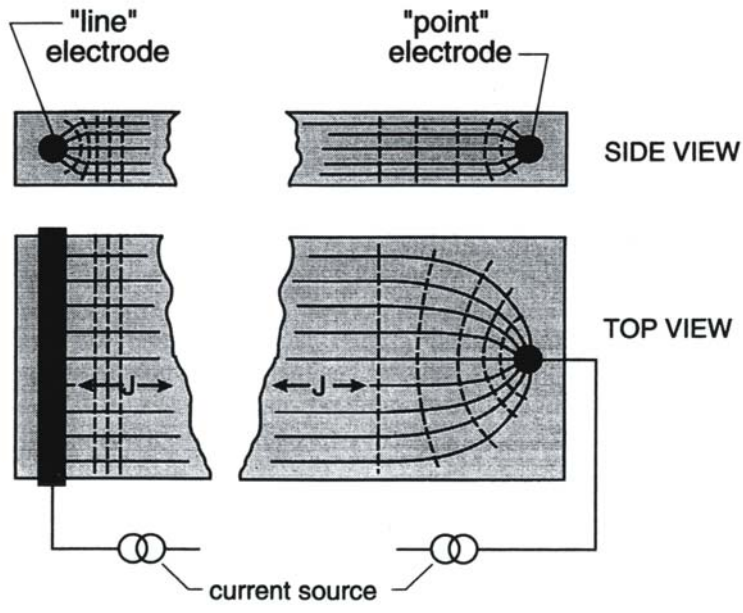

Fig. 5.5. Example of a chamber and electrode configuration for producing a uniform electric field (Misakian *et al*. 1993).

Table 5.3. Key Parameters for *In Vitro* Exposure Experiments.

• Frequency, wave shape (sinusoidal or pulsed, etc)
• Electrode configuration and material
• Measurement and estimation of electric current density in medium
• Conductivity of biological material
• Cell density, cell line
• CO_2, temperature
• Dimensions of cell culture dishes and culture units
• Directional relationship between the magnetic field and cell dish
• Vibration of cell culture dishes
• DC magnetic field (geomagnetic field, strength, direction)
• Conductivity of culture medium
• Ambient magnetic fields power frequency, around cell culture units

tivity σ and exposed to a vertical magnetic field, concentric electric current flow occurs (Figure. 5.6). If r is the radius of the Petri dish, f is the frequency, B is the magnetic flux density in tesla, and the current density of the induced current is J, then $J = \sigma\pi fBr$. This equation shows that the induced current is zero at the center

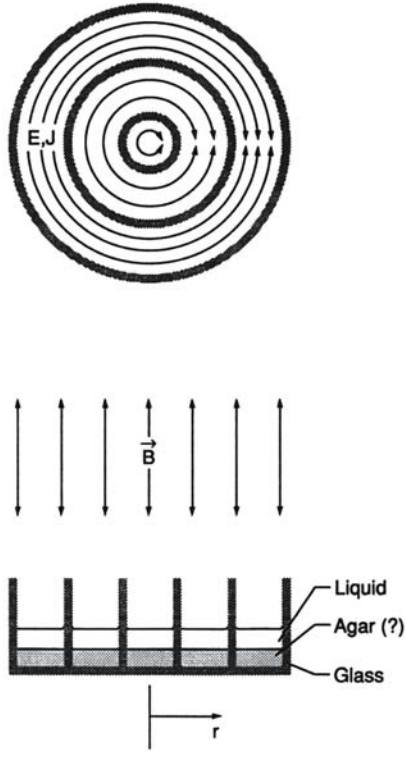

Fig. 5.6. The distribution of the induced electric field and the current density for *in vitro* experiments using a circular dish with applied vertical magnetic field (Misakian *et al*. 1993). The partition of circular walls gives the different exposure conditions.

and becomes greater with increasing proximity to outer edge. Thus, it is possible to conduct experiments with different current conditions using one Petri dish, if the location of cells (distance from the center) is considered carefully.

The magnetically induced electric field (E) is given as $E = J/\sigma = \pi f B r$. E does not depend on conductivity. Induced current density and induced electric field distributions are proportional to the distance from the center of the dish, as shown in Figure. 5.6. Therefore, if circular partitions are added, it is possible to conduct experiments using the induced current or induced electric field as an index of exposure or dose. One of the merits of using a circular dish system (Petri dish) for experiments is that the exposure experiment can be conducted that allow clarification whether observed effects are caused directly by (1) the magnetic field exposure or by (2) the induced electric field. That is, if the results are similar across the Petri dish, then magnetic field effects are being observed. Conversely, if the results vary

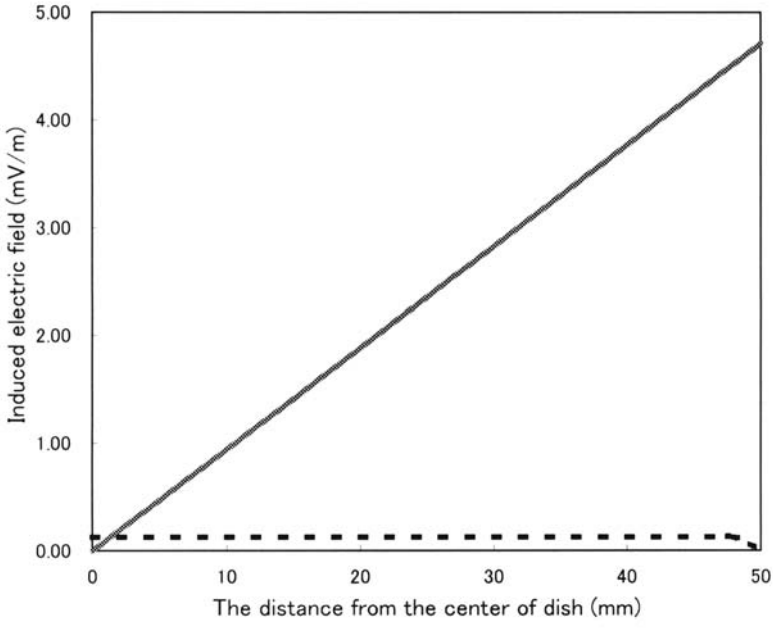

Fig. 5.7. Profiles of calculated magnetically-induced electric field strength as a function of distance from the center of Petri dish. Solid line is the results of vertically polarized magnetic field, and dotted line is for horizontally field. A 500 μT magnetic field at 60 Hz was applied to Petri dish, either vertically or horizontally.

across the cross section of the dish, then the effects of the induced electric field are being observed.

During an *in vitro* study, the direction of magnetic field against circular dish is a key parameter for understanding the effects. If the fluid in a Petri dish is homogeneous and the direction of magnetic field is either vertical or horizontal to the Petri dish, then the magnitude and distributions of the induced electric field from the center of dish can be calculated (Fig. 5.7). Calculation conditions are that the magnetic field is 500 μT at 60 Hz, the circular dish radius (r) is 50 mm. The magnitude of induced electric field can vary depending on the size of the cell culture dish and height of the filled medium (Bassen et al. 1992; Yomori Personal communication).

The solid line in Figure. 5.7 shows that the calculated induced electric field strength increases linearly with the distance from center of dish in the vertically polarized magnetic field perpendicular to Petri dish. The maximum induced electric field was calculated to be 4.7 mV/m (500 μT/60 Hz). Thus the samples, such as cells, will be exposed to different levels of electric field within a single Petri dish. In Figure. 5.7, the dotted line shows that the calculated induced electric field is nearly constant, 0.15 mV/m along the dish radius, with 5 mm height of dish, in horizontally polarized

magnetic field parallel to dish. With an elliptically polarized magnetic field, the computation of the induced electric field becomes more complex. Misakian (1991) made the comparison of induced current densities, electric fields, and rates of energy deposition during *in vitro* studies with linearly and circularly polarized ELF magnetic fields.

5.2.4 Issues related to insufficient consideration of electrical engineering

Biological researchers conducting *in vivo* experiments usually consider many issues, such as species, strain, age and sex numbers of animals, endpoints, seasonal and circadian rhythm, animal care, housing, temperature and humidity, lighting, etc. when planning and evaluating experiments (Table 5.2). Researchers conducting *in vitro* experiments have their own extensive list of factors to be considered, particularly cell line, cell density, addition of growth factors, CO_2/O_2, temperature and humidity, etc (Table 5.3). These aspects of the design of *in vivo* and *in vitro* experiments in bioelectromagnetics are the responsibility of the biologists.

Apart from these issues, in planning, analyzing and evaluating studies on the biological effects of electromagnetic fields with either *in vivo* or *in vitro* experiments, electromagnetic field exposure parameters must be considered carefully with the engineering members of the research team. Five categories of issues require careful attention (Valberg 1995): (1) characteristics of the exposure system, (2) exposure intensity and timing, (3) frequency-domain characteristics, (4) spatial descriptors, and (5) combined EMF exposure.

Magnetic field strengths of *in vivo* and *in vitro* studies range from less 1 μT to greater than 100 mT. Magnetic fields in exposure experiment involve homogeneous, gradient fields, continuous and intermittent fields with on/off cycle ranging from seconds to days, and pulsed fields. Daily exposure ranges between 15 min to 24 h, and the period of exposure can be from minutes to years. It is not easy to evaluate overall results of various experiments, partly because such a wide range of diverse exposure conditions exist. Knowledge often is not sufficient to identify which differences are crucial and which differences are trivial. In this section, difficult issues related to magnetic field exposure conditions will be considered.

There are a number issues associated with magnetic field exposure systems. As exposure systems and exposure conditions have not been sufficiently standardized, the replication of experimental results has been difficult. For example, many reports of experimental studies of magnetic field exposure effects on melatonin have made. The results of these experiments will be compared and issues related to evaluation of these results will be discussed, with an emphasis on electrical engineering parameters.

Although various experimental animal species have been used in magnetic field exposure experiments, the physiological role of melatonin is in the various animal species still is not clear. Also, the engineering aspects of these exposure experiments have differed: individual research teams have developed most of the magnetic field exposure systems themselves. In order to accurately evaluate experimental results, in addition to the biological interpretation, it is necessary to understand the design

Table 5.4. Examples of Key Parameters in Experiments Examining the Effects of Magnetic Fields.

Background data	AC fields: measurement value, frequency, strength, source, time variation
	DC fields: geomagnetic field, strength, direction
Exposure	Coil/electrode: design condition, scale, sample, uniformity
	Applied magnetic fields: strength, rise and harmonics
	Uniformity (calculated and measured values), stray field
	Background

and construction of the magnetic field exposure system, as well as the specifications. However, there are very few reports that are adequate from the electrical engineering standpoint. Thus, it is difficult to compare data from different studies. In the following discussion, the electrical engineering parameters (Table 5.4) will be considered in the comparison of various experiments.

The strength of magnetic fields normally is expressed in terms of Tesla (T) or Gauss (G) (1 G = 100 mT). The values reported in papers usually do state whether the value reported is root mean square (rms) or peak. RMS and peak value have the following relationship: $B_{rms} = 0.707 \times B_{peak}$. Thus, although the values are not the same, they can be compared — but only if the original paper described the measurement procedure used. Also, the reasons for selection of the magnetic field strength often are not clear in published papers.

Many magnetic field experiments are conducted using 50 or 60 Hz (power frequency) fields. Although it is necessary to report the higher harmonic frequencies (100/120, 150/180 Hz, etc.), usually these are not adequately covered. Also, the spectrum associated with power on/off transitions (spike, ramping) typically is not well documented. Although most experiments use rats and mice, reports of experimental studies using seasonally breeding animals, such as sheep and hamsters, are often inadequate in reporting the timing of the magnetic field exposure relative to the maturity and breeding experience of the subjects.

For the cyclotron resonance hypothesis, the orientation (relative strength and direction) of both the DC magnetic and AC magnetic fields is very important. However, reporting of DC magnetic fields, especially characteristics of the geomagnetic field – such as strength, horizontal component and inclination – often is inadequate.

Reporting regarding the uniformity of magnetic fields frequently is inadequate. Currently, many research organizations are using a Merritt-type magnetic field exposure apparatus; typically, these have excellent uniformity. Thus, it is possible to assume a certain degree of uniformity. However, if materials containing iron or other conductive materials are used within or near the coils, then the uniformity of the magnetic field is reduced. This can happen with supporting equipment, such as pumps or fans needed to support cell culture or with placement of measuring or monitoring equipment intend to document cell culture conditions. This means the magnetic field gradient, degree of heterogeneity, etc. must be reported. There is inadequate reporting of the strength of the background magnetic field from air conditioning equipment, etc. as well as the stray magnetic field spillage from exposure system. The latter is

particularly important if exposure system and sham exposure system are installed in a limited space. There are very few electromagnetic field exposure systems that adequately take all of these factors into account.

5.3 Induced Electric Fields and Currents

When considering coupling with ELF electric fields, charges concentrate on the surface of the organism. In electric fields, electric flux lines enter the body surface perpendicularly, and electric currents and electric fields are induced in the body. With regard to coupling of ELF magnetic fields, electric current is induced in organisms in magnetic fields; these currents flow in a loop configuration in the surface perpendicular to the magnetic field orientation. However, the induced current is far too small to account for the associations reported by some epidemiological studies in terms of currently understood phenomena. Therefore, a variety of hypotheses have been proposed – based on the principles of biology, engineering, and physics – about the mechanisms that could be functioning in empirical experiments reporting positive results.

Because the subject size (cell, animal, or human) is very small compared to wavelength in the ELF region, quasi-static coupling analyses are appropriate. A 60 Hz wave traveling at the speed of light has a wavelength of about 5,000 km, which is extremely long compared to either a standard 6 feet human or a 8 cm mouse. Thus, coupling between the electric- and the magnetic field-effects can be calculated separately. The issues involved here have been addressed adequately in the literature (e.g., Durney and Christensen 1999, ICNIRP 2003, NRC 1997). Here, we briefly outline the matter of coupling between the biological system and ELF electric and magnetic fields.

5.3.1 ELF electric and magnetic fields into biological systems and need for scaling

5.3.1.1 Coupling

When an organism enters an electric field, the electric field distribution varies depending on the surface configuration and posture of the organism. Charges accumulate on the surface of raised areas (i.e., protuberant and elevated) of organisms because of electrostatic induction, and thus the electric field strength is increased at these locations. The electric current inside an organism is induced by the electric field distribution inside the organism. Both the concentration of electric fields for a human model and the electric current inside the model has been computed (Figure. 5.8).

In contrast, for magnetic fields, the magnetic permeability (μ) of animals is equal to μ_o, and thus organisms do not disturb magnetic fields. However, based on Faraday's law, electric fields are induced by magnetic fields. As a result, an electric current is induced in organisms exposed to ELF magnetic field. This current induced by

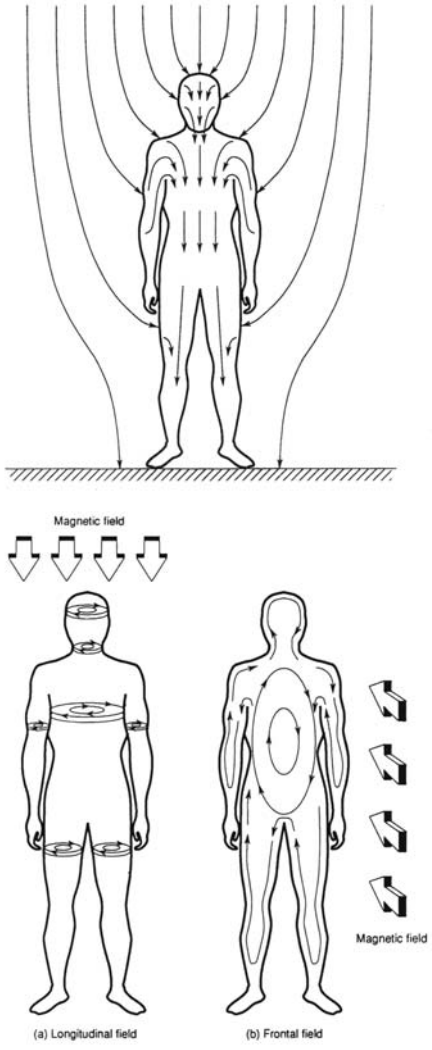

Fig. 5.8. The internally induced electric field in human models in ELF electric and in magnetic field (Reilly p346, p364 1998). In upper figure, perturbation of the electric field by person. External electric field indicated by flux lines outside body. Arrows inside body indicate direction and intensity of induced internal electric field any body current. The distribution of internally indicated electric fields from person exposure to ELF magnetic field is shown in lower figure. Magnetic field direction is parallel to long axis of body (a) and perpendicular to front to body (b).

magnetic fields flows primarily in a circular path that is perpendicular to the applied magnetic field, but flow also involves eddy currents (Figure. 5.8).

To summarize, there are major differences in the size and direction of the currents induced inside organisms by electric fields and by magnetic fields. In an electric field, the induction between the organism and the high voltage causes electric current flow. Vertical components of the electric field on the surface of the organism, beneath a power line, become dominant, which cause current flow to occur. The electric current density in an organism is dependent upon the electrical constant (s) of the body. Electric current induced by magnetic fields form a closed loop, i.e., so-called eddy currents are induced. These eddy currents are zero at the center of plane perpendicular to the magnetic field and become larger with distance from the center.

5.3.1.2 Scaling

One of the key points of animal research on the effects of electromagnetic fields is extrapolation (scaling) of the results from animal experiments to humans. As the strength of the electric field on the surface of the organism and the induced current inside the organism during electric field experiments vary greatly, depending on the morphology (electrical properties), posture, and size of the organism. Thus, the strength of electric fields on the surface of human bodies and the internal induced currents cannot be accurately simulated. Although used frequently as a simplifying assumption for research, the procedure of linking either (1) surface electric field strength, e.g., at the head, or (2) current flow values for specific internal organs for humans and animals, offers no more than an analogy.

For example, the "scaling factor" between human and animal (pig and rat) for maximum surface field is 2.7:1 and 4.9:1, respectively in 1 kV/m vertical electric field (Table 5.5). In a 1 μT, 60 Hz magnetic field, a human model (Table 5.6) would have an average induced current of 1.3 – 1.19 $\mu A/m^2$, while the induced current for a rat in the same magnetic field would be only 0.3 $\mu A/m^2$, about a fourth as much. These results are based on maximizing the average induced currents, with the human model being based on exposure to a horizontal magnetic field and the rat being exposed to a vertical magnetic field.

Bracken (1992) summarizes coupling parameters for ELF electric and magnetic fields and organisms (Table 5.7). It can be seen that the results for scaling of electric field exposure and magnetic field exposure both are heavily dependent on the body shape and size of the subjects. Thus, when the size of the subject (human, rat, pig, baboon, etc.) differs, the coupling is changed greatly. Because the electric field in the head is magnified when a human is standing in an electric field, in order to achieve an equivalent electric field for animals, exposure must occur using a stronger electric field. Also when using animals it is, in principle, possible to measure induced parameters. Thus, it is possible to measure the surface electric field strength and the macro current flow.

Table 5.5. Scaling Factors for Producing Equivalent Electric Field Exposures in Pig and Rat (NRC, 1997)*

		Equivalent exposure field (kV/m)	
Metric	Human	60-kg Pig	0.5-kg Rat
Maximum surface field	18.0 kVm	2.7 (2.7:1)**	4.9 (4.9:1)
Average surface field	2.7 kV/m	1.9 (1.9:1)	2.2 (2.2:1)
Current density			
Neck	0.5 mA/m^2	14.0 (14:1)	20.0 (20.0:1)
Torso	0.25 mA/m^2	7.3 (7.3:1)	12.0 (12:1)
Ankle	2.0 mA/m^2	1.8 (1.8:1)	1.4 (1.4:1)
Short-circuit current	16.0 μA	2.3 (2.3:1)	100 (100:1)

* Comparison of standing human (1.7 m in height) in a 1 kV/m, vertical electric field, plus scaling factors (uniform material model) required for an equivalent field or induced current in a large (pig) or small (rat) model.
** Values in parentheses show the scaling ratios between human and animals.

Table 5.6. Representative Induced Current and Induced Electric Fields for Human, Rat and Mouse in a 1 μT, 60 Hz, Uniform Magnetic Field (Xi and Stuchly 1994, Xi *et al*. 1994)(NRC 1997)

	Current density (μA/m^2)		Electric field (μV/m)	
Subject	Average	Maximum	Average	Maximum
Human, 1.7 m, 70 kg	1.3–1.9*	8 (20)**	14–17.7	161 (296)**
Rat, 0.3 kg	0.3	1.31	4.4	17.7
Mouse, 0.02 kg	0.12	0.4	1.7	5.7

* The average current density depends on the electric properties used for the muscle tissue.
** Values in parentheses obtained from the analysis with an improved resolution of 0.65 cm instead of 1.3 cm.

5.3.2 Models for analysis of induced current inside biological systems

To consider the mutual interaction mechanism for induced electric currents in organisms by ELF electromagnetic fields, research has been conducted including dosimetry to clarify the relationship between electromagnetic fields and induced electromagnetic fields, taking into account the anatomical characteristics of organisms to comprehend induced electromagnetic fields and currents. Previously dosimetry studies were completed using human and animal models, using rotating ellipsoids, oblong shapes, cylinders and other simple geometrical forms to approximate the amount of induced electromagnetic fields. However, in recent years, advances in computer technology with the development of hardware and commercially available software have made it possible to perform calculations using complex models. In addition, MRI has been used to make anatomically accurate images, which are readily available. This means that calculations that take into account the detailed anatomical configuration can be performed.

Table 5.7. Parameters for ELF Electric and Magnetic Filed Dosimetry (Bracken 1992)

	Units	dependence	Scaling	State of measurements		
				Animals	Models	
					Homogeneous	Heterogeneous
A. Electric Field						
Surface electric field	V/m	None	Body shape	Y	Y	Y
Internal electric field	V/m	f, σ	Body shape	N	Y	N
Internal current density	A/m^2	f, σ	Body shape	N	Y	N
Induced whole body current	A	f	Body shape and size	Y	Y	Y
B. Magnetic field						
Internal magnetic flux density	T	None	None	N	N	N
Induced electric field	V/m	f, σ	Body shape and size	N	Y	N
Induced current density	A/m^2	f, σ	Body shape and size	N	Y	N

f, frequency, σ, Local conductivity
Y, measurements performed; N, no measurements available

Table 5.8. Characteristics of Three Human Models Based on MRI Images (Stuchly and Dawson 2000 IEEE ©2000)

Model	United Kingdom (34)	Univ. of Utah (31)	Univ. of Victoria (36)
Height and mass	1.76 m, 73 kg	1.76 m, 64 scaled to 71 kg	1.77 m, 76 kg
Original voxels	$2.077 \times 2.077 \times 2.021$ mm	$2 \times 2 \times 3$ mm	$3.6 \times 3.6 \times 3.6$ mm
Posture	Upright, hands on sides	Upright, hands on sides	Upright, hand in front
Resolution in calculations	2 mm	6 mm	3.6 & 7.2 mm
Frequency in computations	50 Hz	60 Hz	60 Hz

Stuchly and Dawson (2000) compared three contemporary human models developed by three organizations (Table 5.8). In these three models, more than 30 distinct organs and tissues are identified. While the types of human models shown in Table 5.8 are used to obtain the induced current and induced electric field, models that take into account human symmetry are being developed in countries including France (Baraton and Hutzler 1995) and Italy (Bottauscio and Conti 1997).

In calculation of induced electric parameters, conductivity of organs and tissues is a key factor in obtaining the amount of induction using numerical calculations. Because accurate values of conductivity in the ELF region are not available, researchers have assumed various values. Acquisition of good *in vivo* measurement data on conductivity and permittivity of many different tissue types is a serious research need. In addition to the conductivity of various tissues, the anisotropy of tissues (connective tissue, etc.), the position of organ tissue (heart, lungs, etc.), and other factors remain to be considered carefully.

Mathematical modeling methods including the impedance method, scalar potential finite-difference method (SPFD), finite-difference time-domain method (FDTD), boundary element method (BEM), surface charge method, TRIFOU code method and other approaches (See Chapter 7.) Originally the impedance method, which is a vector method, was used. In contrast, the SPFD method is a scalar method. A comparison of analytical research focusing on the human models is shown in Figure. 5.9. Figure. 5.9 shows the external view, the skeleton, and the main vessels and some organs. Also show is two cross-sections of model (Dawson et al 1996: 1997).

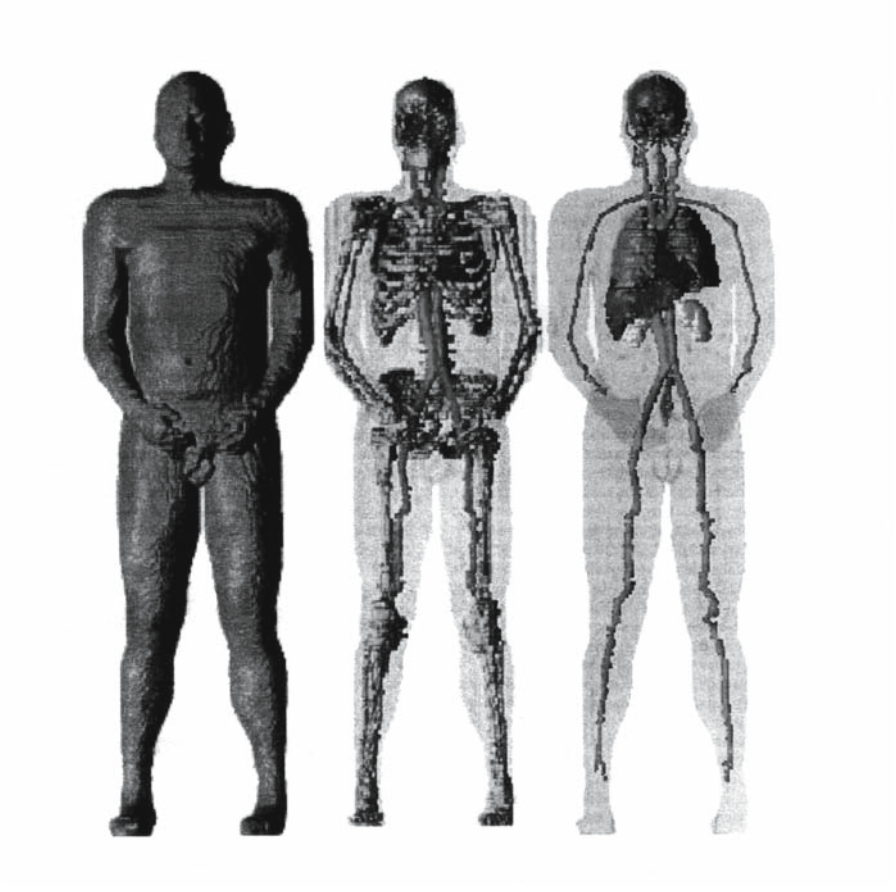

Fig. 5.9. Example of realistically shaped, human ELF current or field computational models. (Stuchly and Dawson 2000 IEEE ©2000). Heterogeneous model of human model: external view, skeleton, major blood vessels and organs.

5.4 Summary

The interaction of ELF electric fields and magnetic fields with organisms differs greatly. For electric fields, organisms perturb the field. In contrast, magnetic fields are not disturbed by organisms and thus remain constant, even with cells, laboratory animals, or human present. However, both ELF electric and magnetic fields induce the electric currents and electric fields in the body. If these electric fields and currents are examined, it is possible to think that the electric field and magnetic field exposure would have the same biological effects. However, the induced electric current distribution inside organisms differs between electric fields and magnetic fields. Thus, for the same tissue, the distribution and strength of the induced electric currents (electric

fields) is different, and clarification of the differences makes clarification of effects mechanisms possible.

Issues that need to be addressed in the future include comparison of the precision of the calculation methods used by each of the models, development of models for cell membranes, and dosimetry from the standpoint of mechanisms of effects, that is comparison of the results of biological experiments and dosimetry research. These related topics will be discussed more detail in Chapter 7.

Other research needs include practical developments like design of better animal exposure cages and cell culture setups. Also, the acquisition of data on tissue conductivity and permittivity with ELF electric and magnetic fields is a pressing need.

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6

What Magnetic Field Parameters are Biologically Effective?

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6.1 Overview

Many laboratories have adopted various exposure designs in order to obtain an answer for the hypothesis raised for a specific experimental purpose. Key features of the exposure designs include (1) the techniques used to construct an exposure facility and (2) the parameters selected for magnetic field exposure, such as strength, duration, polarization, angle to the local geomagnetic field. So far, there is no consensus or guidelines for experimental designs relating exposure conditions.

Although various biological effects have been reported from both *in vivo* and *in vitro* experiments as described in the previous chapters, the mechanisms underlying the effects still are unknown. The flux densities of ELF magnetic field exposure are low, and thus thermal effects can be excluded as a biological mechanisms. Some results have been interpreted based on an assumption that the cell membrane likely is the target of the interaction between physical and biological organism.

Bersani et al.(1997) investigated whether a 50 Hz pulsed magnetic field of 1.88 mT applied for 1, 2, 24, and 72 hours could alter the intramembrane protein (IMP) distribution in cultured Swiss NIH 3T3 fibroblasts. No changes were observed after 1-hour exposure, however 2, 24, and 72 hours of exposure affected the IMP distribution, including significant clustering of the IMP. The authors suggested that, although the underlying mechanisms of clustering required further investigation, their results were useful in studying effects of ELF magnetic fields on cell-membranes. Lisi et al. (2000) performed *in vitro* analysis of morphological changes induced by 50 Hz magnetic field exposure on human B lymphoid cells using atomic force microscopy. The cells were exposed for 64 hours to a sinusoidal vertical magnetic field of 1 mT. The resolution of the microscope is approximately 10 nm in the lateral direction, and around 0.1 nm in the vertical direction. The relative height of the exposed cells was significantly decreased to 44 ± 8% of that of the control after 64 hours exposure because of loss of the microvilli-like structure of the membrane, while no changes in

the volume of cells were identified. These results indicated the cell membrane was affected by magnetic field exposure.

For characterization of *in vivo* and *in vitro* exposure experiments Valberg (1995) categorized key parameters into 18 groups. The parameters included (1) exposure system, (2) exposure intensity and timing, (3) frequency-domain characteristics, (4) spatial characteristics, and (5) combined electric and magnetic field exposure. These parameters have been important in developing laboratory exposure systems and exposure protocols. Often it is assumed that differing outcomes from various experiments are related to the differences in animal species, cell type, exposure duration, exposure conditions etc. Unfortunately, without a clear understanding of basic mechanisms, it is not clear whether particular differences in exposure conditions are relevant or trivial. For example, when trying to understand results from two experiments examining a similar biological endpoint but reporting different results, is the difference between the two power frequencies of 50 and 60 important? Are these to be considered two 'replicates' of one thing or two entirely different experiments?

Many experiments have been conducted with specially designed exposure systems and facilities. Thus, in this chapter, it is investigated how various factors, including polarization and orientation of magnetic fields, exposure duration, application methods of magnetic fields on animals, such as continuous or intermittent exposure, and excessive exposure, are associated with results of exposure experiments.

6.2 Polarization of Magnetic Fields

It has been shown that inversion of either the horizontal or the vertical component of the geomagnetic field can reduce melatonin secretion in rodents (Semm et al.1980, Semm 1983, Welker et al. 1983). These studies stimulated researchers to investigate possible effects of AC magnetic field exposure on endocrine functions. In the earlier years of magnetic field exposure studies, parameters of magnetic fields were not considered critical or important.

In the real world, there are magnetic fields with various profiles. For example, elliptically polarized fields with various ratios of major versus minor axes at different angles, and vertical and horizontal fields can be observed under and near transmission lines. Circularly polarized fields also exist near double circuit transmission lines (see Figure 1.15, Chapter 1).

Shigemitsu et al. (1993) constructed a cubic exposure facility, which was modified from the Helmholtz coil, consisting of five equally spaced coils. This exposure facility can generate magnetic fields of circularly polarized, elliptically polarized, and linear (horizontal and vertical) fields by changing the phase differences, and the amplitude between the two sets of vertical and horizontal coils. The flux density of the field can be controlled by adjusting the electric currents supplied to the coils.

Including the experiments conducted with this exposure facility, the effects of magnetic fields on excretion of melatonin, which are described in Chapter 3, are summarized in Table 6.1 and 6.2. Table 6.1 shows a summary of effects of ELF electric and magnetic fields on melatonin levels in animals, and Table 6.2 shows

the results in humans. Of all the ELF bioeffects, the melatonin database probably is the largest, thus it provides a rich opportunity for attempting to relate the pattern of positive and negative effects to differences in exposure parameters. It is obvious that various parameters, such as magnetic field polarization, strength, and exposure duration, were involved. The subjects of the experiments include rats, mice, hamsters, sheep, baboons, and cows. The strengths of magnetic fields are mostly below 0.1 mT with a maximum of 1 mT. The exposure durations, including 15 minutes, 1–24 hours, a few weeks, 10 months, and a few generations, were set by researchers according to the purpose of each experiment. The levels of melatonin metabolite, 6-hydroxymelatonin sulfate (6-OHMS), secreted from the pineal gland and blood, and secreted into urine, were utilized as indicators to observe the effects of magnetic exposure on the melatonin secretion level. Vertical, horizontal, and elliptic magnetic fields have been applied to animals. Exposure experiments have been conducted under the convolution condition of electric and magnetic fields, which simulated the electromagnetic environment under power lines.

Kato et al. (1993, 1999) reported that exposure to a 50 Hz circularly polarized magnetic field reduced melatonin production in rats. An elliptically polarized field with a 2:1 ratio of major vs minor axes was also effective to reduce the melatonin production, while elliptical field with a 4:1 ratio and horizontal and vertical fields did not influence the pineal function. In a series of experiment, in which young male Sprague-Dawley rats were exposed to 60 Hz horizontal magnetic fields of 1 mT for 6 weeks, 10 days, 20 hours for consecutive 2 days or 1 hour, John et al. (1998) found no significant effects on melatonin metabolite excretion (6-OHMS) in urine. This study showed that horizontal magnetic fields, which were at much higher strength than fields of 1 μT for 6 weeks used by Kato et al. (1994), were ineffective to influence pineal gland function.

Exposure experiments on humans have been conducted on volunteers in chambers with a regulated magnetic field and persons under occupational exposure to magnetic fields. In the experiments with volunteers, the exposure typically is limited only for approximately 8 hours in nighttime, because of the ethical problem associated with a long-term exposure application. In a series of studies by Midwest Research Institute, circularly polarized magnetic fields with continuous or intermittent (1 hour-on/ 1 hour-off) exposure applications were utilized. In the intermittent exposure, the onset/ offset cycle of every 15 seconds during 1 hour of exposure was applied. No effects on melatonin excretion were observed (Graham et al. 1996; 2000; 2001).

Studying persons receiving occupational exposure to magnetic fields, Burch et al. (2000) reported magnetic field dependent reduction in 6-OHMS levels among persons working in substations and 3-phase environments, where elliptically polarized fields were observed. The results suggested that circular or elliptical magnetic field polarization was associated with magnetic-field-induced melatonin suppression in humans.

The results from studies on both animals and humans suggested that polarization of magnetic fields is critical for biological systems. However, polarization of a magnetic field was ignored in studies of magnetic field effects on humans. For linear

polarization, the orientation of a field is constant. When two or more fields are applied, they interfere with each other to create elliptically polarized fields. If two fields of equal amplitude with 90 degree phase difference interact with each other, rotating circular polarization is observed. In a linearly polarized field, the induced current crosses the zero current level, while in an elliptically polarized field, the induced current does not reach zero level. Calculations show that the current density produced by a circularly polarized magnetic field is, when the same maximum magnetic field (B_{max}) is assumed for the linearly and circularly polarized magnetic field, about 40% greater than that induced by either a linearly polarized or elliptically polarized magnetic field of the same density. The absorbed power density is also about twice greater for the circularly polarized magnetic field than for the elliptically polarized or linearly polarized magnetic field (Kato and Shigemitsu 1997)

Recently renewed attention has been placed on the issue of magnetic field polarization when relation between melatonin reduction and magnetic field exposure is discussed (Ainsbury et al. 2005; Henshaw and Reiter 2005). In a study which measured the ellipticity of 50 Hz magnetic fields in a bedroom continuously for 24 hours, it was shown that the ellipticity varied between about 5% and about 20% (Ainsbury et al. 2005). It was considered that a complicated condition, in which magnetic fields were distorted from pure single sinusoids, was involved in the variation of the ellipticity. Thus, they indicated that the magnetic fields at home should not be characterized solely by simple measurements, such as time-weighted average or peak exposure levels.

It seems clear that ELF fields can reduce melatonin in laboratory animals and humans. However, in about 1/3 of the published papers, negative results are reported. This indicates that all of the factors controlling the effect are not understood. The factor that appears to be understood best is magnetic field polarity. In the rat, circularly polarized fields most effectively induce currents and most effectively reduce melatonin. However, additional research is required to place this hypothesis on an incontrovertible footing and to assess its generality. Perhaps the size and shape of the rodent brain and the location of its pineal gland make it particularly susceptible to field effects. It is interesting to note that the proportion of negative publications is higher in studies with larger animals and humans.

Table 6.1. Studies of the Effect of ELF Electric and Magnetic Fields on Melatonin (MT) in Animals

	Exposure	Duration	Response
Rats			
Bakos (1995)	50 Hz, 5, 500 μT_{rms}, Vertical	24 hr	No effect (aMT6s)
Bakos (1997)	50 Hz, 1, 100 μT_{rms}, Vertical	24 hr	Increased night after 100 μT exposure (aMT6s)
Bakos (1999)	50 Hz, 1, 100 μT_{rms}, Horizontal	24 hr	No effect
Bakos (2002)	50 Hz, 50, 100 μT_{rms}, Vertical	1 week	No effect
Chacón (2000)	50 Hz, 10, 100, 1000 μT_{rms}, Vertical	1 hr	Decreased NAT activity at 1000 μT
Fedrowitz (2002)	50 Hz, 100 μT_{rms}, Horizontal	2 weeks	No effect
Grota (1994)	60 Hz, 65 kV/m, Vertical	30 days (dark)	Decreased serum MT, no change in night pineal MT
Jentsch (1993)	10 Hz, 0.03 mT_{p-p}	10–30 min	No effect
John (1998)	60 Hz, 5, 100, 500 μT_{rms}, Horizontal	24 hr	No effect (6-OHMS)
John (1998)	60 Hz, 1 mT_{rms}, Intermittent (1 min on·off), Horizontal	2 days	No effect (6-OHMS)
John (1998)	60 Hz, 1 mT_{rms}, Intermittent (1 min on·off), Horizontal	1 hr	No effect (6-OHMS)
John (1998)	60 Hz, 1 mT_{rms}, Horizontal	10 days	No effect (6-OHMS)
John (1998)	60 Hz, 1 mT_{rms}, Horizontal	6 weeks	No effect (6-OHMS)
Kato (1993)	50 Hz, > 1.4 μT_{rms}, Circular	6 weeks	Decreased pineal and serum MT above 1.4 μT_{rms}
Kato (1994)	50 Hz, 1.4 μT_{rms}, Circular	6 weeks	Night-time MT level reduced
Kato (1994)	50 Hz, 1.4 μT_{rms}, Circular	6 weeks	Night-time MT level reduced, return to normal within one week
Kato (1994)	50 Hz, 1 μT_{rms}, Horizontal/Vertical	6 weeks	No effect
Lewy (2003)	50 Hz, 1 mT	4 hr	No effect
Löscher (1994)	50 Hz, 0.3~1.5 μT_{rms}, Gradient	8–9 weeks	Serum MT reduced
Löscher (1998)	50 Hz, 100 μT_{rms}, Horizontal	1–13 weeks	No consistent effects on MT
Marzinez-Soriano (1992)	50 Hz, 5.2 mT	30 min per day for 15 days	Serum MT reduced
Mevissen (1996)	50 Hz, 100 μT_{rms}, Horizontal	91 days	Decreased pineal and serum MT
Mevissen (1996)	50 Hz, 50 μT_{rms}, Horizontal	91 days	Decreased pineal and serum MT
Reiter (1988)	60 Hz, 10-130 kV/m, Vertical	23 days of conception	Decreased pineal MT at night-time, MT rhythm phase delayed
Rosen (1998)	60 Hz, 0.05 mT, Vertical	12 hr	Decreased in pinealocyte
Selmaoui (1995)	50 Hz, 1, 10, 100 μT, Horizontal	12 hr	Night-time MT, NAT decreased at 100 μT
Selmaoui (1995)	50 Hz, 1, 10, 100 μT, Horizontal	30 days	Night-time MT, NAT decreased at 10 and 100 μT
Selmaoui (1999)	50 Hz, 100 μT, Horizontal	1 week	NAT activity and serum MT decreased (young rat)
Tripp (2003)	50 Hz, 500 μT, Circular	4 hr	No effect
Wilson (1981)	60 Hz, 65 kV/m (1.7–1.9 kV/m), Vertical	30 days	NAT activity and pineal MT decreased
Wilson (1986)	60 Hz, 39 kV/m, Vertical	1–4 weeks	NAT activity and pineal MT decreased

Table 6.1. Studies of the Effect of ELF Electric and Magnetic Fields on Melatonin (MT) in Animals (continued)

	Exposure	Duration	Response
Mice			
De Bruyn (2001)	50 Hz, 2.75 μT_{rms} (0.5~77 μT_{rms}), Elliptical	23 days	No effect
Heikkinen (1999)	50 Hz, 1.3, 13, 130 μT, Vertical	17 months	No effect (6-OHMS)
Picazo (1998)	50 Hz, 15 μT_{rms}, Horizontal	3 generations	Decreased serum MT
Hamster			
Brendel (2000)	50 Hz, $16^{2/3}$ Hz, 86 μT	8 hr	Decreased pineal MT
Niehaus (1997)	50 Hz, 450 μT_{p-p}, 300 μT_{p-p}	56 days	Increased pineal and serum MT depending on wave shape
Truong (1996)	60 Hz, 100 μT, Vertical	15 min (light)	No effect, reduced night-time peak in first study
Truong (1997)	60 Hz, 10, 100 μT, Vertical	15 min (light)	No effect
Yellon (1994)	60 Hz, 100 μT, Vertical	15 min (light)	Decreased pineal and serum MT (first study), No replication
Yellon (1996)	60 Hz, 100 μT, Vertical	15 min (light)	Decreased pineal and serum MT
Yellon (1998)	60 Hz, 100 μT, Vertical	15 min (dark)	No effect
Wilson (1999)	60 Hz, 0.1 mT, Horizontal	15 min (light)	Decreased pineal MT
Sheep			
Lee (1993)	60 Hz, 6 kV/m, 3.37 μT, Powerline	10 months	No effect, seasonal effects
Lee (1995)	60 Hz, 6 kV/m, 4 μT, Powerline	10 months	No effect, seasonal effects
Baboon			
Rogers (1995)	60 Hz, 6 kV/m + 50 μT, 30 kV/m + 100 μT, EF; Vertical, MF; Horizontal, Irregular On·Off	9. 21 days	Serum MT reduced
Rogers (1995)	60 Hz, 6 kV/m + 50 μT, 30 kV/m + 100 μT, Regular On·Off	12 h for 2, 4, 6 weeks	No effect
Bird			
Fernie (1999)	60 Hz, 10 kV/m, 30 μT	70 days	Decreased serum MT
Cow			
Burchard (1998)	60 Hz, 10 kV/m (vertcial) + 30 μT (Horizontal), Powerline	16 h per day for 4 weeks	No effect
Burchard (2004)	60 Hz, 10 kV/m (vertcial), Powerline	16 h per day for 4 weeks	No effect
Rodriguez (2004)	60 Hz, 10 kV/m (vertcial) + 30 μT (Horizontal), Powerline	16 h per day for 4 weeks	Lowered MT during light period, No effect in dark period

Table 6.2. Studies of the Effect of ELF Electric and Magnetic Fields on Melatonin (MT) in Human Being

	Exposure	Duration	Response
Volunteer study			
Ametz (1996)	VDT at work	1 day work vs no work	MT decreased on work day
Akerstedt (1999)	50 Hz, 1 μT, Vertical	8 hr during sleep for 3–5 days	Night-time serum MT no effect
Crasson (2001)	50 Hz, 100 μT_{rms}, Linear, Intermittent	30 min at daytime	No overall effect (plasma MT)
Graham (1996a)	60 Hz, 100 μT_{rms}, Circular, Continuous	8 hr at night	No effect (plasma MT)
Graham (1996b)	60 Hz, 1, 20 μT_{rms}, Circular, Intermittent	8 hr at night	No effect, Possible effect on low MT subjects, no replication
Graham (2000)	60 Hz, 28.3 μT_{rms}, Circular, Continuous, Intermittent	8 hr at night for 4 days	No effect (serum MT and 6-OHMs)
Graham (2001a)	60 Hz, 28.3 μT_{rms}, Circular, Intermittent	8 hr at night	No effect (serum MT and 6-OHMs)
Graham (2001b)	60 Hz, 127.3 μT_{rms}, Circular, Continuous, Intermittent	8 hr at night for 4 days	No effect (serum MT and 6-OHMs)
Graham (2001c)	60 Hz, 28.3 μT_{rms}, Circular, Intermittent	8 hr at night	No effect
Griefahn (2001)	17.6 Hz, 0.2 mT, horizontal	8 hr	No effect
Hong (2001)	50 Hz, 0.7–8.3 μT (Electric blanket)	8 weeks	No effect
Karasek (1998)	40 Hz, 2.9 mT	20 min per day for 3 weeks	Night-time MT decreased
Karasek (2000)	200 Hz, 25~80 μT	16 min per day for 3 weeks	No effect (serum MT)
Kurokawa (2003)	50 Hz, 20 μT (with harmonics and transient)	10 hr	No effect (plasma MT)
Levallois (2001)	735 kV powerline, living close to power line		Trend of decreasing of 6-OHMs with age, No overall effect
Selmaoui (1996)	50 Hz, 10 μT, Horizontal, Continuous, Intermittent	9 hr at night	No effect (serum MT)
Selmaoui (1997)	50 Hz, 10 μT, Horizontal, Continuous, Intermittent	9 hr at night	No effect
Warman (2003)	50 Hz, 200–300 μT, Circular	2 hr at night	No effect (plasma MT)
Wilson (1990)	60 Hz, 0.2–0.6 μT (Electric blanket)	8 weeks	No overall effect, decreased 6-OHMs in 7/28 users (CPW)
Wood (1998)	50 Hz, 20 μT, Circular, Continuous, Intermittent	1.5–4 hrs	No overall effect, delayed MT rise time in subgroup
Occupational			
Burch (1998)	60 Hz, 0.1–0.2 μT at work, home and sleep	3 consecutive workdays	No overall effect, stable MF associated with reduced 6-OHMs
Burch (1999)	60 Hz,	3 consecutive workdays	No overall effect, stable MF associated with reduced 6-OHMs
Burch (2000)	60 Hz, 1-phase and 3-phase conductors,	3 consecutive workdays	Circular or elliptical polarized MF is associated with MT suppression
Juutilainen (2000)	60 Hz, 0.3–1 μT (low), > 1 μT (high)	3 consecutive workdays	No effect
Pfluger (1996)	16.7 Hz, 20 μT at work, Control: 50 Hz, 1 μT		Evening 6-OHMS decreased, no effect on morning level
Touitou (2003)	50 Hz, 0.1–2.6 μT, Control: 0.004–0.092 μT	Chronic exposure up to 20 yr	No effect

6.3 Orientation of Magnetic Fields

When considering magnetic fields, the phrase "orientation of a magnetic field" can have two different meanings on living organisms: (1) ability of migrating and/or homing animals, such as birds and reptiles to detect and discriminate directional properties of magnetic fields, or (2) the direction of an applied magnetic fields. Discussing the former meaning Phillips and Deutchland (1997) discussed that sensitivity to the direction of magnetic fields, which is widely observed among birds, reptiles, and some other terrestrial organisms, may derive geographic position ('map') information from spatial gradients in one or more parameters of the magnetic field. In the study, it was shown that shoreward compass orientation of newt was influenced by light of certain wavelength. In the study to examine the latter meaning of orientation, Yasui (1994) measured magnetic fields under double circuit transmission lines. As shown in Fig 1.15, orientation of magnetic fields under transmission lines was mainly vertical, and the strength and ellipticity of magnetic field changed gradually from the center. In laboratory experiments, mutual directions of a body, such as frontal-dorsal axis and axis of the applied magnetic field, were occasionally influenced biological functions (Welker et al. 1983)

Effects of different orientation of magnetic fields on cells also have been investigated (Simko et al. 1998). In the experiment, genotoxic effects of continuous application of a magnetic field of 50 Hz and 1 mT for 24, 48, and 72 hours on human amniotic fluid cell (AFC) were investigated by observing the formation of micronucleus. Merritt-coil and Helmholtz-coil exposure systems were utilized for application of exposure to the surface of the culture medium vertically and horizontally. A formation of micronucleus was increased under horizontal application of the magnetic field in the Helmholtz-coil system, while no increase was observed in the horizontally generated Merritt-coil system. In the application of vertical magnetic fields, a micronucleus formation after 72 hours was induced in the Merritt-coil system, while no induction was observed in the vertically generated Helmholtz-coil system. The authors indicated that the differences described above were closely related to the orientation of the magnetic field to the cell culture dish, and the differences of physical conditions between the exposure systems, in certain cell types. With the action mechanism which is the current induced by a magnetic field applied, the induced current is generally increased with the application of a magnetic field vertically to the culture dish. Genotoxic effects on chromosomes also were investigated in an experiment with the application of a magnetic field of 50 Hz and 300 μT for 72 hours on AFC (Nordenson et al. 1994). No increased incidence of chromosomal aberration was observed with the continuous application of a horizontal magnetic field in the Helmholtz-coil system in the study.

It has not been fully explained why results obtained from studies using different magnetic field generating systems varied. In both Helmholtz-coil and Merritt-coil systems, it is possible to produce both horizontal and vertical magnetic fields by altering the arrangement of the system. Larger uniform space of a magnetic field in a system can be obtained better with a Merritt-coil than with a Helmholtz-coil. Thus, in order to clarify the reason to have obtained different results in different

exposure systems, other factors should be considered for linking the difference in the results in the difference of systems. This is also one of the reasons which make the interpretation of results of exposure experiments difficult.

6.4 Exposure Intensity of Magnetic Fields

Magnetic field strength is referred to the magnetic flux density, and described as a Tesla in SI unit. One tesla is equal to 10^4G in CGS unit. Field strength is usually expressed in terms of rms (root-mean-square) values, effective values, than peak values. The relationship between two values is $B_{peak} = 1.4 \times B_{rms}$. Magnetic field strength is usually described as an effective value. However, some reports do not have a clear description of effective values and peak values. Thus, it is required to take this into consideration when the effects of magnetic fields obtained from various reports are compared.

Most studies have been conducted using strength of magnetic fields under 0.1 mT (i.e., $< 100\ \mu$T) to observe effects of low-level magnetic fields. For example Selmaoui and Touitou (1995) investigated the effects of low-level horizontal magnetic fields of 50 Hz and 1, 10, and 100 μT on 9-week old rats for 12 hours and 30 days (18 hours a day) in terms of serum melatonin and NAT activity. With a exposure duration of 12 hours, the highest intensity (100 μT) was effective to depress melatonin synthesis in rats, while exposure at 10 μT did not show any effects (Selmaoui and Touitou 1995). Results showed a reduction of melatonin synthesis and the NAT activity with 100 μT exposure in a 12-hour-exposure experiment. Several reports indicated that the intensity of magnetic fields was a factor to be taken into account, as many researchers observed the effects at some intensity. Usually the effect is a step-function effect on melatonin suppression (Kato et al. 1993).

Selmaoui and Touitou (1999) further investigated the effects of a vertical magnetic field of 50 Hz and 100 μT on 9-week-old and 23-week-old rats in terms of various factors, such as melatonin secretion. Reduction of the level of serum melatonin and NAT activity was observed in the 9-week-old rats, while no effects of the magnetic field were observed with the 23-week old rats; thus, it was assumed that old rats had no sensitivity to magnetic fields. In many experiments, young animals of few-weeks old were utilized; thus, observation of no magnetic effects on old animals described in the report cannot be fully explained based on verified data. Reduction of melatonin secretion according to aging, which has been confirmed, may be involved in this phenomenon. On further investigation with an extended exposure duration of 30 days on melatonin secretion, it was shown that weaker stimulus at 10 μT and 100 μT were effective (Selmaoui and Touitou 1995). It was indicated that exposure strength and duration of a magnetic field were critical in the results. In exceptionally long (multi-generation) period of exposure experiments, Picazo et al. (1998) reported radical decrease of melatonin levels and loss of the circadian rhythm in the third-generation mice. These reports indicated that exposure period was also critical upon exposure evaluation.

Cumulative or interactive effects of duration and intensity also were suggested in several experiments (Kato et al. 1993; Selmaoui and Touitou 1995; Lai and Carino 1999). Kato et al. (1993), Kato and Shigemitsu (1997) reported that both circularly polarized magnetic field, and elliptically polarized magnetic field at the ratio of major versus minor axes of 2:1, effectively decreased melatonin production. However, it was found that 6-week exposure to magnetic fields of vertical, horizontal, or elliptical (with 4:1 ratio) polarization was ineffective.

It was reported that effects of magnetic exposure on an activity of the central cholinergic nervous system of rats were affected by exposure strength and duration (Lai and Carino 1999). Effects of exposure to different intensities of the magnetic field and durations of exposure on the central cholinergic activity in the frontal cortex and hippocampus of the rat were evaluated. In the first experiment, rats were exposed to a 60 Hz magnetic field for 60 min at a flux density of 0.5, 1.0, 1.5, or 2.0 mT. In another experiment, effects of exposure to a 1.0 mT magnetic filed for 30, 45, 60 and 90 min were investigated. A significant decrease in cholinergic activity was observed after exposure to 2.0 mT. Only after 90-minute exposure, a decrease in cholinergic activity was observed. The authors concluded that the combination of intensity and duration of exposure influenced the effects of magnetic field exposure. By increasing the duration of exposure, effects can be observed with a lower intensity of a magnetic field.

Dose-response relationships are so ubiquitous in biology that it seems obvious that ELF fields also should show dose-response relationships. Furthermore, field intensity is the most obvious candidate for dose. However, the available literature contains few examples that convincingly demonstrate the presence of dose-response. Two general interpretations are possible. Some scientists conclude either that ELF field-bioeffects do not exist or that they might exist, but that the domain is hopelessly messy. Alternatively, one can argue that the complexity of the existing data base merely shows that Mother Nature is complicated. If scientists in bioelectromagnetics thought more carefully, and if they conducted more appropriate experiments so that general mechanisms could be understood, it is likely that order would emerge.

6.5 Exposure Duration of Magnetic Fields

In vivo exposure experiments have been conducted with various exposure durations ranging from 5 minutes to 3 generations of hamster. Yellon (1996), Truong and Yellon (1997), and Wilson et al. (1999) observed suppression of melatonin secretion in hamsters after linearly polarized magnetic field exposure for 15 minutes. Wood et al. (1998) reported that 1.5–4 hour exposure of circularly polarized magnetic fields prior to the rise of melatonin secretion at night delayed the rising phase in humans. Selmaoui et al. (1996) observed no effects after 9 hour exposure on human volunteers. As mentioned above, very short exposure duration was effective to influence the pineal function under a certain experimental condition although it seems to be difficult to draw a line between the effective zone and ineffective zone.

Graham et al. (1996, 2000, 2001) examined the effects of magnetic field exposure for 1 to 4 consecutive nights, and observed no significant changes in melatonin secretion levels of human volunteers. Selmaoui and Touitou (1999) reported that a melatonin concentration was depressed after one week exposure on young rats. Kato et al. (1993, 1994) found that 6 week exposure resulted in decrease of melatonin concentrations in rats. Löscher et al. (1994) reported that a serum melatonin concentration was suppressed after 8–9 weeks of magnetic field exposure. Picazo et al. (1998) performed exposure experiments with the very long duration of third generations on mice, and observed a radical decrease of melatonin production in the third generation. The summary of experimental conditions and results is shown in Table 6.1 and 6.2. In the studies on short-term (30 minutes to 8 hours) exposure at different flux densities on human melatonin excretion, no significant effects were observed (Crasson et al. 2001; Graham et al. 2001; Griefahn et al, 2001). In these studies, continuous and intermittent (15 seconds on/off interval) exposure of linear and circular magnetic fields with frequencies of 50, 60, and 17.6 Hz were exposed as shown in Table 6.2, although some effective value description of magnetic field strength is missing. Even in the experiments with the same purpose of investigating effects of magnetic field exposure on melatonin secretion, various experimental conditions have been used; thus, the differences among utilized conditions should be taken into consideration upon comparison of results among these studies. On the other hand, it may be possible to infer that there are no effects of magnetic field exposure on melatonin secretion as a whole, since experiments have been conducted under various conditions. It may also be possible that only specific exposure conditions can induce exposure effects, although it has not been clearly indicated.

Exposure experiments on mice for 23 days, 17 months, and three generations have been reported (De Bruyn et al. 2001; Heikkinen et al. 1999; Picazo et al. 1998). Differences of experimental conditions among these studies are shown in Table 6.3. De Bruyn et al. measured the magnetic fields under power lines of 88–400 kV, and developed and utilized a system which can recreate a magnetic field of the strength which was 11-times stronger than the measured values in a laboratory. The system, with the variation of the magnetic field strength was between 0.5–77 μT with a mean of 2.75 μT, required 3 minutes intervals before moving to the next field level with the maximum rate of 0.43 μT/s. The experiment, in which lifetime exposure of 3 generations was conducted, indicated the disappearance of a circadian rhythm and reduction of nighttime melatonin; thus, accumulated effects of magnetic fields over a few generations were identified (Picazo et al. 1998). No magnetic field effects on melatonin secretion were observed both in the short-term (23 days) exposure experiment which utilized a recreated under-power-line environment, and the chronic exposure experiment for over 17 months in a vertical magnetic field.

In behavioral studies, rats and mice were exposed to magnetic fields for 45 minutes, and effects were investigated using maze trials (Lai 1996; Sienkiewicz et al. 1996). For the study to assess receptors of chemical transmitter substances in the central nervous system, 15 to 60 minutes exposure to magnetic fields was commonly used in many laboratories.

Table 6.3. Comparison of Three Different Exposure Experiments with Different Protocols

Researcher	Heikkinen	De Bruyn	Picazo
Magnetic field	Vertical polarized magnetic field 50 Hz, 1.3, 13, 130 μT (20 min On-Off; 24 hr/day)	Elliptically polarized magnetic field 50 Hz, 2.75 μT (0.5–77 μT) (24 hr/day)	Horizontal polarized magnetic field 50 Hz, 15 μT
Strain	Female CBA/S (3–5 weeks old) mice	Male AKR and BALB/c (19 days old) mice	Male OF1 mice
Exposure	Chronic over 17 months	23 days	third generation until 14 weeks old
Biological Endpoints	Noctural 6-OHMS production	Noctural plasma melatonin	Plasma melatonin and day-night rhythm

In the experiment by Lai, effects of magnetic field exposure on the learning ability of adult rats (250–300 g), which were in a learning program of 45 minutes per day for 10 days using a 12-arm radial maze, were investigated. It was shown that exposure to a magnetic field of 60 Hz and 0.75 mT for 45 minutes on the rats immediately before each training session retarded their learning ability significantly. However, rats which were pretreated with a cholinergic agonist, physostigmine, showed an inverse effect of magnetic field exposure on learning. In the experiment for investigating effects of magnetic field exposure on 12-week-old adult mice, they were in a learning program of 10 minutes per day for 10 days (Sienkiewicz et al. 1996). It was indicated that exposure to 50 Hz vertical magnetic fields of 5 μT, 50 μT, 0.5 mT, and 5.0 mT did not have any effects on learning regardless of the magnetic strength. Researchers have adopted different durations of exposure to the subjects depending on the end point of the study. Differences among results may be associated to differences in experimental conditions.

The effect of duration as an important variable in ELF bioelectromagnetics is not clear. Intuitively, if field strength is the first most likely measure of dose, then duration of exposure would be the second. Then one would ask about intensity x time as a joint factor: is the product of intensity x time a constant, is the relationship a power law etc. would be obvious equations.

6.6 Time-weighted Average and Dose

In many studies of humans, time-weighted average (TWA) is used as the magnetic field dose. In epidemiological studies, time-weighted average is used implicitly for the studies based on a characterization of homes or jobs, such as wire-code and job-title analysis. However, these factors may be too crude to result in a meaningful characterization of the fields.

For exposure evaluation in epidemiological research, in addition to TWA, many alternative metrics have been proposed, such as time above a threshold, time within an exposure range, a rate of change, field stability, and the maximum magnetic field strength (Lee et al. 2002; Li et al. 2002). It was reported that there was no significant correlation between a time-weighted average above 0.3 μT, which was the exposure scale, and the incidence of spontaneous abortion, while increased incidence of spontaneous abortion was observed as the maximum magnetic field strength with the threshold level of 1.6 μT was increased in a prospective cohort study (Li et al. 2002). The study that utilized TWA, the maximum magnetic field level (MAX), a rate of change metric (RCM), and a wire code as exposure scales was conducted (Lee et al. 2002). Results indicated that there was no significant correlation between the high wire code and the incidence of spontaneous abortion, and no dose-response relationship nor significant correlation between TWA and a risk, while the correlation between the incidence of spontaneous abortion and MAX, and RCM, and the dose-response relationship, in which the odds ratio increased as exposure increased, were observed.

Mezei et al., Savitz et al. reexamined Lee et al's and Li et al's study on magnetic field exposure and spontaneous abortion (Mezei et al. 2005, Savitz et al. 2006). They summarized that 50 Hz, 1.6 μT maximum magnetic field is not associated with spontaneous abortion risk but the association is related to the result of behavioral differences between women with healthy pregnancies and women who experience miscarriages.

In epidemiological studies, relationships between magnetic field exposure and diseases have been mainly discussed using a time-weighted average, which was the mean value of magnetic field measurements for 24–48 hours, as an exposure indicator. For example, when the magnetic field exposure included exposure to a magnetic field of 10 μT for 1 hour a day and a very low-level magnetic field for the rest of the 23 hours, the exposure in time-weighted average would be equivalent to exposure to a constant magnetic field of low-level, like 0.2–0.3 μT. However, in recent years, as described above, epidemiological results from some studies, which utilized MAX and RCM as exposure indicators, have been accepted. This may be leading to distrust of using time-weighted average as an appropriate exposure indicator in epidemiological research evaluation, and a further decrease in detectability of magnetic field exposure.

It has been tacitly assumed that a magnetic field exposure scale with time-weighted average is associated to the concept of "dose." As described in this chapter, externally applied electric and magnetic field strengths commonly are utilized as an indicator, referred to as "exposure," for comparison of results in electromagnetic field studies. Thus, exposure is described as both field strength and exposure duration.

For ionizing radiation exposure, the amount of exposure is expressed as "dose," which is the absorbed radiation by an exposed living organism, and an effective dose, which is dependent on various factors, such as the size of the exposed living organism, body organization, and sensitivity, is defined. "Dose" in electromagnetic field research should involve various factors relating to the concept of "exposure" as described above. The main factors modifying the effective dose include (1) shape and

size of an exposed living organism, (2) the electrical properties of the organisms, (3) characteristics of applied electric/ magnetic fields, and (4) orientation of the fields with respect to the exposed living organism. If externally applied fields are referred to as exposure, "dose" can be referred to as the resulting internal field and current distribution. This concept does not allow simply connecting a time-weight average and "dose".

As described in Chapter 5, in which the interaction between electric and magnetic fields and living organisms was described, and the concept of dosimetry was introduced, examining "dose" requires dosimetry of electromagnetic fields as an important factor. In Chapter 7, one of the concepts, which can clarify biological effects, and the concept of dosimetry, which involves induced current, were described.

Knowledge on relevant metric and period of exposure still is lacking in epidemiological study. If such knowledge were obtained, then laboratory experimenters would be able to construct exposure facility mimicking the conditions. In reverse, if laboratory experiments firmly established any adverse health effects on experimental animals, then epidemiologists would be able to use such conditions for searching pathological outcomes of either residential or occupational populations.

In laboratory studies, the experimenters are able to strictly characterize the exposure parameters such as frequency, strength of the field and duration. However, it is necessary to investigate for "dose" with higher efficacy in electromagnetic research.

6.7 Intermittency or Irregularity of Magnetic Fields

Perfect forms of sinusoidal or rectangular magnetic fields rarely exist in the natural environment. However, in laboratory experiments, researchers tend to utilize both continuous and intermittent magnetic fields with perfect sinusoidal or rectangular form, since these fields are easier to produce. Experiments have been conducted with the aim to clarify differences in exposure effects by continuous and intermittent exposure. Various conditions for intermittent frequency and scheduling were utilized to suit each purpose. Although there is no clear definition for "intermittency" or "irregularity," they indicate a chronological repetitive change, which is a spontaneous chronological change, of magnetic field.

Exposure experiments on humans have been conducted on both (1) volunteers in chambers with a well controlled conditions and regulated magnetic field or (2) persons under occupational exposure to magnetic fields. In controlled experiments with volunteers, the exposure is limited to only approximately 8 hours in nighttime due to the difficulty of a long-term exposure application upon the ethical problem. In a series of studies by Midwest Research Institute, circular magnetic fields with continuous and intermittent (1 hour-on/ 1 hour-off) exposure applications were utilized. In the intermittent exposure, the on-off cycle of every 15 seconds during 1 hour of exposure application was applied. No effects on melatonin excretion were observed (Graham et al. 1996; 2000; 2001). The intermittent pattern profile in this experiment was derived from conditions with high efficiency, which were obtained from results

of investigation of influences on electroencephalogram and electrocardiogram in human, heart-rate variation, and event-related potential (Cook et al.1992).

Levels of melatonin secretion of electric company workers, who were occupationally exposed to magnetic fields, were investigated (Burch et al. 1998; 1999). The exposure was measured at 15-second intervals over 3 consecutive 24-hour periods. With a magnetic field intensity, intermittence, temporal stability as exposure indicators, RCM and the standardized RCM (RCMS: RCM/standard deviation) were calculated to estimate intermittence and temporal stability, respectively. Effects of magnetic field exposure on total melatonin metabolites (6-OHMS) secreted into urine during nighttime, and creatinine-adjusted concentration of nighttime 6-OHMS (6-OHMS/cr), were investigated. Residential RCMS magnetic field exposures were associated with lower nocturnal 6-OHMS/cr concentration. The greatest reductions occurred when RCMS exposure both at work and at home were combined. This result indicated that temporally stable magnetic field exposures were associated with reduced nocturnal 6-OHMS excretion in humans. Furthermore, it was shown that light exposure modified the magnetic field effects. A progressive decrease in the mean 6-OHMS/cr concentration in response to temporally stable magnetic fields was observed in persons with low workplace light exposures, whereas those with high ambient light exposure showed negligible magnetic field effects.

Effects of intermittent and continuous exposure on cells were examined in several studies (Ivancsits et al. 2002; 2003; 2005; Schuderer et al. 2004). These studies were conducted as a part of "REFLEX" project (Risk evaluation of potential environmental hazards from low energy EMF exposure using sensitive *in vitro* methods), which is a part of the 5th European Framework Program "Quality of Life and Management of Living Resources." Genotoxic effects of intermittent application of a vertical magnetic field of 50 Hz and 1000 μT on human diploid fibroblasts were observed as increase of DNA strand break levels. On the other hand, no effects of continuous application of the magnetic field exposure were observed. In the study, the maximum level of DNA strand break was observed with intermittent exposure of 5/10 (5 minutes field-on/ 10 minutes field-off), and no difference was observed in 5/25 compared to the control. It was assumed that DNA damage caused during the on-periods was repaired by the intrinsic DNA repairing mechanism above the 15 minutes of off-periods. Therefore, the degree of DNA damage depends on the exposure duration and the length of repairing time. (However, the author mentioned that this explanation is speculative and requires further evaluation.) It was also confirmed that the induction of DNA strand breaks by intermittent exposure was cell-type specific (Ivancsits et al. 2005). Since intermittent exposure induced the DNA strand breaks, it was reported that ELF magnetic fields possessed genotoxic effects. The experiment, in which the effects of intermittent exposure of a vertical magnetic field of 50 Hz and 30 μT, with a 15-second on-off cycle, and a cycle of 2-second on and 20-second off, for 72 hours on human amniotic fluid cell (AFC) were investigated with chromosomal aberration as an indicator, provided a result indicating increased incidence of chromosomal aberration by intermittent exposure (Nordenson et al. 1994). In the study, it was also reported that no effects of continuous exposure of a magnetic field of 50 Hz and 300 μT for 72 hours were observed. Although several discussions have been made re-

garding the difference in effects of continuous and intermittent exposure, sufficient explanation has not yet been provided.

Niehaus et al. (1997) investigated the effects of shape of magnetic fields on the reproductive and physiological function in Djungarian hamsters. They used two types of weak magnetic fields (sinusoidal magnetic field; 50 Hz, 450 μT_{p^-p}, max.dB/dt = 140 mT/s; rectangular magnetic field; 360 μT, max.dB/dt = 2.5 T/s). Stronger effects were seen when animals were exposed to rapidly changing magnetic field than to sinusoidal ones. They reported a significant increase in serum melatonin levels due to high dB/dt magnetic field exposure. The dB/dt ratios of the rectangular magnetic field were approximately 17 times larger than with the sinusoidal magnetic field. The effects of magnetic fields are dependent on the field's characteristics. This is one of example for *in vivo* effect by exposure of rat to different shape of magnetic fields.

Although the suggestion is intriguing, the evidence that intermittency or irregularity of field exposure is an important determinant of ELF bioeffects is weak. The possibility that waveform is important, especially high dB/dt, has somewhat more support. However, the relevant database is small. Thus additional research certainly seems desirable.

6.8 Transients of Magnetic Fields

The term "transients" indicates rapidly decaying high-frequency electric and magnetic fields. In the usual living environment, numerous variations of magnetic field profiles are assumed to exist; such as fields which are originated primarily from switching, and field frequencies ranging from below 50 Hz to about 500 MHz. Magnetic fields from transients ranged from 0.001 to 10 μT, with a very strong spatial dependence (EPRI, 1994).

It is assumed that rapid rise-and decay time signals induced internal electric current and eddy current in living systems, and were expected to be an effective biological stimulus. Wilson et al. (1990) reported that an electric blanket, which produced transients, and with relatively frequent on/off, was more likely to influence urinary melatonin concentrations of humans than did electric blankets with no production of transients and relatively infrequent on/off.

"Transient" does not refer to a periodically repeating phenomenon. In exposure experiments, magnetic fields are obtained with the application of an electric current in a magnetic field generating system. When a transitional electric current occurs, a transitional magnetic field and transitional electric field occur within the exposed subject. Especially with inductive load, such as involving large coils, this problem should be taken into consideration. In the laboratory exposure experiments, however, no researchers have ever adopted scheduled transient exposure paradigms to study any effects on biological systems. There seems to be two main reasons; 1) so far no reliable epidemiological studies have been reported indicating "transients" caused any kind of outcome, and 2) therefore researchers don't know how to produce what kind of "transients" paradigms.

Several exposure experiments using regular and irregular exposing schedules have been reported. Rogers et al. reported a significant decrease of melatonin concentration in baboons after regular and irregular exposure of an electric and magnetic field of 60 Hz. When slow electric and magnetic fields (E/MF) onsets/ offsets were used in exposure experiments in order to avoid occurrence of a transitional electric current, no effects of daytime exposure for 6 weeks in nighttime melatonin secretion were observed (Rogers et al. 1995a). On the other hand, reduction of nighttime melatonin secretion was observed with rapid E/MF onsets/ offsets in the same experimental condition (Rogers et al. 1995b), which may indicate a transitional phenomenon. Combination exposure of vertical electric fields and horizontal magnetic fields of 6 kV/m+50 μT and 30 kV/m+ 100 μT, respectively, and 60 Hz is shown in Table 6.1. The results indicated that occurrence of spontaneous transient electric current and eddy current *in vivo* upon "rapid" onsets/ offsets may be involved in melatonin secretion. In order to identify whether the difference in electric phenomenon described above influenced melatonin secretion, further investigation is required.

The phenomenon of "transients" still is a good candidate for explaining the obtained results under a certain experimental conditions. The results of the study by Rogers et al. are consistent with results from other studies in terms of suggesting that transients produced by rapid onset/offset of electric and/or magnetic fields might be critical in melatonin reduction in baboons. It was reported that 'rapid' reversal of the Earth's magnetic field orientation in dark caused melatonin suppression, but 'slow' reversal did not (Lerchl et al. 1991). This report discussed reduction of the NAT activity and the melatonin level was induced by effects of rapid changes, which were associated with the eddy current generated *in vivo* upon transient electric current application by a rapid repetition of onsets/ offsets at the msec level, on the pineal gland.

6.9 Conclusion

Many experiments have been conducted with originally designed exposure systems and facilities. Thus, in this report, it was investigated how various factors, including polarization and orientation of magnetic fields, exposure duration, application methods of magnetic fields on animals, such as continuous or intermittent exposure, and excessive exposure, associate with results of exposure experiments.

A magnetic field is described by its frequency and strength. Exposure studies of low-frequency magnetic fields mainly utilized 50 and 60 Hz, and occasionally 10 and 17.6 Hz. Real-world field exposure includes various frequencies with the main frequencies of 50 and 60 Hz.

Many studies have been conducted with a constant frequency and magnetic field strength in a continuous exposure condition. Since some researchers believe that intermittent and transient field application is more effective for observation of biological effects, some studies have been performed in intermittent and transient magnetic fields. Various conditions have been utilized for exposure experiments on animals

and humans. Some studies were performed with exposure conditions which were established based on results of examination on physiological changes, heart-rate variation, and event-related potential.

Genotoxic effects of magnetic fields at around 0.1 mT have been investigated for more than 30 years. Studies with sister chromatid exchange (SCE), micronucleus formation, and DNA damage as indicators did not indicate any effects of magnetic field exposure; however, it was indicated that results could vary by exposure systems and experimental protocols.

A number of studies have been reported on the effects of magnetic field exposure. Most of the studies utilized various properties of magnetic fields and exposure profiles according to protocols, which were developed by researchers to match the specific purpose of a study. The variation in exposure conditions among exposure studies is considered to be involved in differences among results, and other conditions, such as cell lines and physiological conditions of cells, may also be involved. In order to identify the properties of magnetic fields, which are more biologically effective upon exposure, further investigation of currently available data and experiments based on the analysis is required.

6.10 References

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7

Induced Current as the Candidate Mechanism for Explanation of Biological Effects

Kenichi Yamazaki

7.1 Background

When exposed to a time-varying magnetic field, both electric fields and resulting currents are induced inside a human body. These phenomena are described by the well-known Maxwell equations. The amplitude of the induced current is proportional to the (1) amplitude of the outer magnetic field, (2) frequency of the outer magnetic field, and (3) electric conductivity of the medium. Generally, the amplitude of the induced current is higher at the circumference of the cross-section of the body that is perpendicular to the direction of the outer magnetic field.

The induced current has been considered to be a key index of the interaction mechanism between ELF magnetic fields and human bodies (Bernhardt 1988). The amplitude of the magnetically induced current is compared with the amplitude of the endogenous electric current inside the human body. In general, if the induced current is "small" relative to the endogenous current, the situation is regarded as "safe". Protection guidelines for exposure of the human body to magnetic fields are being established by various guideline-setting bodies, e.g., the International Commission on Non-ionizing Radiation Protection (ICNIRP 1998). Most of these guidelines have adopted an induced-current approach. Typically, an induced current density of 10 mA/m^2 is considered to be the threshold level not to be exceeded. This value is selected because nerve stimulation might occur at a greater current density.

Because the induced current density inside the human body is not directly measurable, a magnetic field to which a population can be exposed safely is estimated using a computational model of the human body. The human body is a complex structure composed of many tissues and organs, and the electrical conductivities of these biological structures differ greatly. As noted above, the electrical conductivity of a target is a major determinant of induced current. Thus, modeling efforts have focused on how an accurate assessment of the distribution of induced currents inside the body can be achieved. Because recent progress in computational methods has enabled development of more convenient, accurate and precise human models, many

numerical calculations have been conducted in recent years. This chapter provides an overview of efforts to estimate induced currents inside the human body.

7.2 Methods for Estimating the Induced Current Inside the Human Body

7.2.1 Direct measurement with a miniature probe

Direct measurement of the induced current is attempted using a miniature probe with either experimental animals or "phantoms", i.e., surrogates composed of materials with an electrical conductivity equivalent to that of the living organism. The approach is to measure the difference in electrical potential between two closely located positions. The current density can be obtained from the relation $J = \sigma V_m/d$, where J is the induced current density, σ is the conductivity of the medium, and V_m is the potential difference between the two electrode tips of a probe. To reduce artifacts due to interference by outer noise, the measurement is performed using a differential probe and a lock-in amplifier.

This method was firstly adopted by Kaune et al. (1985), and Miller and his colleagues (Miller 1991ab, Robertson-De Mers and Miller 1992, Miller 1994: 1996: 1997) developed the method considerably. These studies include induced current measurements conducted in (1) scaled human and rat phantom models made of agar-formed saline, (2) euthanized rats, and (3) anesthetized rats. Spatial resolution was 2 mm.

Another study on the measurement of induced current in experimental animals was conducted by Bourdages and Nguyen (1998) of Hydro-Quebec (IREQ). They developed an orthogonally arranged, three-axis probe that enabled 3-D measurement of induced current inside recently deceased rabbits.

In general, the induced current density is higher at the position close to the surface of the phantom or animals, and it decreases as the probe moving to the center of the objects.

7.2.2 Analytical formulae describing induced current in a spherical model

The amplitude of the magnetically induced current density in a homogeneous spherical object, or an object with circular cross section, can be easily described as $J = \pi f B r \sigma$, where f is frequency, B is magnetic flux density, r is distance between the center of the model to the measurement position, and σ is the conductivity of the object. This equation indicates that the current density increases in proportion to the (1) frequency, (2) magnetic field, (3) conductivity, and (4) distance from center of the model. The maximum current density exists in the periphery of the circular cross section. Because of its simplicity, this model sometimes has been adopted in field-limiting guidelines, such as that of the National Radiological Protection Board (NRPB) in the United Kingdom, as a conversion model between outer magnetic field and inner induced current density (NRPB 1993).

On the other hand, the standing human can be simulated better by an ellipsoid rather than a spherical model: an elliptical cross section is more realistic than a circular cross section. There is also a simple formula to describe the induced current density in elliptical cross section: $J = 2\pi fB\sigma(b^4x^2 + a^4y^2)^{1/2}(a^2 + b^2)^{-1}$, where a and b are the semi-major and semi-minor axes on the x- and y-axes, respectively. This formula also has been adopted by some guideline-setting bodies, such as the American Conference of Governmental Industrial Hygienists (ACGIH) and the Institute of Electric and Electronic Engineers (IEEE): see ACGIH (1995) and IEEE (2002).

7.2.3 Numerical calculation of induced current

Recent development in computing ability has enabled large-volume numerical computation of induced currents using an anatomically accurate human body with finer resolution. Several calculation methods for estimating the induced current inside the human body have been developed based on Maxwell equations.

7.2.3.1 Finite element method

The finite element method (FEM) is a standard method of numerical calculation used in many scientific fields. The advantage of this method for calculation of induced currents inside the human body is that the FEM is suitable for simulation of the complex shape of the human body. At Electricite de France (EdF), the TRIFOU code has been applied to a human model with simplified internal organs (Baraton and Hutzler 1995). The code is a combination of FEM with the boundary element method (BEM). The FEM is applied to the human body, and the BEM is applied to the boundary between human model and surrounding air.

7.2.3.2 Impedance method

An impedance method is a computational procedure used to solve a circuit equation for 3-D impedance meshes that represent the human body. Gandhi and his colleagues (Gandhi and Chen 1992; Gandhi et al. 2001) at the University of Utah and Stuchly and her colleagues at the University of Victoria used this method in the earlier stages of their studies (Xi and Stuchly 1994ab), applying it to an anatomical human model having a resolution of 3.6 millimeters.

7.2.3.3 Scalar-potential, finite-difference method

A scalar-potential, finite-difference (SPFD) method is a finite-difference method where a scalar potential is an unknown parameter. A human body is modeled by voxels, and node equations are solved. The outer magnetic field is expressed as a vector potential. With this approach, the amount of calculations required is relatively small. The method is used in the studies conducted by Stuchly and her colleagues (Dawson et al. 1997ab) and by Dimbylow (1998) at NRPB. In the NRPB study, calculation was conducted with minimum resolution of 2 mm.

7.2.3.4 Finite-difference, time-domain method

A finite-difference, time-domain (FDTD) method is used for the frequencies of the microwave region. In the ELF region, this method is disadvantageous because of the large number of repetitions required in the calculation. Therefore, Furse and Gandhi (1998) introduced a frequency-scaling technique. Here calculation of induced current is performed at 10 MHz, and the results then are converted into that of power frequency, using the linear relationship between induced current and frequency. Also, this approach can be applied to scenarios where both magnetic and electric fields exist simultaneously. The same method was also applied by Gustrau et al. (1999). They used 5 MHz as the scaling frequency.

7.2.3.5 Boundary element method

A BEM (boundary element method) is used by Bottauscio and Conti (1997) at IEN (Istituto Elettrotecnico Nazionale, Italy) and CESI (Centro Elettrotecnico Sperimentale Italiano) for a simplified human model. The advantages of a BEM are less input data and good accuracy, provided the internal medium is simple.

7.2.3.6 Calculation method for an electrostatic problem

Accurate calculation methods that were developed originally for electrostatic problems can be applied to the problem of ELF magnetic induction in the human body, because the fundamental equation (Laplace's equation) to be solved is the same for both problems, provided that the quasi-static approximation is valid. That is the condition for which displacement current can be neglected, i.e. $\sigma \gg \omega\varepsilon$ where ω is angular frequency and ϵ is permittivity of human model. The charge-simulation method (CSM) and surface-charge method (SCM) are used by Yamazaki et al. (2001) of the Central Research Institute of Electric Power Industry (CRIEPI) in Japan. These methods also are boundary-dividing methods, and they have the advantages over volume-dividing methods like FEM or Impedance Method in the amount of input data.

In the CRIEPI study, a simple human model constructed with axis-symmetric objects representing several major organs was used (Fig. 7.1). The effect of the organ conductivity values assigned to each organ was investigated (Fig. 7.2), by comparing the amplitudes of the induced currents at respective organs. As expected, large differences occur in the values of the induced current for each organ, depending on the assumed conductivity of each organ. Example of induced field in the horizontal crosssection of the heart is shown in Fig. 7.3.

7.3 Human Models, Field Uniformity, and Frequency Domain

In this section, unsolved problems relating to estimation of induced current inside the human body are discussed briefly. The three main issues are (1) human model used for induced current calculation, (2) exposure condition, and (3) frequency concerns.

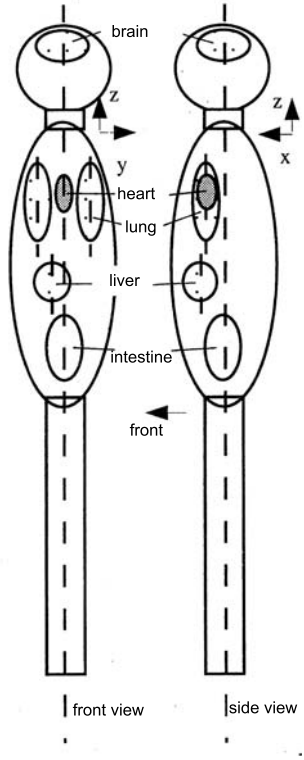

Fig. 7.1. Human model used by CRIEPI. This is a simple human model constructed with axis-symmetric objects representing five major organs (brain, heart, lung, liver, and intestine).

7.3.1 Human models

Human models used for numerical computation of induced current distribution inside human bodies are classified into two categories. The first is an anatomically accurate human model based on an image obtained by magnetic resonance imaging (MRI) and a medical atlas of an anatomy. An output (http://www.nlm.nih.gov/research/visible/visible_human.html) of the US Visible Human Project is sometimes used; it can be segmented into a resolution of 2 – 5 millimeters for computation (see Fig. 5.9). In addition, Japanese male and female realistic models with 2 mm resolution have been developed (see Fig. 9.2, Nagaoka et al. 2004). The second type of human model is a simplified one composed of a relatively simple shape of an outlook and internal organ (Yamazaki et al. 2001).

The conductivity value allocated to each tissue or organ is essential for accurate induced current calculation, because the induced current density is proportional to the conductivity of the tissue concerned. However, the published conductivity values for each tissue or organ differ considerably, depending on the biological citation

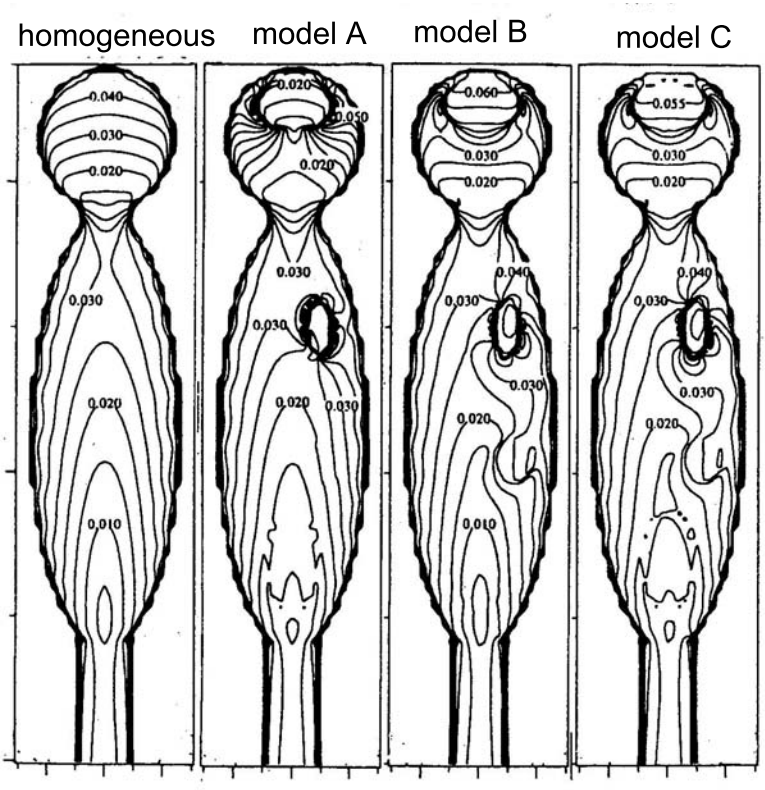

Fig. 7.2. Example of induced field distribution on the cross sections of four human models perpendicular to side-to-side uniform magnetic field. These models differ in assigned electric conductivities (homogeneous and inhomogeneous models A,B,C).

selected. Moreover, in some reports, the anisotropic character of conductivity is considered for muscle.

A comprehensive investigation of tissue conductivity measurements of biological tissues was conducted by Gabriel and co-workers (Gabriel 1996, Gabriel et al. 1996abc). The output of this important work has become the standard reference for tissue conductivity values used in modeling efforts. The use of a standard source of tissue conductivity values reduces variability, based on choice of conductivity values, among different models. It remains to be determined whether improved measurements providing more accuracy and increased spatial specificity can be obtained.

7.3.2 Field uniformity

In the previously mentioned studies, magnetic fields were assumed to be uniform, allowing for easier computation and for easy comparison of the results. In addition, in protection guidelines such as ICNIRP's, the reference levels of magnetic exposure

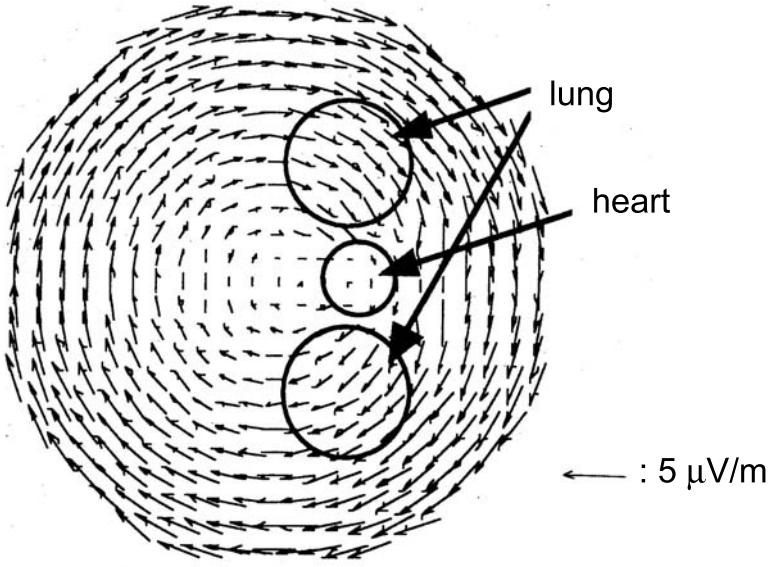

Fig. 7.3. Example of induced electric field in the horizontal cross section at the center of the heart when exposed to 1 μT, 50 Hz vertical magnetic field. The length of the arrow in the legend indicates electric field of 5 μV/m. The induced currents can be calculated by multiplying conductivity at every position with the electric field.

are derived by assuming the magnetic field is uniform, because the coupling between outer magnetic field and inner induced current is maximum under this condition.

On the contrary, the real-world exposures to intense magnetic fields mainly occur in the position very close to a field source, such as (1) near a power line conductor, in the case of a worker near a "live" line, or (2) near an electrical appliance. In these situations, in general, the magnetic field is highly non-uniform. With a non-uniform field, the coupling between the field and the human body is relatively weak, compared to that occurring with a uniform field.

There are several reports, in the ELF range, that take into account non-uniformity when performing their calculations. Some reports dealt with power lines (Baraton and Hutzler 1995; Stuchly et al. 1996; Dawson et al. 1999abc), and others describe electrical appliances, such as a hair dryer (Baraton and Hutzler 1995; Cheng et al. 1995; Kaune et al. 1997; Tofani et al. 1995ab). Considerable work remains to be done in the description of real-world exposure situations and in numerical modeling of the induced currents and fields occurring in models of the human body under these situations.

7.3.3 Expansion of frequency range studied

Resent development of appliances using magnetic field with a frequency higher than that of the 50 or 60 Hz power system has raised a new interest in health effects. Induction heating (IH) cookers are one of the newer appliances that utilize a higher frequency, typically 20 kHz to 100 kHz, for heating of ferromagnetic pans. Another concern is electric article surveillance (EAS) systems installed at the entry of buildings, such as grocery stores and libraries. These devices also use these ranges of frequency. These frequency ranges are sometimes called "intermediate frequency (IF)" mainly in the European organizations (COST 1998; Matthes et al. 1999).

Because higher frequency fields induce higher induced currents inside the human body, with the increase proportional to the frequency, existing protection guidelines use more strict regulation of field levels for higher frequency ranges. For example, in the ICNIRP (1998) guideline, the reference level of magnetic exposure for the public is 0.1 mT at 50 Hz. However, the permissible exposure is reduced greatly, to 0.00625 mT, at frequencies of a few tens of kHz. So far, only a few reports have been published that focus on currents induced in the human body by magnetic fields in this frequency range (Kaune et al. 1997; Gustrau et al. 1999; Gandhi and Kang 2001; Yamazaki et al. 2004).

7.4 Challenges to Interpretation of Biological Outcomes

One of the aims of estimation of the induced current occurring inside biological object is to contribute to interpretation of the outcomes of biological experiments with animals or cells. Stuchly and her co-workers have analyzed induced currents in cell culture dishes considering cell membranes and gap junctions (Stuchly and Xi 1994; Fear and Stuchly 1998ab). These efforts contribute to clarifying the microscopic dosimetry of induced current in the experimental conditions used in cell exposure. Biological investigators are coming to understand that the exposure conditions applied to their cells are not uniform: cells at the center of a culture dish can be at relatively low field exposure conditions while cells at the periphery of a dish, or near the liquid interface with dish or with air, can be at relatively high field exposure conditions.

Another concern is that the biological effect caused by magnetic field can be dependent on polarization of the field (Kato et al. 1993). In this experiment, a circularly polarized field caused the biological effect, suppression of melatonin, while linearly polarized fields, either horizontal or vertical, did not (with the field intensities examined). Furthermore, elliptically polarized fields of various degrees of circularity produced intermediate effects. The magnetic field near an ordinary overhead transmission line is elliptically or circularly polarized. Thus, consideration of polarity might clarify the literature on biological effects of power-frequency magnetic fields.

There are some reports dealing with the induced currents produced by circularly polarized currents (Misakian 1991, 1997; Yamazaki et al. 1996; Wake et al. 2000). It should be noted that circular polarization of outer magnetic field does not necessarily mean circular polarization of induced current inside a biological object: the polarity of the induced current can depend on the location within the organism.

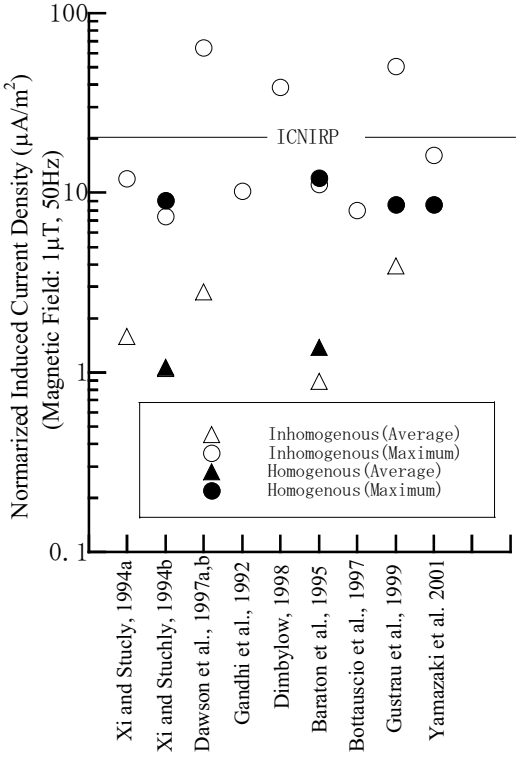

Fig. 7.4. Comparison of estimated current density inside the human body when exposed to uniform magnetic field of 1 μT, 50 Hz. Among the nine studies examined, values varied by almost two decades, and some exceed the ICNIRP standard.

7.5 Inter-laboratory Comparison Studies

In general, the result of numerical calculation must be verified by comparing it with analytical or experimental values. The difficulty in the present problem is the inability to verify results with *in situ* measurements. (Very little data will be available from electrodes placed in human bodies under controlled exposure conditions.) Because of this limitation, many guideline-setting bodies have not adopted the recent, highly advanced numerical calculation as their rationale for deriving limiting magnetic field values.

Some efforts have been conducted to compare the results of numerical calculation among different research group (Stuchly and Dawson 2000; Stuchly and Gandhi 2000; Caputa et al. 2002). The comparisons showed good agreement, provided that similar anatomical models and conductivity values were used. However, another effort used data from nine reports and showed large variation in induced current results among the several studies. To allow comparison, the induced current values from each study were converted to a standard intensity of the outer uniform magnetic field (Fig. 7.4).

7.6 Summary

For a variety of reasons, induced current has long been considered to be the most important measure of dose when dealing with biological effects of applied or induced currents and fields. Electrostimulation has a long history in biology, dating back to the work of Galvani and Volta in the early 19th century. Furthermore, the basic mathematical tools for describing fields and currents were developed in the 18th century, especially by Faraday, Laplace, and Maxwell. Two centuries of biomedical research have supported the view that current density is a prime independent variable in bioelectromagnetics.

Given the importance of current as a basic mechanism, it has been the key component of efforts to set safety standards to protect the public against any possible adverse health effects from environmental exposures to electric and magnetic fields. Thus, a variety of computational models have been developed in recent years to compute the induced current produced in the body by an external electric or magnetic field. Furthermore, the development of anatomically correct images of the human body, coupled with assignment of accurate conductivity values to all tissues and organs, has moved the discipline from simplistic, general, back-of-the envelope computations to what are assumed to be highly accurate and precise computational models. The tools now available handle anatomic detail down to voxels of about 2 mm per side.

It now is relatively straight-forward to implement commercially available or investigator-developed codes on personal computers and minicomputers. Available approaches now include (1) finite-element methods, (2) impedance methods, (3) scalar-potential, finite-difference methods, (4) finite-difference, time-domain methods, (5) boundary element methods, and (6) electrostatic-based computations. Using a basic standard of 10 mA/m^2 as a safety threshold for the current induced in the human body by an external field, one can compute the induced current in all of the various tissues or organs in the body and therefore determine if a given external field is likely to be safe or not.

Despite dramatic progress in the last few decades, important challenges remain. Validation is a key issue with any computational model. Direct measurement of induced current is difficult, and acquisition of additional good empirical data under a variety of exposure conditions would be very helpful. Additionally, a 2 mm voxel is biologically large (or even huge) for some targets; thus, finer resolution might be very useful. Comparison of the results from various models is just beginning: sometimes agreement is good, which is encouraging. However, sometimes agreement is not so good, which is discouraging. It is important to understand how models agree and disagree, and it also is important to understand the causes for similarities and differences in results from various models.

Standards would be improved by inclusion of such real-world factors as field inhomogeneity and polarity. Also, investigations need to be conducted over a wider variety of frequencies. Because of the commercial importance of ELF power and microwaves, these two regions have been studied more extensively than the intermediate frequencies between these extremes. As new technologies operating at different

frequencies are developed and applied, the need for safety information continues to develop.

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8

Electromagnetic Fields, Biophysical Processes, and Proposed Biophysical Mechanisms

Tsukasa Shigemitsu

As described in previous section 1.3.3, there are many sources of ELF electromagnetic fields. Humans are exposed daily to these naturally and artificially originated fields. During the past three decades, questions have been raised about whether the exposure to human-made ELF electromagnetic fields might be linked to adverse health effects. Since the paper of Wertheimer and Leeper (1979), there have been many reports on the question of an association between seemingly small increased exposure to ELF magnetic fields in the environment and an approximately two-fold increase in the incidence of the rare disease state of leukemia in children.

Although ELF magnetic field interactions with biological systems have been proposed, no biophysical mechanism that explains biological effects produced by low-level magnetic fields, those less than 0.1 mT, has been established. There are several hypotheses about interactions of ELF magnetic fields with living organisms. Proposed mechanism include (1) induced current, (2) direct effect, (3) force on biomagnetic materials, (4) effects on free radicals, (5) ion cyclotron resonance, (6) charge transfer processes, and (7) stochastic resonance, *etc*.

8.1 Electromagnetic Fields and Electromagnetic Waves

It is well known that high-frequency electromagnetic waves, such ultraviolet and microwave can damage biological tissue by damaging molecular structure. At these frequencies, electromagnetic waves behave like photons, i.e., they are like particles at discrete energy units, with the energy based on frequency. Power frequency (50/60 Hz) fields are, in principle, electromagnetic waves (see Fig. 2.1). However, when power frequency fields are considered as particles, energy levels are extremely small (Table 8.1).

When electromagnetic fields are considered as particles, i.e., as photons, energy $E(J)$ is defined as follows:

$$E(J) = hf = hc/\lambda$$

Here, h is Planck's constant ($= 6.626 \times 10^{-34}$ JS), $\lambda(m)$ is the wavelength of the electromagnetic wave, $f(Hz)$ is the frequency, c is the speed of light ($= 3 \times 10^8\ m/s$). En-

Table 8.1. Comparison of Particle Energies at Different Frequencies

	Wave length	Energy (eV)
Soft X ray	$\lambda = 1.2$ nm	1000
Visible light (green)	$\lambda = 0.5\,\mu$m	2.5
Far-infrared	$\lambda = 30\,\mu$m	0.04
Transmission (50 Hz)	$\lambda = 6,000$ km	10^{-13}

Table 8.2. Examples of Energies of Chemical Bonds

Bond type	kcal/mol	Energy (eV)
O-O	32.8	1.43
O=O	118	5.16
C-C	83	3.60
C=C	144.5	6.29
C-O	83.5	3.64
N-H	92.6	4.03
ATP hydrolysis	7.3	0.3
Hydrogen bond	2.4	0.1
$3/2kT$	0.9	0.04

ergy is inversely proportional to wavelength, i.e., long wavelengths (e.g., those at 60 Hz) have little energy but short wavelengths (e.g., those at 1 GHz) have considerable energy. One electron volt (1 eV) is 1.60×10^{-19} J. An electromagnetic wave exceeding 12.4 eV, or wavelength of less than 100 nm (ultraviolet region) is called ionizing radiation. This energy can cause ionization, i.e., dividing neutral molecules into electrons and positive ions and has the capability to break chemical bonds. Gamma (γ) rays, X rays, and parts of the ultraviolet region fall into this category; their energy is sufficient to break chemical bonds. Energy of 3.6 eV is required to break a covalent bond, and 0.1 is required to sever a hydrogen bond (Table 8.2).

Power frequency electromagnetic waves have wavelengths of 6,000 km at 50 Hz and 5,000 km at 60 Hz. Thus, with such very long wavelengths, they do not have the energy to break chemical bonds by causing ionization (Tables 8.1 and 8.2). Given the inability to break chemical bonds, they do not appear to be an issue. ELF electromagnetic fields, including power frequency fields, do not have the power to cause heating or ionizing.

As mentioned above, the physical properties of power-frequency fields have no fundamental interaction with tissues; the energy of such fields is so small that is much less than the energy required to break chemical bonds. This means that exposure to such fields will not result in disruption of the chemical bonds of key biological molecules, such as DNA, proteins, or small molecules. Furthermore, as expected from this mechanistic viewpoint, experimentally it has been demonstrated that power-frequency fields have no direct effect on such key biological processes as cell death or gene mutation.

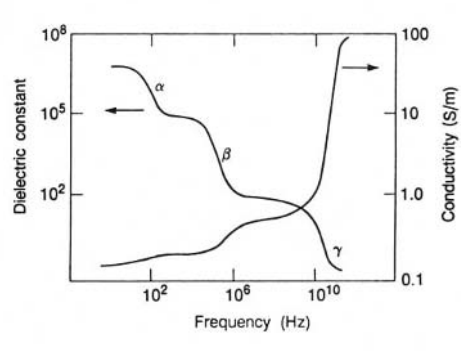

Fig. 8.1. Frequency dependence of the relative dielectric constant (permittivity) and electrical conductivity of biological tissue.

Table 8.3. Three Types of Dielectric Dispersion in Biological Systems

Type	Frequency	Mechanism
α	100 Hz (m Hz–1 kHz)	Polarization of counterions near the membrane surfaces, polarization of the membrane and intracellular plasma structure, ionic diffusion
β	1 MHz (0.1–10 MHz)	Maxwell-Wagner effects, charging of cell membrane though the intracellular and extracellular media.
γ	20 GHz (0.1–100 GHz)	Polarization of tissue water, cellular protein polarization and bound water.

8.2 Electrical Characteristics of Organisms

8.2.1 Fundamental units and properties

The electrical characteristics of organisms are given by (1) the dielectric constant ($\varepsilon = \varepsilon_\gamma \varepsilon_0$: F/m), (2) the electrical conductivity (σ: S/m), and (3) the magnetic permeability ($\mu = \mu_\gamma \mu_0$: H/m). In free space, the dielectric constant is ε_0 and the magnetic permeability is μ_0, where $\varepsilon_0 = 8.8542 \times 10^{-12}$ (F/m) and $\mu_0 = 4\pi \times 10^{-7}$ (H/m). The entities ε_γ and μ_γ are the relative dielectric constant and relative magnetic permeability of a particular medium. The magnetic permeability of organisms is almost the same as the value in free space and does not change with the frequency. In contrast, the dielectric constant and the electrical conductivity of organisms change with frequency, which is referred to as the frequency dispersion or the frequency dependence (Fig. 8.1).

For both relative dielectric constant and electrical conductivity, there are three break points; they occur at (1) several tens of Hz, (2) several MHz, and (3) about 20 GHz. At each of these frequencies, a dispersion phenomenon of a rapid decline of relative dielectric constant and rapid increase in electrical conductivity occur (Fig. 8.1). These are referred to, respectively, as the α, β and γ dispersions (Table 8.3).

The high dielectric constant observed at low frequencies is a result of the complex and non-uniform structure of biological organisms. The origin of the α dispersion is a relaxation phenomenon of cell membranes; it is related to the permeability of membranes and to the diffusion process of ions in these complex structures. Although the origin of the β dispersion is less well understood, it is due to inability of the polarization of cellular structural components, including cell membrane, which act as barriers of ion flow. The β dispersion also comes from the polarization of organic polymers and proteins. The γ dispersion is cause by the polarization of water molecules, both free and bound, which are common in biological systems.

8.2.2 Cells and tissues

Organisms are composed of a number of different tissues, and a fair amount of data on the detailed electrical characteristics of these tissues has been collected (Gabriel et al. 1996). For example, the electrical characteristics of muscle tissue vary substantially with frequency. However, at 50 or 60 Hz, ε is on the order of 10^6 and σ is 0.1 S/m. The ratio of relative dielectric constant and electrical conductivity at 50 or 60 Hz is $\omega\varepsilon_\gamma\varepsilon_0/\sigma \fallingdotseq 3 \times 10^{-2}$, with conductivity being dominant. Thus, in the 10 Hz − 100 kHz range and with biological organisms, it is possible to consider only electrical conductivity (σ).

At the organ, tissue or cellular levels, organisms have a highly non-uniform structure. Cellular membranes are highly important, for both cellular physiology and electrical interactions. Thus, when electrical effects are considered, the membrane structure of organisms is an important issue. In general, for current densities of less than 1 mA/cm^2 the biological organisms can be considered to be linear in electrical character. However, greater current densities, they are considered to be non-linear, because both excitation and electrochemical phenomena occur.

Efforts have been made to model cells from an electrical perspective (Fig. 8.2). Cell membranes are near-perfect insulators with a thickness of about 5–10 nm. Inside and outside the cell there are electrolytic solutions consisting of Ca^+, K^+, Na^+, Cl^-, and other ions. From an electrical standpoint, the cell is a combination of series and resistance components; to the electrical engineer, this is a series-parallel, resistive-capacitive (R-C) combination. Therefore, in principle, the organism's structure can be modeled as a complex structure composed of many of these R-C circuits. Capacity (C) is 1–10 $\mu F/cm^2$, and resistance (R) is about 0.5–10 $k\Omega/cm^2$. Also, in the low frequency range, the impedance of membranes is very high, meaning electrical current flows in the "gaps" between cells. However, in high frequency ranges, membranes are electrically short-circuited, meaning the electrical properties of the tissue become uniform inside and outside of cells. Therefore, the electrical distinction between intra-cellular and extra-cellular is lost.

Cells have electrical potential differences that are mediated by cell membranes. The electrical potential inside cells is negative relative to the outside of cells, and the potential difference ranges from several mV to c. −100 mV, depending on the cell type and the biological circumstances at the time of measurement. In electrically excitable tissues, this potential is called the "resting" membrane potential. The

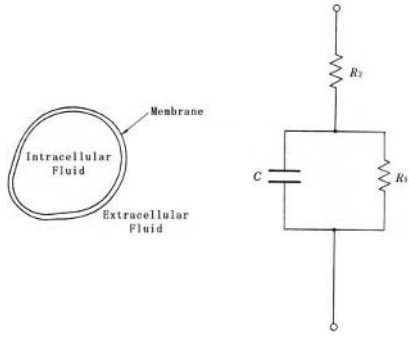

Fig. 8.2. A Conventional Electrical Circuit Model of a Single Cell (C ≒ 1–10 $\mu F/cm^2$, R_1 = 0.5–10 $k\Omega/cm^2$, and $R_2 \doteq 1\Omega m$).

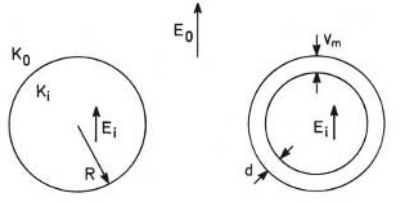

Fig. 8.3. A Solid Sphere and a Membrane-surrounded sphere, Each exposed to an alternating Electric Field (E_0) (based on Schwan (1985)).

electrical potential of nerve, muscle and other excitable cells drops greatly when stimulated. If the net stimulation exceeds a threshold value, which might be −60 to −70 mV, the ion permeability of the membrane suddenly increases greatly. (See section 1.2.1.) An impulse is generated when this change occurs, and the impulse is transmitted along membranes to distant points. This is called an action potential, and it is maintained for about 1 *ms*.

Schwan (1985) considered the types of interactions, including those at the cellular level, that could occur between organisms and external electric fields (Fig. 8.3).

If a spherical object, with isotropic internal conditions is assumed, is within an external electric field (E_0), the internal electric field (E_i) can be described in relation to the external field by the following equation (Schwan 1985):

$$E_i = \frac{3E_0K_0}{K_i + 2K_0}$$

Here, K_i, K_0 are complex specific admittances. It is given that $K = \sigma + j\omega\varepsilon_\gamma\varepsilon_0$. As noted previously, σ is the electrical conductivity, and ε_γ is the relative dielectric constant. Here, if the surrounding medium is air and the sphere has tissue like electrical properties, $K_0 = j\omega\varepsilon_0$; $|K_i| > |K_0|$, the internally induced electric field and

induced electric current can be obtained from the following equation:

$$E_i = \rho f E_0/6 \times 10^{10}, \quad J_i = f E_0/6 \times 10^{10}$$

J_i is the internal induced current density (A/cm^2), f is the frequency (Hz), $\rho = 1/\delta$ and is specific resistance (Ωcm). At 10 Hz, where the muscle tissue value of ρ is about 600 Ωcm, $E_i = 10^{-7} \times E_0$.

The induced electric field strength is proportional to the external applied electric field. In the low-frequency region, the internal induced electric field becomes very small compared to the external electric field. Also, the induced electric field strength is proportional to the frequency. Here if we assume that the size of the sphere is relatively small compared to the wavelength of the external applied electric field, electrically this can be handled using a quasi-static formulation. The previous equation has been extended to other shapes, such as columns and prolate spheroids.

The fundamental properties of biological organisms are the dielectric constant and the electrical conductivity; which are influenced by frequency has been described. Using simple models, equations based on these properties have been developed to calculate fields and currents induced in organisms by exposure to electromagnetic fields.

8.3 Electromagnetic Fields and Organisms

8.3.1 Fundamental

The effect of relatively high-level electromagnetic fields on organisms is based mainly on the induction of electric charges, i.e., electric currents, within the exposed organism. Macroscopically, the effects can be divided into three categories: (1) non-thermal effects, (2) thermal effects, and (3) other effects.

Non-thermal effects, which usually are called electrical stimulation effects, involve the excitation of nervous and muscular tissues by the electric current induced inside the exposed organism. The electrical stimulation effects vary with the amount of current flow and duration of the stimulus (Reilly, 1998). The effects of current stimulation also depend on frequency, with effects of low frequencies being relatively large; at higher frequencies, effects are comparatively smaller. Generally, at frequencies greater than about several tens of kHz, the stimulation effects are inversely proportional to the frequency (Fig. 8.4).

Thermal effects are caused by conversion of the electromagnetic fields absorbed in organisms into heat. Also, depending on whether the heat generation is caused by electric fields or magnetic fields, heating is classified into dielectric heating or induction heating. Dielectric heating occurs at relatively low frequencies (several kHz to MHz), and induction heating occurs even at higher frequency from RF to microwaves. The dielectric heating is the heating of an insulting material due to its own dielectric losses when the material is placed in a time-varying electric field. The induction heating is the heating of a conductive material by causing eddy currents inside the material when the material is placed in a time-varying magnetic field.

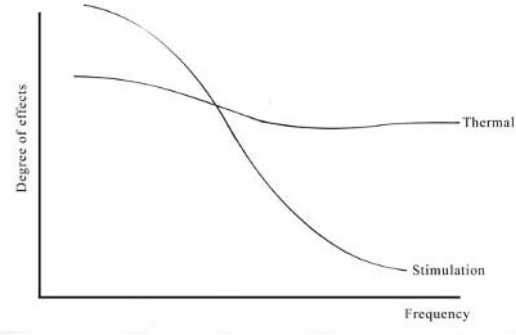

Fig. 8.4. The Relationships between Frequency and both Thermal, and Non-thermal Effects. Frequency makes a Modest Difference for Thermal Effects, but the Influence of Frequency is Considerable for Stimulation Effects.

Other effects, including formation of so-called 'pearl chains' have been addressed. In a homogeneous external field with a local gradient, two dielectric particles will be attracted to each other. They will be oriented in the direction of the electric field. In a homogeneous field, cells will tend to form pearl chains in the direction of the electric field.

In summary, electrical stimulation and non-thermal effects are large at low frequencies and decline with increasing frequency (Fig. 8.4).

8.3.2 Non-thermal effect

For this discussion, a non-thermal effect is the stimulation received by nerves and muscle when an organism touches a charged object; electric current flows, and an electric shock is received. Depending upon the amount of the current, effects can be divided into sensation, involuntary movement, and ventricular fibrillation. This range of effects ranges from trivial to lethal. For adult males, the threshold for perceiving stimulation is about 1 mA; the value for females is about two thirds of that amount. Profound disruption of cardiac function occurs at levels about 1,000-fold greater (Table 8.4). Table 8.4 shows the summary of the range of induced current density that produces possible effects (WHO 1987). It should be noted that current-induced injuries can involve considerable tissue heating, and severe burns can be a consequence of electrical current accidents. Electric shock hazards, mechanisms of injury and safety codes intended to prevent or minimize current-based injuries are understood (Reilly 1998).

Magnetic field stimulation effects at low frequency include the magnetophosphene. This involves the perception of light flashes caused by exposure of the head and/or eyes to an ELF magnetic field. The threshold for perception is about 10 mT at 20 Hz (Lövsund et al. 1980). This stimulation effect is thought to be caused by the induced electric current density in the retina: the electric current density required for occurrence of the phenomenon is about 10 mA/m^2.

Table 8.4. Summary of Induced Current Density Ranges between 3 and 300 Hz for Producing Biological Effects (WHO 1987).

Current density (mA/m^2)	Effects
< 1	Absence of established effects
1–10	Minor biological effects reported
10-100	Well established effects are reported; magnetophosphene possible nervous system effects, and facilitation of bone fracture reunion occur.
100-1000	Changes in central nervous system excitability are established; stimulation thresholds and possible health hazards are recognized.
> 1000	Extrasystoles and ventricular fibrillation are possible; definite health hazards exist.

8.3.3 Thermal effect

Thermal effect occur when the energy of electromagnetic fields absorbed in an organisms are convertes into heat. If the absorbed heat energy is greater than the heat released by the organism into the environment, the temperature of the interior of the organism will rise. (See section 10.8.1.) When the body temperature of the exposed organism rises from its normal value, important biological effects can occur.

When the whole-body-averaged specific absorption rate (SAR) exceed 4 W/kg (within 1 hour), the rise of core body temperature will result, possible producing serious effects. Here, the 4 W/kg is a "limit value" from the changes of the activity of animals and is related to the basal metabolism of organisms. The averaged-basic metabolic rate for humans is about 1 W/kg. Heating levels less than 4 W/kg are regarded as a minor burden that can compensated for readily by normal physiological changes, such as sweating and increased blood flow. Taking into account a 10-fold safety factor when setting the limit value for humans, ICNIRP's basic restriction for occupational exposure indicates a permissible whole-body-averaged SAR of 0.4 W/kg for 6 min for frequencies of 100 kHz to 10 GHz (ICNIRP 1998).

When thermal effects are localized and concentrated because exposure is not uniform over the entire body, e.g., in the side of the head when using a cellular phone, it is necessary to consider the localized SAR. Even if energy is being delivered locally, important thermal changes in the body can be avoided: the thermal regulatory system in the organism can be effective; the body temperature is regulated, and the heat introduced by exposure is released. For example, a cellular phone producing an exposure of a few μW to the scalp and musculature delivers a very small amount of energy, but the normally present blood flow is sufficient to prevent any local macroscopic heating, and overall body temperature also is not affected.

At the power frequency of 50 or 60 Hz, the energy is about 10^{-13} eV. In order to deliver energy equivalent to the human basal metabolic rate of 1 W/kg, an electric field of 10^7–10^8 V/m, or a magnetic field between 10^4–10^5 T, would be required. In general, typical electric fields experienced by humans are a few tens of V/m; a

Table 8.5. Established Biophysical Mechanisms of Magnetic Fields, with Thresholds for Biological Effects and Thresholds for Health Effects (Bernhardt 1997).

Mechanism	Effect	Lowest threshold for effects	Thresholds for health effects
Magnetic induction a) static fields: forcing on moving charges	Electric streaming potential; induced fields and currents in moving tissues (i.e., heart)	(Earth's magnetic field: Orientation with special organs) −10mT	 −5T
b) Time varying field: Faraday currents	Magneto-phosphenes; Cellular and membrane response Stimulation of excitable cells, VEP, cardiac muscle	50–100 mV/m or 10 mA/m^2 0.1 V/m > 5 V/m	 > 5 V/m $\left(\frac{dB}{dt} > 20\ T/s\right)$
Magneto-mechanical interactions	Orientation and translation effects, dia and paramagnetic molecules and cells (e.e., retinal rods, sickle RBC)	> 100 mT	Unknown for B < 2 T
	Torques on magnetic particles Forces on ferromagnetic objects	(5 μT) (Earth's magnetic field: orientation of special organism) > 5 mT	 > 50 > 100 mT*
Electronic interactions	Nuclear spin effect	Earth's magnetic field	Unknown
	Influence on charge transfer processes (bacterial photosynthesis; reaction of enzymes)	> 10 mT	Unknown for B < 2 T

(∗) Persons at risk: ferromagnetic implants

"strong" electric field is about 10^5 V/m. In general, typical magnetic fields experienced by humans are several μT, and a "strong" magnetic field is 10 or 20 μT.

In the atmosphere, under real world conditions, dielectric breakdown occurs when electric fields exceed about 10^6 V/m. Also, if the electrical potential difference across a typical cell membrane (10 nm thickness) is about 100 mV, the electric field across the cell membrane is about 10^7 V/m. The energy level of ELF electromagnetic fields is very low, especially when compared to the much higher levels present as a part of normal physiology.

In summary, at lower frequencies, exposure to electromagnetic fields can produce electrical stimulation through processes that are fundamentally non-thermal in their initiation. At higher frequencies, the primary mode of interaction of electromagnetic field with biological organism is mediated by heating of tissue.

In organisms, the stimulation effect of electric current on nerves and muscles and the magnetic field effect of magnetophosphenes are well known and well-explained phenomena. Bernhardt (1997) reviews the modest number of magnetic field bioeffects for which mechanisms have been established and minimum threshold values have been reported (Table 8.5).

8.4 Proposed Biophysical Mechanisms

The problem of biological and health effects of ELF electromagnetic fields originated from the results of epidemiological studies. The EMF RAPID Program's Working Group Report (NIEHS 1999) and the IARC carcinogenicity assessment (IARC 2002) concluded that magnetic fields are "possibly carcinogenic to humans" (Group 2B).

The basis for this conclusion was the about 2-fold, statistically significant association between estimated residential magnetic field exposure and doubled incidence of childhood leukemia; the evidence from humans, despite the lack of positive data from animals, was judged to be "sufficient" (Ahlbom et al. 2001).

However, despite an extensive amount of *in vivo* and *in vitro* research and numerous epidemiologic studies, an explanation for the slightly increased (c. 2-fold) risk of childhood leukemia has not emerged. It still is uncertain whether the association is related to ELF magnetic field exposure only and/or to some other factor. In order to explain this situation, the fundamental mechanism by which very weak magnetic fields could cause a biological effect must be clarified. However, neither the biophysical mechanism(s) nor the resultant series of biological changes leading to the adverse health effect are almost known.

8.4.1 A framework for understanding bioeffects

No matter what the initial interaction mechanism by which electromagnetic fields affect tissue, the process from interaction to injury must occur through well established biological mechanisms of integration, regulation, injury, and repair. For at lest two decades, biologists have speculated about how electromagnetic fields might interact with biological processes, as they currently were understood.

8.4.1.1 Background

From the initial reaction when an organism receives some external stimulus from electromagnetic field exposure to the final outcome of disease, a number of stages of biological response must be completed (Fig. 8.5). When organisms are exposed to an ELF magnetic field, a chain of biological reactions must occur, and the principles that underlie these reactions can be described in general (Valberg et al. 1997). Normally, changes in an organism occur within a restricted "normal" range (homeostasis), and long-term effects on the overall function of the organism do not occur. However, if a complete sequence of responses is completed, an adverse health effect can be reached. The many alternative outcomes are also shown in Figure. 8.5.

When an organism is exposed to external electromagnetic field, mechanical forces are applied on the body surface and induction of electric currents occurs within the organism. Potentially, this allows for explanation of a number of phenomena, if the field strength is great enough to produce resultant stimuli of sufficient magnitude. However, the events that have been observed during exposure to low-level electromagnetic fields cannot explain these phenomena. The energies involved are much too small, by at least several orders of magnitude. Thus, novel mechanisms based on a variety of biological and physical principles have been proposed.

Although possible effects on cells are being addressed, because of the dielectric properties of cells there is very little induced current flow inside cells. Therefore, it is thought that effects on organism must be caused by the electric currents, which are induced by external electromagnetic fields, flowing around cells. Presumably such factors as change in conformation of the membrane, alteration in distribution

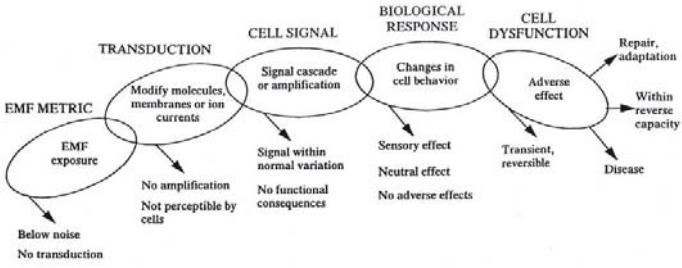

Fig. 8.5. Links of Causal Steps Connecting Electromagnetic Field Exposure to a Disease (Valberg et al. 1997).

of electrical charges on the membrane, altered chemical status within cells as a consequence of changes in membrane function, etc. bring about changes in the internal function of cells.

8.4.1.2 Some early examples of potential mechanism

Tenforde and Kaune (1987) provide an example of a proposed mechanism of action based on the coupling of ELF electromagnetic fields into cells (Fig. 8.6). This proposed hypothesis is based on the transductive coupling between induced electric current in the extracellular medium and the intracellular events occurring in living cells. The key events are the transductive signaling events within the cell membrane. In a similar manner, Adey (1990) has hypothesized an interaction between electromagnetic field energy and the receptor proteins on the surface of cell membranes involved with signal transmission to cell interior. Mechanisms also have been proposed involving an interaction between electromagnetic field energy and movement of ions through cell membranes.

The outer surface of cells is composed of a double layer of phospholipids and cholesterol that from a thin membrane (Fig. 8.7). In this lipid structure there are "floating" giant proteins that span the membrane from exterior to interior. These intra-membrane proteins perform important roles in the functioning of cells. Adey (1990) proposed a three-staged model for the interaction of low electromagnetic fields and the complex structure of cells involving the signal transmission from the cell surface receptors to the cell interior. The proteins floating in the two layer lipid structure of cell membranes have a huge negatively charged sheet oriented toward the exterior of the cell. These are linked to hydrogen and calcium ions, which serve as counter ions.

The mosaic structure of the membrane changes when a chemical interaction occurs; after realignment occurs, vertically oriented coating forms on the cell membrane. Small spaces between cells form channels for flow of electric current. In other words, instead of the high resistance pathway of cell membrane, electric current flows across cell membrane surfaces. This is the first linkage between electromag-

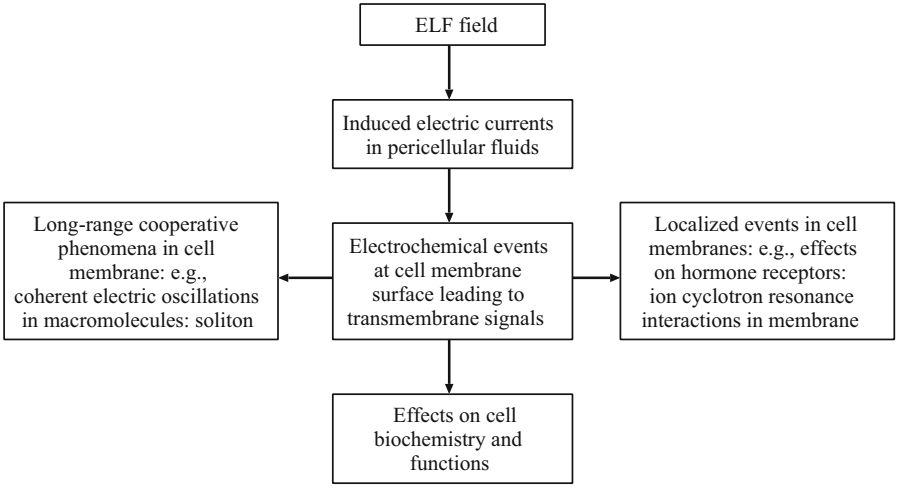

Fig. 8.6. Various hypothesized Mechanism through which ELF fields are coupled with Living Cells. The Induced Electric Currents in the Extracellular Fluid are proposed to interact with Membrane Structures either in a long-term Coherent Manner, or at Localized Sites on the Cell surface (Tenforde and Kaune 1987).

netic fields and enzymes, because the activation of single molecules by the calcium-dependent activation of communication among all cells occurs at this point.

A simplified outline (Fig. 8.7) follows: (1) From the lipoproteins forming cell membranes, intra-membrane glycoprotein chains protrude from the outer surface of the cell membrane to link with neurohumoral molecules, hormones, antibodies, sugars, etc. In principle, when this process is affected by an electromagnetic field, a cell can "sense" weak electrochemical events. (2) The trans-membrane portion of the intra-membrane proteins transmits the event on the cell surface as a signal to the interior of the cell. (3) This signal couples with the enzyme activity inside the cell. This signal "on" the cell couples with microtubules, filaments, etc., which are intracellular membranes that are connected with the exterior cell membrane. The trans-membrane signal pathways provide communication from receptor sites to intracellular organelles. Thus the signal now is "in" the cell. (4) At various stages, these interactions are dependent on calcium ions.

In the interaction between ELF electromagnetic fields and organisms, the first event that occurs is the induction of electric current that circulates in the medium outside of the cell. This current possibly affects ion transport through membranes, voltage-sensitive receptor channels, membrane proteins, membrane surface hormones, and/or proteins promoting cell division. That is, this is the point where electromagnetic fields interact with cells to change the structure and function of cells, which respond to stimulation by altering cell growth. Thus, investigating the structure of cells exposed to electromagnetic fields is important for understanding the interaction between cells and electromagnetic fields. Effects at the organism level

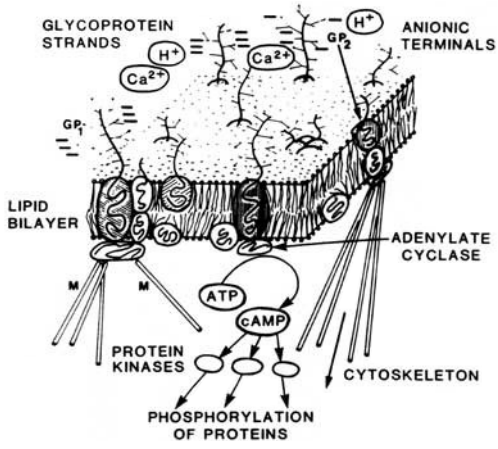

Fig. 8.7. Fluid Mosaic Model of Cell Membrane (From Adey (1990), Wilson et al. (1990))

are much more complex, because of such factors as the self-regulatory systems of cells and signal transmission pathways between cells.

In summary, to be meaningful and real, electromagnetic fields must interact with the extremely complex sets of processes that characterize living organism. The initial biophysics of the independent variable interaction with an organism might be novel, and they certainly are not yet understood. However, the dependent variable processes from that point of interaction onward are understood and must be obeyed.

8.4.2 Forces acting on ions and molecules

In order to clarify the biological effects of electromagnetic fields, the forces and energy acting upon organisms in electromagnetic fields will be analyzed using electromagnetic field theory. Phenomena will be analyzed at the molecular level using various theories, and those phenomena for which the mechanism of ELF electromagnetic field effects on organisms have been clarified, and several of the theoretical models that have been proposed for the various effects of very weak electromagnetic fields will be discussed (NIEHS 1999). Issues related to several electromagnetic field effects discussed by Valberg et al. (1997) and the EMF RAPID Program Working Group (NIEHS 1999) also will be discussed.

8.4.2.1 External field

The force acting on the molecules making up the structure of an organism when electric and magnetic fields of 1 kV/m and 0.1 mT, respectively, act on an organism

Table 8.6. External 50/60Hz Electromagnetic field forces compared to biological forces (Valberg et al. 1997)

Externally Applied Force	Force produced (pN = 10^{-12} N
Electromagnetic Force	
Electric-field (1,000 V/m) force on a charged molecule (10^+)	6×10^{-11} pN (a)
60 Hz, 0.1 mT induced force on a molecule in the cell membrane	2×10^{-5} pN (b)
Force on a moving molecule (10^+) produced by magnetic field (0.1 mT)	1×10^{-7} pN (c)
Biological entity	
Activation of a single hair cell in the inner ear	1 pN
Single kinesin molecule acting against a microtubule	3 pN
Single muscle myosin molecule pulling on an actin filament	4 pN
Force required to open a mechano-receptive, cell-membrane ion channel	14 pN
DNA transcription (RNA polymerase force)	14 pN
Charged molecule (10^+) in cell membrane, at resting potential (50 mV)	16 pN (d)
Force required to stretch out a DNA molecule	20 pN
Protein receptor to molecular ligand forces (molecular recognition)	90 pN
DNA stand-to-strand binding force, per each complementary base pair	70 pN
Force generated in flagellar motor (10-nm radius) of bacteria	100 pN

(a) An electric field of 1,000 V/m outside the body yields an internal E field of 4×10^{-5} V/m; if the charged (10^+) molecule is within a cell membrane, the force is 3,000 times larger, i.e., 1.8×10^{-7} pN.

(b) The maximum electric field induced by a 0.1 mT magnetic field is 4.810^{-3} V/m (i.e., at the body periphery); the consequent E field produced in the cell membrane is taken to be $0.0048 \times 3,000 = 14.4$ V/m.

(c) Lorentz force on a charged ion (10^+) with thermal average velocity (37°C) of calcium ions (~450 m/s).

(d) With 50 mV across a distance of 5 nm is an electric field of 10^7 V/m.

externally can be described (Valberg et al. 1997). The force exerted by electric and magnetic fields at environmental levels is extremely small (Table 8.6). For example, a 0.1 mT magnetic field would induce electric fields of about 14 V/m inside cells, and the force exerted on a protein molecule with 10 electric charges would be only 2×10^{-5} pN. In contrast, a cell membrane with a resting potential of 50 mV would result in an electric field of 10^7 V/m that would exert a force of 16 pN. From this comparison, it is obvious that the force exerted by external electromagnetic fields inside organisms is very small in value, especially compared with the naturally occurring forces.

8.4.2.2 Electrical noise

The minimum electric field level of cell membranes has been described (Weaver and Astumian 1990). For an interaction between electromagnetic fields and organisms to occur, an electric field greater than the thermal noise (Johnson-Nyquist noise) is required. In the low frequency region in the vicinity of cell membranes, the minimum value of the electric field required to exceed the Johnson-Nyquist noise level would

Table 8.7. Threshold Field strength in tissue due to electrical noises and other sources (Bernhardt 1997)

Source of noise	Threshold field strength in tissue	Trans-membrane voltage shift
Johnson-Nyquist thermally generated noise	100–1000 mV/m	3–10 μV
1/f noise	100–1000 mV/m	10 μV
Counter-ion polarization	4–10 mV/m	< 10 μV
Endogenous, biological background field strength	5–50 mV/m	up to several μV
External 50 or 60 Hz field (worst case, humans) B $\approx$ 100 μT; E $\approx$ 5–20 kV/m	< 10 mV/m	< 1 μV

be 100 mV/m. For a cell with parallel circuits, with resistance R and capacitance C, the effective bandwidth is calculated using $\Delta f(= 4RC^{-1})$). However, if a bandwidth of 10 Hz is assumed, the threshold value for a cellular response to an electric field is 0.1 mV/m and others have applied this type of simple model to *in vitro* cell systems. However, further considerations must be considered for the more complicated responses of *in vivo* systems.

In the environment surrounding cells, ions randomly moving because of Brownian movement are subject to electrical noise from the following four sources of electrical noise within cell membranes (Valberg et al. 1997): (1) Thermal noise (Johnson-Nyquist noise) is related to temperature; at physiological temperatures, a maximum of voltage of 3 μV is possible. However, at tissue membrane levels, the threshold for thermal noise that causes effects to occur in cells is about 1 V/m, a difference of more than 30-fold. (2) The $1/f$ noise is related to the flow of ions in the membrane channels, and a voltage of about 10 μV is created. (3) Shot noise is dependent on the ion charge and carrier properties, and it is considered to be a small source of membrane electrical noise. (4) Tissue (nerve, muscle, heart) electrical activity can cause electrical fields reaching 50 V/m. Bernhardt summarized the different sources of electrical noise in biological membranes (Table 8.7).

The background provided above clearly demonstrates the formidable challenge to both biology and physics. The energy involved in ELF fields typically is very small, so the mere demonstration of a biological effect presumably is difficult and surprising. In addition, if the occurrence of bioeffects is granted, the challenge of explaining how these effects might occur is substantial. At this point in history, the three main classes of proposed biophysical theories involve (1) resonance, (2) magnetite, and (3) free radicals. These three topics are discussed in the following three sections.

8.4.3 Resonance models

Resonance phenomena have been proposed as mechanisms for the action of low-level electromagnetic fields in the low-frequency region. Resonance phenomena, including ion cyclotron resonance, electron spin resonance, and nuclear magnetic resonance, have been proposed as mechanisms for explaining biological responses to electromagnetic fields. The most widely discussed model is the ion cyclotron resonance model. Therefore, only the ion cyclotron resonance model and the ion parametric resonance model will be discussed. In addition, stochastic resonance has been proposed as a model, and it is briefly summarized here as well.

8.4.3.1 Ion cyclotron resonance model

The ion cyclotron resonance (ICR) model was proposed by Liboff (1985) to account for low-frequency electromagnetic field effects based on ion cyclotron movement of ions along circular or helical paths in relation to cell surface receptors or membrane channels. For a freely moving charged particle in a DC magnetic field (B_0), the relationship with the Lorentz force, which is described by $F = q(vxB_0)$, is the basis of the argument. When a charged particle (q) moves at velocity (v) through a B_0, the Lorentz Force draws the path of the charged particle to move perpendicular to the direction of a magnetic field. Therefore, a charged particle moves in a circle when the direction of charged particle movement is perpendicular to the magnetic field.

If an AC magnetic field (B_1), is given in parallel with B_0, the electric field induced as described by Faraday's law will cause a charged particle to gain energy. Therefore, it will move in a circular path of increasing radius. The frequency (f_c) for a charged particle moving in a circle in a uniform magnetic field B_0, can be obtained using the following equation:

$$\omega = 2\pi f_c = (q/m)B_0$$

Here ω is the angular frequency, f_c is the cyclotron frequency, q is the net charge, and m is the mass.

If there are no collusions between particles, this movement will continue for a long time. However, in a dense medium, collisions occur between particles, meaning energy is lost. Therefore, to maintain the process, energy must be added from an external source. In the ion cyclotron resonance model, energy applied by a time-varying magnetic field in a horizontal plane and parallel to the motion. If the frequency of the magnetic field meets the conditions of the above equation, energy is supplied continuously.

The relationship derived from the above equation for important ions and DC magnetic field strength can be described (Fig. 8.8). Cyclotron frequencies for ions of less than 100 Hz are important to organisms. For example, the cyclotron frequency for $^{40}Ca^{2+}$ in a 50 μT DC magnetic field is 38.4 Hz. Biologically calcium is particularly important, along with Cl^-, K^+, Mg^{2+}, and Na^+.

At first glance, the ion cyclotron resonance model as a mechanism for interaction of magnetic fields and organisms seems difficult to believe. Furthermore, research

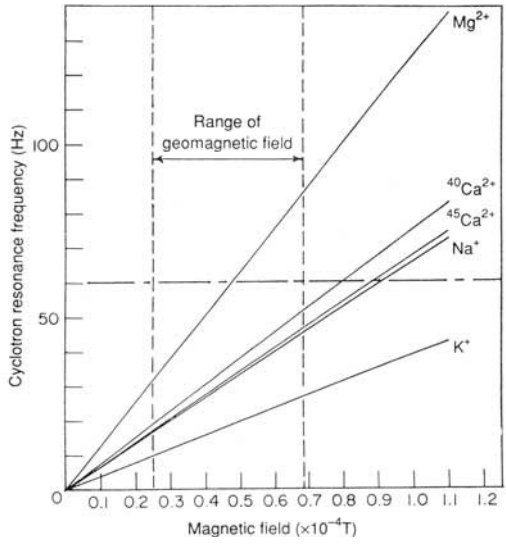

Fig. 8.8. The Relationship between the Ion Cyclotron Resonance Frequency and the Strength of DC Magnetic Field, B for Various Physiologically Important Ions. The Solid Lines give $f = (1/2\pi)(q/m)B$; q/m is charge-mass ratio (Liboff 1992).

has been conducted superimposing DC and AC magnetic fields for various physiologically important ions: results compatible with the ion cyclotron resonance model have been suggested (Table 8.8). Table8.8 shows the earlier observations claiming that the interaction of ELF electromagnetic field and biological systems. The meaning of suggestive in Table 8.8 is that the result in endpoints is consistent with the prediction by ion cyclotron resonance (ICR) conditions. On the other hand, the negative means that no detectable result can be seen when the ICR conditions are applied for biological systems. Following these observations, many applications with ion cyclotron resonance in various biological systems are reported. Many reports have suggested that DC and AC magnetic fields combination are effective in biological responses. However, there is no replication of the effects and no definitive evidence.

Perhaps the failure of the theory to be confirmed biologically is because ion cyclotron resonance motion is not possible in high viscosity media or in a fluid where collisions are dominant. Both of these conditions are characteristic of biological systems. Durney et al. (1988) analyed to identify and explain the fundamental characteristics of the physical mechanisms that result in a resonance response similar to cyclotron resonance. His model indicated that no resonant response is possible. Furthermore, Halle (1988) was unable to explain the observed biological effects by the ion cyclotron resonance model. In particular, he pointed out that the ion cyclotron resonance model violates the laws of classical mechanics.

Table 8.8. Early experimental results from tests of the ion cyclotron resonance model

Researchers	experiment	endpoint
Bawin (1975)	Calcium efflux	Suggestive
Blackman (1982, 1985)	Calcium efflux	Suggestive
Thomas (1986)	Rat operant behavior	Suggestive
Smith (1987)	Diatom motility	Supportive
Liboff (1987)	Lymphocytes	Supportive
Rozek (1987)	Lymphocytes/ Ca blocker	Supportive
Parkinson (1989)	Cellular	Negative

Valberg et al. (1997) noted that the ion cyclotron theory is relevant only in a vacuum and thus does not apply to living organisms. Furthermore, a number of other problems for the ion cyclotron theory were identified: (1) Ion activity in cells and channels differs greatly from that in a vacuum. (2) The orbit of Ca^{2+} ion cyclotron motion in the geomagnetic field is 1 m, which is far bigger than any cell. (3) Molecular collisions occur at a rate of 10^{12} every second. (4) A time-varying magnetic field changes its direction every half cycle, becoming electrically zero. (5) Ions exist within membrane channels for less than 1 μsec, and thus it is not possible for an ion to resonate at 50 or 60 Hz, where the cycle duration is much slower (20 msec at 50 Hz). In summary, the ion cyclotron resonance model is not in accord with either physical principles or biological reality.

Although there are no reasonable understanding to explain the ICR mechanisms at microscopic level and the interaction between ELF magnetic field and biological systems are as yet poorly understood, Liboff (2005) summarized the body of experimental evidence that weak ELF magnetic fields interact with biological systems through an ICR coupling mechanism. This evidence is gathered from studies on wide variety of biological systems, bone, rat behavior, diatom motility, cell culture, neural cell culture, complex biological systems, plants and cell-free biochemistry.

8.4.3.2 Parametric resonance model

Lednev (1991) developed a new model – the parametric resonance model – based on ion cyclotron resonance for ions weakly bonded to proteins, especially for calmodulin weakly bonded to Ca^{2+} and serving as an oscillator (NIEHS 1999). If the state of Ca^{2+} in calmodulin changes, it would affect enzymes and induce physiological changes. This model also is dependent on the interaction between DC and AC magnetic fields. Especially for the oscillation of an ion induced in an AC magnetic field (B_1), the oscillation frequency, depends on both the oscillation frequency (ω_1) and the frequency of Zeeman splitting (ω_2). The cyclotron resonance frequency is $\omega_c = \omega_1 - \omega_2$.

If in a parallel magnetic field (B_s), B_1 is added, due to the changing of frequencies ω_1, ω_2, then the oscillation level shifts from excitation level to ground level (ω_0) with an ion transition probability (P). The probability is given by $P = K_1 + (-1)^n K_2 J_n(x) \cos\delta$. Here, K_1 and K_2 depend on the infrared oscillation amplitudes from the transitions from ω_1, ω_2 to ω_c. Also, δ is the difference in phase of radiation from two sublevels.

The argument of the Bessel function $J_n(x)$ is $x = nB_1/B_s$. If the frequency of applied AC magnetic field is $f = \omega_c/2\pi n = f_c/n$, the Bessel function $J_n(x)$ leads to maximum and minimum. Finally, n is an integer.

In addition to predicting the resonance at cyclotron frequency and its fundamental, the Lednev model also expresses the relative oscillation amplitudes for B_1 and B_s that are expected to occur at the maximum resonance. The value corresponding to the extreme value $J_n(x)$ can be obtained from the independent variable x. For example, $J_n(x)$ has its first maximum at $x = 1.84$. For this model, if B_s and the resonance frequency f_c are constant, oscillation amplitude B_1 varies and $J(x)$ oscillation amplitude system variation can be predicted (NIEHS 1999).

Calmodulin is necessary for normal cell function: it interacts with Ca^{2+}-dependent enzymes to control their activity. In the calmodulin molecule, there are four binding sites for Ca^{2+} ions. As Ca^{2+} bind to one or more of these sites, it successively affects the shape of the calmodulin molecule and thus controls the interactions between calmodulin and various enzymes. If magnetic fields can affect the bonding of Ca^{2+} and calmodulin, a wide range of physiological processes could be affected (NIEHS 1999).

8.4.3.3 Ion parametric resonance model

Blackman et al. (1994) stimulated PC-12 cells with nerve growth factor and then exposed the cells to superimposed AC and DC magnetic fields. Compared to unexposed cells, neurite outgrowth, i.e., the axial extensions of the cells, was reduced as a function of the AC/DC magnetic field amplitude. Because this effect was confined to a narrow frequency region, which was determined by the amplitude of the applied DC magnetic field, it suggested a "resonance" phenomenon. Furthermore, if the following dependency is assumed, they argued that the experimental data fit the Bessel function.

$$P = K_1 + K_2(-1)^n J_n(2nB_1/B_0)$$

In this Bessel function, the factor (2) takes into the ratio B_1/B_0 that differs greatly from the value predicted by the Lednev equation. It should be stressed that data from the PC-12 experiments have shown resonance for H^+, Mg^{2+}, Mn^{2+}.

Furthermore, Blackman et al. (1994) showed from these experimental results that (1) the oscillation amplitude of the electric field induced by the magnetic field was not related to this phenomenon, and (2) perpendicular – not parallel – AC and DC magnetic fields were responsible for eliciting the various biological responses.

The experimental results on neurite outgrowth obtained from experiments with PC-12 cells provided a basis for the ion parametric resonance theory of Blanchard

and Blackman (1994). However, the results of experiments with snails were consistent with Lednev's initial statement (NIEHS 1999).

Despite these experimental results, there still are concerns regarding the soundness of the ion parametric resonance theory. For example, Adair (1992) has pointed out that if the theory were to be effective, it would be necessary for radiating ions to be shielded from collisions and other interactions with nearby matter. Other concerns also regarding the effectiveness of the ion parametric theory are provided in NIEHS (1999).

The ion parametric resonance theory appears to be an elegant and useful model, but considerable work remains to be done before its utility can be established. It does appear to be a substantial advance over its two predecessor models.

8.4.3.4 Stochastic resonance model

The interaction between very weak, low frequency electromagnetic fields and cells is not well understood. This is especially true given that the electromagnetic fields inside cells induced by external electromagnetic fields are very small compared to those induced in biological system by thermal noise and $1/f$ noise. From this perspective, it often is suggested that there can be no effects on organisms from very weak, low frequency electromagnetic fields. However, there are experimental reports of effects from very weak low frequency electromagnetic fields. Thus, understanding the manner by which cells detect extremely weak electric signals and the nature of the energy source necessary to amplify these signals are two issues of concern.

In non-linear system, in artificial physical systems, stochastic resonance is a process whereby a weak signal is amplified by system noise itself. It has been proposed that biological noise is responsible for amplifying the electric signal in the form of stochastic resonance (Kruglikov and Dertinger 1994). If noise is present to expand the input noise, both the output S/N and the output signal strength are increased. Extremely low intensity signals that by themselves are beneath the threshold are raised above the threshold by the presence of the background noise. Also, it has been shown for systems without thresholds, i.e., systems that respond to narrow oscillation amplitude signals, that stochastic resonance is occurring (Bezrukov and Vodyanog 1997).

Using mechanoreceptor cells in the tailfin of crayfish, Douglass et al. (1993) showed that amplification of an electrically applied signal by stochastic resonance was occurring. An extremely small movement of a cuticular hair is converted by neurons into a spike. This experiment showed that stochastic resonance was occurring as the receptor cells responded to a below-threshold signal from mechanical stimulation, with Gaussian-distributed noise superimposed.

Galvanovskis and Sandblom (1997) investigated the effects of weak, square-wave signals on the opening and closing of ion channels by measuring changes in ion current flow. If the number of ion channels is taken to be N = 1000, a 50 Hz signal frequency is used, and a circular state with a 10 cm radius (human model) is assumed, it is shown – under optimum conditions – that even for extremely weak, low frequency electromagnetic signals (less than 100 Hz and 100 μT), many cells with ion channels are able to detect the signal.

Starting from a different perspective, one based on the signal processing capabilities of the nervous systems of organisms rather than from a biophysical interaction of charged particles with electromagnetic fields, the concept of stochastic resonance provides another potential answer to the conundrum of how incredibly weak signals can produce biological changes. Although the concept of stochastic resonance has some experimental support for nervous tissues, its generality as a biological mechanism for other processes, such as carcinogenicity are not known.

Four different models based on the general concept of resonance have been offered to provide a fundamental biophysical mechanism to explain examples of biological effects produced by exposure to relatively weak ELF magnetic fields. The ion cyclotron resonance model proposed by Liboff (1985) was the first recent effort. It was successful in the sense that it stimulated biological experiments and further theoretical efforts. However, the model quickly was found wanting in terms of both data and theory.

Lednev (1991) developed the parametric resonance model. Compared with its predecessor, this theory is somewhat improved in terms of the mathematics and physics, but criticisms of the biological relevance of the model persist. Biologically, the model is restrictive, focusing only calmodulin weakly bonded to Ca^2. Although Ca^{++} and calmodulin are important biological regulators, there are many other biological molecules and processes with which ELF fields could interact.

Blackman et al. (1994) offered the ion parametric resonance model based on neurite outgrowth experiments. Based on its immediate predecessor (parametric resonance model), there is more biological data to support the model. Also, with respect to biochemistry, the ion parametric resonance model is more general, explicitly making predictions about many common and important ions that are involved in all biological processes. Although ion parametric resonance mechanism has been investigated, the conclusion is not plausible. It requires narrow vibrational energy levels, a fixed phase relationship between the vibrational states and the externally applied fields.

Kruglikov and Dertinger (1994) proposed that biological noise is responsible for amplifying biologically weak electric signals through the process of stochastic resonance. Interestingly, this theory is developed primarily from physics rather than from biology. Also, there is modest experimental support for the concept that stochastic resonance does function in biological systems. In summary, both additional theoretical development of resonance theories and biological experiments designed explicitly to evaluate these theories are very necessary.

8.4.4 Biogenic magnetite

Ferromagnetic materials in biological organisms interact with the earth's magnetic field to produce torque that can be sensed by some organisms and can provide a mechanism for sensing direction (Blakemore 1975). This interaction between ferromagnetic materials and magnetic fields is well known for magnetic bacteria; the magnetic material here is magnetite crystal (Fe_3O_4). The biophysical mechanism based on biogenic magnetite is well known.

The magnetic properties of magnetite crystal are related to the size and configuration of the material. If the balance between static magnetic energy and the wall energy in the magnetic domain of large crystals is considered, the situation becomes that of a multiple, magnetic-domain structure. However, for small crystals of less than 1 μm, a single-domain structure is more stable. If the crystals are much smaller, the anisotropic energy becomes about the level of thermal energy (kT) and the crystal becomes super-paramagnetic because of Brownian movement.

Magnetite has a single-domain structure. In the geomagnetic field, bacteria sense the magnetic field, because it has been reported that their behavior is influenced by geomagnetic fields (magneto-taxis). Honey bees, birds, salmon, and other organisms also have biogenic magnetite in their bodies, and the geomagnetic field can affect their orientation and migration. After the calculation of limits imposed by thermal noise on the effects of 60 Hz, less than 5 μT on biological structures holding magnetite, Adair showed that the effects of weak field will be masked by thermal noise energies and can not be expected to affect biological responses at cellular level, or the human health (Adair 1993: 1994).

In summary, although biogenic magnetite clearly is involved in some specialized processes, such as orientation and navigation, of a few species, such as bees and birds, the degree to which magnetite could underlie interactions with widely used, key cellular processes, such as signal transduction, control of the cell cycle and carcinogenicity is not known.

8.4.5 Free radical reaction

In the last two decades, biologists have increasingly recognized that both the formation of free radicals and the processes by which they are controlled and scavenged once formed are critical determinants of cellular injury highly relevant to processes such as aging and cancer. Highly reactive intermediates, i.e., molecules deficient in an electron and thus highly proficient at stealing electrons from other macromolecules, can damage membranes, DNA, and RNA, among other targets. For example, in recent years it has been recognized that melatonin (see section 3.4) is a potent scavenger of free radicals. Thus, if ELF electromagnetic field exposure reduces melatonin, increased free radical damage could be a step involved in carcinogenicity. In addition, physical chemists have demonstrated that very strong magnetic fields can influence free radical chemistry. These intriguing connections have made free radicals a hot topic in many areas of science, including bioelectromagnetics.

8.4.5.1 Background

Molecules are made from atoms; atoms are based on electrons, and their properties are determined by their electron shells. Besides their negative electric charges, electrons also have spin properties. There are two types of electron spin. The electron spin has a magnetic moment. When two electrons have different types of spin, they form a pair. Although stable molecules have an even number of electrons, there are molecules that have an odd number of electrons. In this case, pairs are not formed;

this situation is referred to as an unpaired electron. Molecules that have unpaired electrons are called free radicals.

Because free radicals have magnetic properties, it is thought that chemical reactions related to free radicals might be affected by magnetic fields. Biologically, free radicals are very important causes of cellular damage. A free radical molecule is highly reactive chemically, readily acquiring its needed electron by stealing it from another biological molecule, such as a protein. The affected molecule is damaged, and in turn becomes reactive, seeking an electron form another molecule. Each step is very short lived. However, this chain (or cascade) is a primary source of cellular damage and thereby is a primary contributor to cellular aging. Thus, cells have free radical scavenger molecules as a defense mechanism. Therefore, if magnetic fields affect free radicals, by promoting their formation or increasing their persistence, it could have very important biological consequences.

Active research on this topic has been conducted, particularly in Japan, and it has been shown that chemical reactions can be affected by magnetic fields (Hayashi 2004). Particularly for free radical reactions, nuclear spin and electron spin are key factors in determining the elementary process of reaction, and the spin conversion process is affected by magnetism (NIEHS 1999).

The orientation of the electron spin of a radical pair is either a singlet (S) or triplet (T), and the energy difference is determined by the electron conversion interaction (2J). As J decreases, as an index constant with distance between radicals, the energy difference between S and T for radicals is large immediately after formation; however, as radical diffuse and become about 10 Å apart, S and T become degenerate (J~0) radical pairs. If the pairs become further separated, they become dissipated radicals (J = 0). Usually, the inter-system crossing between S and T occurs in terms of energy when the two states are almost completely degenerated. The inter-system crossing of radical pairs occurs because of the Δg mechanism, and the hf (ultra structure) mechanism, and relaxation – with magnetic fields acting on each process.

8.4.5.2 Free radical interaction

Scaiano et al. (1995) superimposed 140 mT DC and maximum 60 mT AC (60 Hz and 120 Hz) magnetic fields to investigate the changes in benzophenone triplet reaction products (dissipation products) as a reaction model in living organisms. Under DC magnetic fields, for a given AC magnetic field strength, the amount of dissipation products collected increased along with the DC magnetic field intensity. When the DC field intensity was kept constant, an increase and a decrease was observed with increasing AC magnetic field intensity. Effects on free radical concentration were observed at AC field intensities greater than 0.01 mT. Benzophenone radical reactions also can occur in sodium dodecyl sulfate micelles. Although this condition differs from those found in living organisms, it has been argued that this is an appropriate model system to consider effects of magnetic field on free radical chemistry in biological systems. This type of experiment can be conducted using relatively high magnetic fields. There are very few reports indicting that low-level magnetic fields

have any effects on free radicals in organisms. Thus, the ability to operate at very high magnetic field intensities is a distinct advantage of this simple, model, free-radical chemistry system.

The frequency of low frequency magnetic fields acting on free radical reactions has not been confirmed. This is because the diffusion time for free radicals is much shorter than the cycle for low frequency magnetic fields. The lifetime of free radical is extremely short; the cycle for the reaction ranges from nanoseconds to microseconds. On the other hand, the cycle time for a 50 Hz is 20 msec. Therefore, some have argued that power-frequency magnetic fields can have no free radical effects, based on these temporal considerations. Therefore, a 10^5 Hz magnetic field is thought to have almost the same effect as a DC magnetic field.

Migratory birds have the ability to sense the geomagnetic field and to use it as a source of compass information. The two candidates for the biophysical mechanism underlying magnetoreception are (1) magnetite in animals, and (2) magnetically sensitive chemical reactions. Ritz et al. (2000) postulated the possibility that magnetoreception involves radical-pair processes as a biophysical mechanism. They first considered a system of radical pairs as a model for a magnetic sensory organ and evaluated the influence of the geomagnetic field on radical-pair systems. Then, Ritz *et al*. (2004) conducted the experiments using European robins (*Erithacus rubecula*). Results showed disruption of magnetic orientation behavior of robins resulted when they were exposed to a vertically aligned broadband of 0.1–10 MHz and 0.085 μT, and the single-frequency of 7 MHz and 0.47 μT together with the geomagnetic field. Disorientation was found to depend on the angle between the 7 MHz oscillating field and the geomagnetic field. The robins oriented in the migratory direction when the oscillating field was parallel to the geomagnetic field. The authors suggested the magnetic compass was based on a radical-pair mechanism involving a resonance effect on singlet-triplet transitions in an oscillating RF magnetic field.

In summary, magnetic field effects on radical-pair reactions have been established, both theoretically and experimentally. Free radicals do play an important role in many biological processes. For example, free radicals are thought to cause genetic damage. Experiments in physical chemistry have shown that extremely strong applied magnetic fields can modulate free radical chemistry. Thus, there is considerable interest in whether the lower intensity magnetic fields which are present environmentally can modulate free radical processes in living systems. However, to date, there is no evidence that such effects do occur with relevant magnetic field intensities and frequencies.

8.5 Discussion

This chapter summarizes the interaction between ELF electromagnetic fields and biological tissue from the viewpoint of electromagnetic waves, reviews electrical characteristics of cells and tissues, and briefly compares thermal and non-thermal effects. In addition, a framework for the entire process from application of an electromagnetic stimulus to the production of a disease state is provided. These basic

concepts lead to an increased understanding of the biophysical mechanisms underlying the biological and health effects of low-level electromagnetic fields in animals and humans.

Low frequency electric fields induce electric charges on the surface of organisms. Therefore, in strong electric fields, biological effects, such as muscle stimulation, can be perceived by humans. This can produce discomfort, but it does not result in chronic health problems. Also, if a human in a strong electric field touches an object that has different electrical potential, an electrical current will flow. Furthermore, low frequency electric fields and magnetic fields will induce electric fields and current flow inside organisms. In addition, low frequency magnetic fields can cause magnetophosphenes. These all are examples of a relatively high-level electromagnetic field effects that have been well confirmed (e.g., see Table 8.5). However, effects of magnetically induced low-level ELF electric fields or low-level ELF magnetic fields can not be explained at this time.

Epidemiology is one way to obtain knowledge about the health effects of ELF magnetic fields in humans. Despite the publication of many studies, it still is uncertain whether or not a relatively small (2-fold), but repeatedly found; increased risk of childhood leukemia has been caused by magnetic field exposure or by some other factor. Although theories regarding the health effects of low-level electromagnetic fields have been offered, they have not been confirmed by experimental data.

A number of mechanisms for effects of low-level electromagnetic fields have been proposed. (Brief descriptions of representative mechanisms are discussed in section 8.4.) If mechanisms are considered from the standpoint of energy, the energy associated with low frequency electromagnetic fields is very small. The larger-magnitude currents and fields that exist naturally in organisms often have been proposed to interact with DC magnetic fields (geomagnetic fields, etc.). This proposed interaction has been used to explain the mechanism for bird navigation during migration, and navigation and prey location by sharks and rays. (See section 1.3).

Unpaired electrons have magnetic properties, and free radical reactions have been identified as being affected by very strong magnetic fields. However, these are DC magnetic fields, and it is thought that low-frequency magnetic fields, especially 50/60 Hz fields, are unlikely to affect free radical processes, because the free radical transition time is on the order of nanoseconds to microseconds. However, adequate experimental data to support this contention do not exist.

The power and energy involved in biochemical processes are much greater than that related to the induced electric fields in tissues and cells. The magnetic permeability of tissue is almost equal to that in free space. Thus, other than biologically produced magnetite, it is unlikely that internal magnetic fields greater than external magnetic fields will occur. However, many biochemical processes can be described by non-linear state equations. Thus, with non-linear conditions, phenomena such as stochastic resonance are occurring.

In order for exposure to electromagnetic fields to affect biological organisms, a number of connected reactions are required (Fig. 8.5). Thus, it is necessary to consider biophysical mechanisms for changes in organisms induced by exposure to electromagnetic fields. In order for a mechanism to be believable, the following con-

cerns need to be addressed (Repacholi and Greenebaum 1999): (1) The physics and biophysics underlying the mechanisms must be logical. That is the linkage between the electromagnetic field exposure and the physical phenomenon occurring inside cell must be strong. (2) There must be a possibility of a linkage between the physical phenomena and some meaningful biological change. (3) The biological endpoint must be believable, i.e., the physiological phenomena must be linked strongly to the cellular communication system.

For the numerous physical interactions that might be considered, as it is thought that the electrical effects are overshadowed by noise and endogenous electrical signals. In order for mechanisms to be clarified, a strict approach based on realistic physical and biological hypotheses is necessary.

The NIEHS EMF-RAPID Working Group report proposed the following experiments for building and validating theoretical models about the action of low-level electromagnetic fields (NIEHS 1999).

- Duration of the electromagnetic field exposure required for effects to occur must be determined for different exposure periods.
- Although the ultimate interest is the effects of power-frequency electromagnetic fields, experiments should be conducted at other exposure frequencies.
- Experiments should be conducted at several electromagnetic field intensities; it is desirable to use intensities identified by models. Determination of threshold values is not the primary objective; clarification of the dependency of effects on electromagnetic field intensity is more important.
- Experiments to clarify the effects of several different types of electromagnetic noise are needed, observing the various biological changes occurring when the different treatments are conducted.
- Physical demonstration of detection of electric and magnetic fields by animals.
- Experiments to assess the temperature variation during exposure, to assess the temperature-dependence of effects of electromagnetic field exposure, and to examine joint effects of exposure to chemical substances.
- Experiments to assess whether effects are produced by induced electric fields only, magnetic fields only, or both electric and magnetic fields in combination.
- Measurement of AC magnetic fields versus DC magnetic fields and assessment of field directionality.
- Possibility of contaminating magnetic particles with *in vitro* experiments.
- For occupational epidemiological studies, the existence of transient phenomena related to electromagnetic fields, the assessment of field levels relevant to phenomena investigated *in vitro*, description of rate of rise, duration of a particular exposure intensity, and frequency of occurrence all should be investigated.

How electromagnetic fields could influence on organisms is still unknown. In this paper, issues related to biophysical aspects of exposure of organisms used in the study of the biological effects of electromagnetic fields are reviewed. Also, several proposed mechanisms-of-action for low-level electromagnetic fields on organisms are discussed briefly. Based on these issues, the more work to understand the in-

teraction mechanisms of low-level effects at the biophysical level is needed by the biological research community.

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Part III

Radiofrequency Fields

9

Radiofrequency Dosimetry and Exposure Systems

Osamu Fujiwara, Jianqing Wang

In general, RF dosimetry indicates quantifying the SAR, or the temperature rise caused by the SAR, in a biological body. It generally is difficult to measure the SAR directly in a living biological body, and therefore dosimetry efforts are forced to rely on computer simulation with numerical biological models or experimental simulation with biological-equivalent phantoms. Dosimetry also is an essential task for the design and use of exposure systems for *in vivo* or *in vitro* experiments in investigating possible biological effects of RF exposure.

This chapter first describes the basic computational and experimental dosimetry techniques. It then describes basic dosimetry considerations for design of exposure systems, introducing some representative exposure systems.

9.1 Computational Techniques

9.1.1 Anatomically based biological models

An anatomically based biological model is essential for numerical dosimetry. Such a numerical model is developed commonly from magnetic resonance imaging (MRI) or computed tomography (CT) scans. MRI or CT provides gray-scale image data as many transverse slices, at a designated spacing, from the head to feet of the biological body; the resolution in each slice is on the order of several millimeters. In general, it is easy to identify bone from CT images, but it is more difficult to segment interior organs. MRI data generally are superior to CT data in identifying interior tissues, because of their high contrast. Consequently MRI data are used more often in the development of numerical models. The drawback of MRI is that the image acquisition is time-consuming and automatic identification of various organs is difficult.

In order to develop a model for numerical dosimetry, original gray-scale data must be interpreted into tissue types, which is known as a process of segmentation. However, the gray scales in magnetic resonance images do not correspond to tissue types directly. Different tissues can have the same gray-scale values, which makes it impossible to automatically identify the original images into corresponding tissues

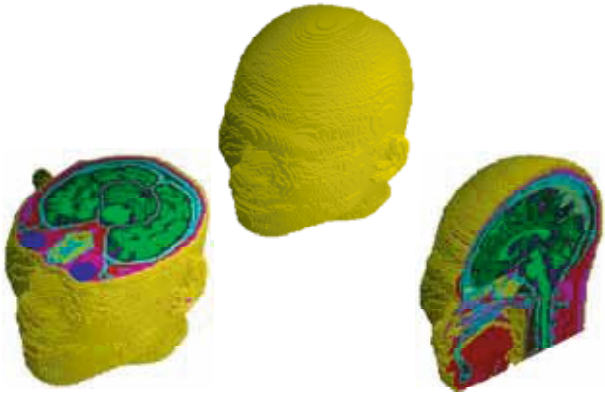

Fig. 9.1. A numerical human head model based on magnetic resonance imaging (Wang and Fujiwara 2002). The model consists of 17 tissue types. It has a resolution of 2 mm, meaning voxel size is $2 \times 2 \times 2$ mm.

or organs with sufficient accuracy using presently available image-processing techniques. All tissue- and organ-identification processing therefore is performed manually, to a large extent. A manual segmentation must be performed under the guidance of medical specialists.

The gray-scale images first are rescaled to produce appropriate voxels. Each voxel in the images then is identified rigorously as belonging to one type of tissue by assigning each voxel a red-green-blue code that identifies the discrete tissue type of that particular voxel. This process can be performed with commercial software such as Adobe Photoshop. All identified transverse images then are combined to obtain a three-dimensional numerical model. A fine adjustment generally is required to smoothly connect each slice in the three orthogonal planes (axial, sagittal and coronal).

Wang and Fujiwara (2002) provide an example of numerical human-head model that was developed from MRI data using the method just described (Fig. 9.1). This head model is composed of more than 520,000 cubic voxels, with a voxel size of $2 \times 2 \times 2$ mm, meaning voxel volume is 8 mm^3. Each voxel was assigned to one of 17 different tissue types, such as skin, fat, bone, muscle, etc.

Nagaoka et al. (2004) developed a whole-body adult-male model that consists of 51 tissue types and has a voxel size of $2 \times 2 \times 2$ mm (Fig. 9.2). Completion of this whole-body human model required more than 3 years.

The highest complexity used in contemporary models of the whole human body is about 50 tissue types, and the maximum resolution is about 1 mm (Fig. 9.3). It is worth noticing that higher resolution allows an increase in the tissue complexity of the model.

Compared to a numerical human voxel model, the difficulty in developing a numerical rat or mouse voxel model is smaller organs sizes. Lower resolution can make it impossible to identify the interior structure of a small animal. Watanabe et al.

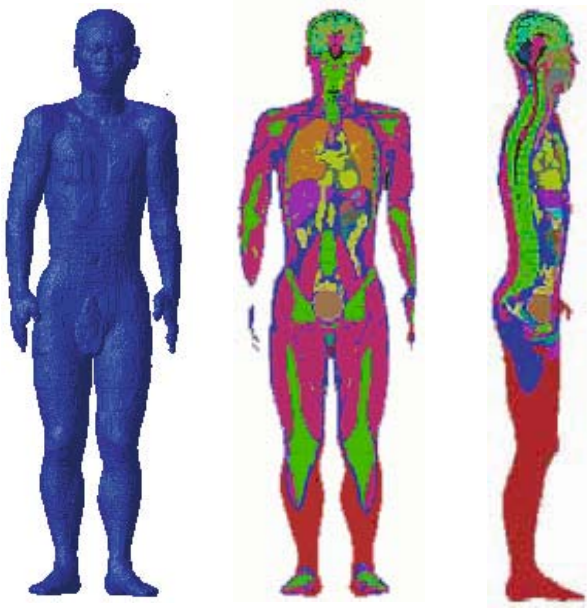

Fig. 9.2. A numerical whole body model based on magnetic resonance imaging data (Nagaoka et al., 2004). The model consists of 51 tissue types and has a resolution of 2mm.

(2000) developed a numerical rat model that has been used in the design of exposure systems (Fig. 9.4).

For dosimetry with the numerical voxel models, proper permittivity and conductivity values must be assigned to each tissue. The data from 10 MHz to 6 GHz, which were derived from 4-Cole-Cole extrapolation (Gabriel 1996) based on measurements for small animals, constitutes the most widely accepted database for this information. The data are recommended by various international standardization organizations and can be accessed from the Web site http://www.fcc.gov/fcc-bin/dielec.sh. An analysis of the variability in Gabriel's and other published dielectric property data shows a difference up to 40% for some tissues. This suggests that an uncertainty concerning dielectric properties of tissues should be considered in numerical dosimetry.

It is evident that the above-described approach is cumbersome and time-consuming when developing a numerical voxel model. Another approach is to develop an anthropomorphic numerical model, which is known as the dielectric anatomical model (Sandrini et al. 2004). The gray-scales in magnetic resonance images are correlated to the permittivity and conductivity values by continuous transfer functions based on mixture theory, and the model is obtained semi-automatically from the raw MRI data. Because discrete organs are not identified, the model is only valid for whole-body SAR calculation.

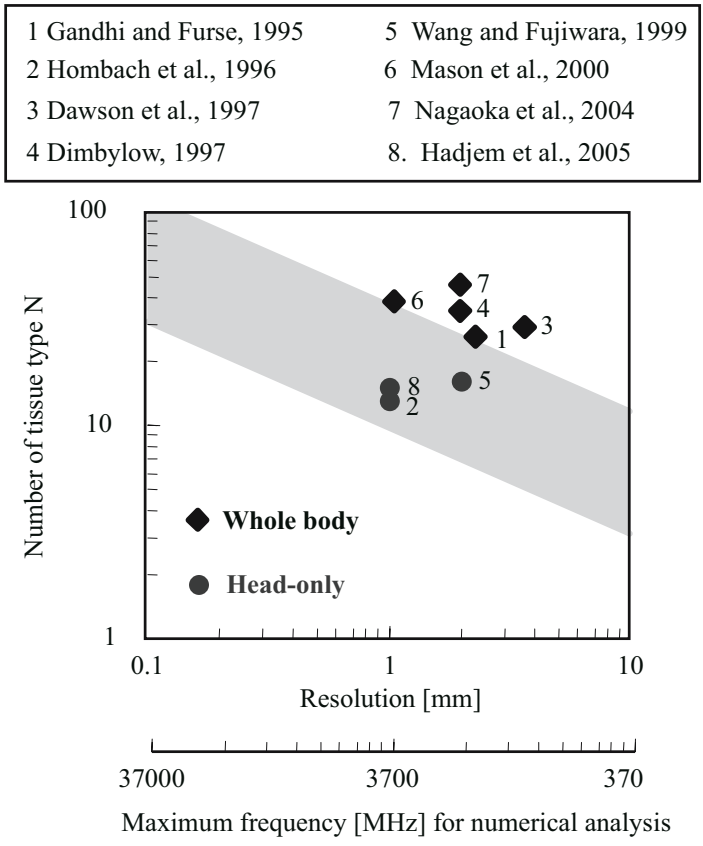

Fig. 9.3. A review of eight anatomically based human voxel models used for human dosimetry. The data were taken from Gandhi and Furse (1995), Hombach et al. (1996), Dawson et al. (1997), Dimbylow (1997), Wang and Fujiwara (1999), Mason et al. (2000), Nagaoka et al. (2004) and Hadjem et al. (2005.) The horizontal axis represents the resolution or the maximum applicable frequency, which is calculated from the condition that the resolution, or voxel size, be 1/10 of the shortest wavelength in tissue. The vertical axis represents the tissue complexity, i.e., the number of tissues types included in the model.

9.1.2 Finite difference time domain method

9.1.2.1 FDTD formulation

Rapid progress with computers has enabled conduct of high-level numerical dosimetry with the aid of high-resolution biological models. The finite difference time domain (FDTD) method (Taflove and Hagness 2000) is currently the most widely accepted means for numerical RF dosimetry. The FDTD formulations are derived from the following Maxwell's time-domain equations:

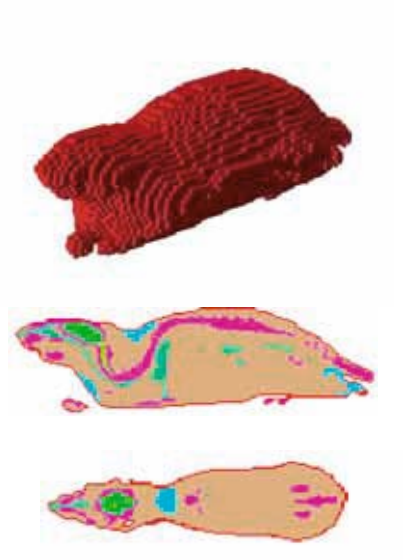

Fig. 9.4. An anatomically based numerical model of the rat (Watanabe et al. 2000). The model consists of 8 tissue types and has a resolution of 1 mm.

$$\nabla \times E = -\mu \frac{\partial H}{\partial t} \tag{9.1a}$$

$$\nabla \times H = \varepsilon \frac{\partial E}{\partial t} + \sigma E \tag{9.1b}$$

where E is the electric field, H is the magnetic field, ε is the permittivity, μ is the permeability and σ is the conductivity. Under rectangular coordinates, we can write Eqs. (9.1a) and (9.1b) as

$$\frac{\partial H_x}{\partial t} = \frac{1}{\mu}\left(\frac{\partial E_y}{\partial z} - \frac{\partial E_z}{\partial y}\right) \tag{9.2a}$$

$$\frac{\partial H_y}{\partial t} = \frac{1}{\mu}\left(\frac{\partial E_z}{\partial x} - \frac{\partial E_x}{\partial z}\right) \tag{9.2b}$$

$$\frac{\partial H_z}{\partial t} = \frac{1}{\mu}\left(\frac{\partial E_x}{\partial y} - \frac{\partial E_y}{\partial x}\right) \tag{9.2c}$$

$$\frac{\partial E_x}{\partial t} = \frac{1}{\varepsilon}\left(\frac{\partial H_z}{\partial y} - \frac{\partial H_y}{\partial z} - \sigma E_x\right) \tag{9.2d}$$

$$\frac{\partial E_y}{\partial t} = \frac{1}{\varepsilon}\left(\frac{\partial H_x}{\partial z} - \frac{\partial H_z}{\partial x} - \sigma E_y\right) \tag{9.2e}$$

$$\frac{\partial E_z}{\partial t} = \frac{1}{\varepsilon}\left(\frac{\partial H_y}{\partial x} - \frac{\partial H_x}{\partial y} - \sigma E_z\right). \tag{9.2f}$$

The FDTD discretization for the above equations is based on a Yee cell approach (Fig. 9.5). A special feature of the Yee cell is that the electric field (E) and magnetic field (H) components are staggered one half space-cell apart. That is to say, the E field generally is assigned at the edges of the Yee cell, and the H field is assigned on the faces of the Yee cell, which facilitates the differencing scheme. Denoting the field F (E or H) at spatial location ($i\Delta x$, $j\Delta y$, $k\Delta z$) and time step $n\Delta t$ by

$$F(i\Delta x, j\Delta y, k\Delta z, n\Delta t) = F^n(i, j, k) \tag{9.3}$$

and using central difference approximations of the derivatives such as

$$\frac{\partial F}{\partial x} \approx \frac{F^n\left(i+\frac{1}{2}, j, k\right) - F^n\left(i-\frac{1}{2}, j, k\right)}{\Delta x} \tag{9.4}$$

$$\frac{\partial F}{\partial t} \approx \frac{F^{n+\frac{1}{2}}(i, j, k) - F^{n-\frac{1}{2}}(i, j, k)}{\Delta t} \tag{9.5}$$

It is possible to discretize Eq. (9.2) and consequently derive the electric and magnetic field components. Their z components, for example, are as follows:

$$\begin{aligned} E_z^n\left(i, j, k+\frac{1}{2}\right) &= \frac{1 - \frac{\sigma(i,j,k+1/2)\Delta t}{2\varepsilon(i,j,k+1/2)}}{1 + \frac{\sigma(i,j,k+1/2)\Delta t}{2\varepsilon(i,j,k+1/2)}} E_z^{n-1}\left(i, j, k+\frac{1}{2}\right) \\ &+ \frac{\Delta t}{\varepsilon(i, j, k+1/2)} \left[\begin{array}{c} \frac{H_y^{n-1/2}\left(i+\frac{1}{2}, j, k+\frac{1}{2}\right) - H_y^{n-1/2}\left(i-\frac{1}{2}, j, k+\frac{1}{2}\right)}{\Delta x} \\ - \frac{H_x^{n-1/2}\left(i, j+\frac{1}{2}, k+\frac{1}{2}\right) - H_x^{n-1/2}\left(i, j-\frac{1}{2}, k+\frac{1}{2}\right)}{\Delta y} \end{array} \right] \end{aligned} \tag{9.6}$$

$$\begin{aligned} H_z^{n+1/2}\left(i+\frac{1}{2}, j+\frac{1}{2}, k\right) &= H_z^{n-1/2}\left(i+\frac{1}{2}, j+\frac{1}{2}, k\right) \\ &+ \frac{\Delta t}{\mu(i+1/2, j+1/2, k)} \left[\begin{array}{c} \frac{E_y^n\left(i+1, j+\frac{1}{2}, k\right) - E_y^n\left(i, j+\frac{1}{2}, k\right)}{\Delta x} \\ - \frac{E_x^n\left(i+\frac{1}{2}, j+1, k\right) - E_x^n\left(i+\frac{1}{2}, j, k\right)}{\Delta y} \end{array} \right]. \end{aligned} \tag{9.7}$$

For applying the FDTD method to numerical dosimetry, the Yee cells correspond completely to the voxel in biological models. By assigning each voxel to the corresponding permittivity and conductivity, we can easily model the anatomical tissues and organs, and calculate the interior electric and magnetic fields.

A noticeable problem in the assignment of permittivity and conductivity is that, referring to Fig. 9.5 and Eq. (9.6) or Eq. (9.7), the permittivity and conductivity are required at the edges of the voxel. In other words, the permittivity and conductivity are required at the boundary of different tissues, because each voxel is identified rigorously as belonging to one type of tissue.

Let us see the permittivity and conductivity at the boundary of four voxels as shown in Fig. 9.6. According to Ampere's law, along the closed curve denoted in dotted lines with an area of $S = S_1 + S_2 + S_3 + S_4$, we have

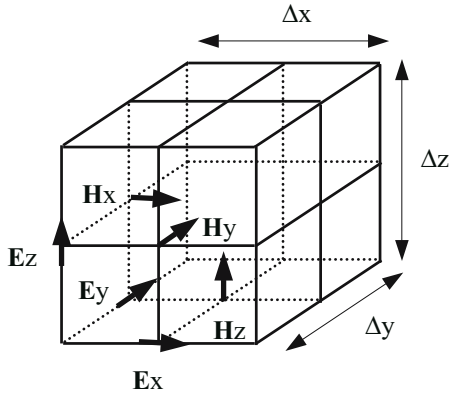

Fig. 9.5. A Yee cell used in finite difference time domain discretization.

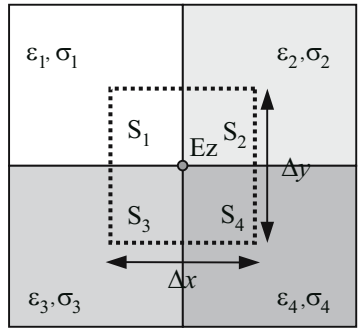

Fig. 9.6. A view at the x-y plane of four voxels with different permittivities and conductivities.

$$\begin{aligned}
&\int_S \varepsilon \frac{\partial E}{\partial t} \cdot ndS + \int_S \sigma E \cdot ndS \\
&= \left(\frac{\varepsilon_1 + \varepsilon_2 + \varepsilon_3 + \varepsilon_4}{4}\right) \frac{\partial E_z}{\partial t} \Delta x \Delta y + \left(\frac{\sigma_1 + \sigma_2 + \sigma_3 + \sigma_4}{4}\right) E_z \Delta x \Delta y \\
&= \oint_C H \cdot ds
\end{aligned} \tag{9.8}$$

which means the averaged permittivity and conductivity of the four neighboring voxels should be used at the boundary.

9.1.2.2 Absorbing boundary conditions

The FDTD method requires discretization of the entire domain over which the solution is to be calculated. However, it is impossible to discretize an infinite space, because of the finite memory capability of computers. The calculation domain, therefore, must be truncated to a finite size, even for an open-region problem. Once the infinite space of the open-region problem is truncated to a finite size, absorbing bound-

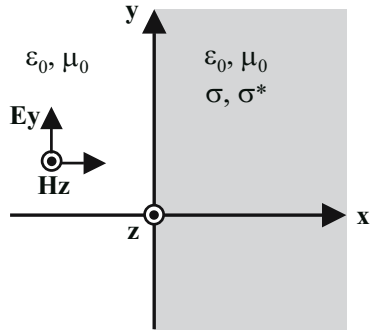

Fig. 9.7. A plane wave traveling normal to a perfectly matched layer medium.

ary conditions must be applied to the outside boundaries of the calculation domain in order to simulate the non-reflective nature of open space.

One of the most popular and effective absorbing boundary conditions (Berenger 1994) is known as the perfectly matched layer (PML). The basic concept of PML is based on impedance matching to minimize reflections. For a plane wave traveling normal to a PML medium (Fig. 9.7), the characteristic impedances in the free space and in the PML medium are

$$Z_0 = \sqrt{\frac{\mu_0}{\varepsilon_0}} \tag{9.9}$$

and

$$Z = \sqrt{\frac{\mu_0 + \frac{\sigma^*}{j\omega}}{\varepsilon_0 + \frac{\sigma}{j\omega}}} \tag{9.10}$$

respectively. Here σ^* is called the magnetic conductivity. From the impedance matching condition, i.e., $Z = Z_0$, we have

$$\frac{\sigma}{\varepsilon_0} = \frac{\sigma^*}{\mu_0} \tag{9.11}$$

That is to say, the reflection from the medium is zero for a normal incidence as long as Eq. (9.11) is satisfied.

For an incident wave of any angles, each electric or magnetic field component generally is split into two non-physical components along the traveling direction. A respective electric and magnetic conductivity value is assigned to each of the new field components, and then the relationship between the material properties characterizing the PML medium is derived from impedance matching condition.

Theoretically speaking, the PML provides a perfect absorption for traveling waves with any angle of incidence. However, in practice, the PML must be terminated, because of finite computer memory. Typically termination is accomplished using a perfect electric conductor, which introduces a reflection back into the calculation domain. The performance of PML therefore is characterized by three parameters: (1) thickness, (2) conductivity profil, and (3) the reflection coefficient at normal incidence.

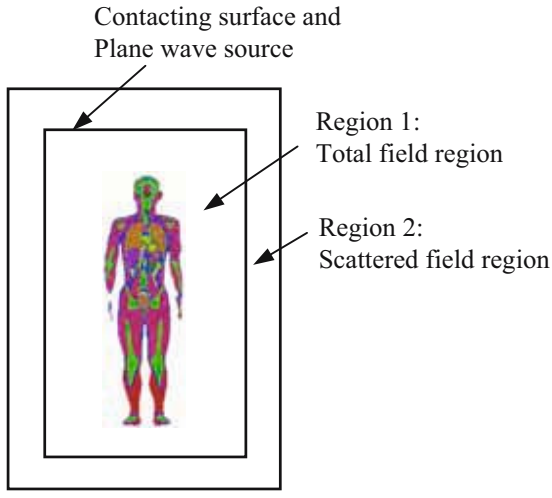

Fig. 9.8. Total-field / scattered-field "zoning" of the calculation domain.

9.1.2.3 Field excitation

In the FDTD calculation, an energy source must be couple into the algorithm to excite the electric and magnetic fields. Popular energy sources in numerical dosimetry are either an antenna or plane-wave excitation. For an antenna, a voltage source V is commonly impressed at the feeding gap of the antenna and then is converted into the electric field via $E = V/\Delta$, where Δ is the voxel size. For a plane wave excitation, the total-field/scattered-field (TF/SF) technique commonly is used. The TF/SF formulation is based on the linearity of Maxwell's equations. It assumes that the total electric and magnetic fields (E and H) can be decomposed in the following manner:

$$E = E_i + E_s \tag{9.12a}$$

$$H = H_i + H_s \tag{9.12b}$$

where (1) E_i and H_i are the incident fields, which are assumed to be known, and (2) E_s and H_s are the scattered fields resulting from the interaction of the incident field with the biological body. Because FDTD formulations are applicable with equal validity to the (1) incident fields, (2) scattered fields, and (3) total fields, one can "zone" the entire calculation domain into two regions: Region 1, the total field region and Region 2 (surrounding Region 1), the scattered field region (Fig. 9.8). Regions 1 and 2 are separated by a non-physical virtual surface that serves to connect the fields in each region, thereby impressing the incident plane-wave fields. The provision of Region 2 permits the application of the absorbing boundary conditions to act only to the outgoing scattered fields.

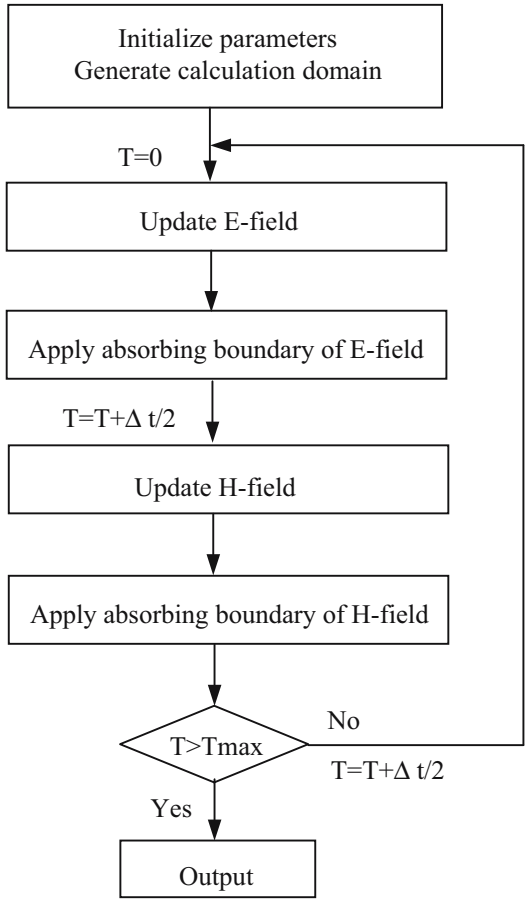

Fig. 9.9. A flow chart of the FDTD calculation process.

9.1.2.4 Finite difference time domain flow chart

Figure 9.9 shows a flow chart of FDTD calculation. From the FDTD-computed electric field (E) inside the biological body, the SAR is determined as

$$SAR = \frac{\sigma}{2\rho}|E|^2 \tag{9.13}$$

where ρ is the density of tissue.

9.1.3 Bio-heat equation and temperature calculation

Because the SAR is the physical quantity that causes tissue heating with RF exposure, it is useful in numerical dosimetry to calculate the temperature rise in a biological body. This is especially true for the brain, which includes the central control circuits governing the regulation of body temperature.

When a biological body in a thermal equilibrium state is exposed to the RF fields, the resultant temperature rise can be calculated from a bio-heat equation which takes into account such heat exchange mechanisms as heat conduction, blood flow and RF heating. The bio-heat equation is given by Pennes (1948):

$$\rho C_p \frac{\partial T}{\partial t} = \nabla(k\nabla T) + \rho SAR - b(T - T_b) \tag{9.14}$$

with the boundary condition

$$k\frac{\partial T}{\partial n} = -h(T - T_a) \tag{9.15}$$

where T is the temperature, C_P is the specific heat, K is the thermal conductivity, b is a constant related to the blood flow, T_b is the blood temperature, T_a is the ambient temperature, h is the convective heat transfer coefficient, n is the unit vector normal to the body surface, and the SAR is the input RF heating source into the bio-heat equation.

The discretization of the bio-heat equation follows that of the FDTD voxel body in the SAR calculation (Wang and Fujiwara 1999). According to the notation in Eq. (9.3) and expanding the bio-heat equation in its finite difference approximation, Eqs. (9.14) and (9.15) can be written as

$$\begin{aligned} T^{m+1}(i,j,k) &= T^m(i,j,k) + \frac{\Delta t}{C_p(i,j,k)} SAR(i,j,k) \\ &- \frac{\Delta t}{\rho(i,j,k)C_p(i,j,k)} b(i,j,k)\cdot\left[T^m(i,j,k) - T_b\right] \\ &+ \frac{\Delta t \cdot K(i,j,k)}{\rho(i,j,k)C_p(i,j,k)\Delta^2}\cdot\begin{bmatrix} T^m(i+1,j,k) + T^m(i,j+1,k) + T^m(i,j,k+1) \\ +T^m(i-1,j,k) + T^m(i,j-1,k) + T^m(i,j,k-1) \\ -6T^m(i,j,k) \end{bmatrix} \end{aligned} \tag{9.16}$$

$$T^m(i,j,k) = \frac{K(i,j,k)\cdot T^m(i+1,j,k)}{K(i,j,k) + h\Delta} + \frac{T_a h\Delta}{K(i,j,k) + h\Delta} \tag{9.17}$$

where the voxel is assumed to be a cubic with a size of Δ, and the finite difference approximation of Eq. (9.15) is given only along the x-directed line. Similar approximations can be obtained along y- and z-directed lines. In order to ensure the numerical stability, Δt should be chosen to satisfy

$$\Delta t \le \frac{2\rho C_p \Delta^2}{12K + b\Delta^2} \tag{9.18}$$

which is derived from Von Neumann's condition.

The thermal parameters C_P, K and b can be found in some physiological textbooks, e.g., Guyton and Hall (1996), but a comprehensive database of thermal parameters does not exist at present.

As for the convective heat transfer coefficient (h), two values are required. One is h_a, the convective heat transfer coefficient from the biological body surface to

the ambient temperature, and the other is h_b, the convective heat transfer coefficient from the internal surface to the cavity, which is general larger than h_a. By updating Eqs. (9.16) and (9.17) with SAR as excitation, the temperature distribution inside a biological body as a function of time can be obtained.

The steady-state temperature rise due to RF exposure can be calculated from the difference between the temperature T and T_0 where T_0 is the normal temperature in the unexposed body (with SAR = 0) at a thermal equilibrium.

It should be noted that the present temperature-calculation technique has a considerable uncertainty, primarily because of the lack of a comprehensive database for thermal parameters. Another problem is the change of blood flow that is not easy to be included in the bio-heat equation, which makes estimation difficult.

9.2 Measurement Techniques

9.2.1 Tissue-simulating phantoms

Experimental dosimetry is focused mainly on homogeneous tissue-equivalent phantoms, because of the difficulty in developing a heterogeneous biological body model. Phantoms generally simulate a single tissue, such as muscle, or a mixed-tissue, such as the head, by having the same permittivity and conductivity as the actual tissue values. Phantoms mainly can be classified into three types: liquid, solid, or gel.

9.2.1.1 Liquid phantoms

A liquid phantom permits scanning the electric fields inside it using an electric field probe, which can provide high-precision SAR measurement. The main materials in most liquid phantoms are deionized water, sugar and sodium chloride. Also other materials, such as hydroxyethyl cellulose (HEC), bactericide, and diethylene glycol butyl ether often are used to adjust the permittivity and conductivity.

Table 9.1 gives a formulation for simulating a brain-equivalent tissue at 900 MHz (ARIB 1998). The permittivity is adjusted mainly by varying the percentage of sugar, and the conductivity is adjusted mainly by altering the percentage of sodium chloride. Because it is based on water, a liquid phantom generally is good at simulating a high water-content tissue. A mixture of a physiological salt solution and ethylene glycol enables the liquid phantom also to simulate a low water-content tissue. The drawback of the liquid phantoms is the evaporation of water, which changes the permittivity and conductivity. This, however, can be managed by the appropriate replenishment of water.

9.2.1.2 Solid phantoms

One representative solid phantom is the TX-151 phantom. A TX-151 phantom is applicable to simulation of a high water-content tissue in the microwave band. Ito et al. (1998) gives a formulation for simulating a brain-equivalent tissue at 900 MHz

Table 9.1. Formulation of a liquid phantom for simulating a brain-equivalent tissue at 900 MHz (ARIB 1998)*

Material	(Weight ratio %)
Deionized water	41.45
Sodium chloride	1.35
Sugar	56.50
HEC	1.00
Bactericide	0.10

* εr = 41.5 and σ = 0.97 S/m

Table 9.2. Formulation of TX-151 phantom for simulating a brain-equivalent tissue at 900 MHz (Ito et al. 1998)*

Material	(Weight ratio %)
Deionized water	82.12
Sodium chloride	0.56
Polyethylene powder	13.33
TX-151	1.39
DASS	0.05
Agar	2.55

* εr = 43.0 and σ = 0.83 S/m

(Table 9.2). The permittivity is adjusted mainly by the amount of polyethylene powder mixed in agar, and the conductivity is adjusted mainly by the amount of sodium chloride. TX-151 acts as adhesive in the agar liquid, thus, it forms a solid phantom. Required materials are easily obtained, and it is easy to make TX-151 phantoms of differing shapes. If the phantom is wrapped with vinyl film, it is possible to keep constant permittivity and conductivity for 1 month, at room temperature.

Glycerin-based phantoms have been developed to provide a longer life span than is possible with TX-151. Its life span can be extended to 6 months by wrapping the phantom with vinyl film for preservation, because glycerin acts to maintain humidity. The permittivity in a glycerin phantom is adjusted mainly with deionized water, and the conductivity is adjusted by the sodium chloride. The polyethylene powder is used in fine adjustment of the permittivity and conductivity. Okano et al. (2000) provides a formulation for simulating a brain-equivalent tissue at 900 MHz (Table 9.3). Compared to a TX-151 phantom, the drawback of glycerin phantom is the narrower frequency range for a single composition: at different frequencies the percentages of various materials have to be changed.

Other solid phantoms include the dry phantom, which is molded from paraffin, resin and silicon rubber with mixture of the powder of carbon and graphite

Table 9.3. Formulation of a glycerin phantom for simulating a brain-equivalent tissue at 900 MHz (Okano et al. 2000)*

Material	(Weight ratio %)
Deiozized water	36.31
Glycerol	53.48
Sodium chloride	1.12
Polyethylene powder	3.74
Agar	5.35

* $\varepsilon r = 41.0$ and $\sigma = 0.88$ S/m

(Kobayashi 1993). The dry phantom does not contain water and therefore avoids the problem of evaporation. Its advantage is that the permittivity and conductivity do not change with time; its drawback is the difficulty of manufacture.

It is evident that scanning the electric fields in a solid phantom for SAR measurement is impractical. Solid phantoms are more appropriate for measuring the temperature rise.

9.2.1.3 Gel phantoms

Gel phantoms can be manufactured from a physiological salt solution and polyethylene powder mixed with TX-150 or agar. It generally is used to simulate a high water-content tissue. With a gel phantom, it is difficult to have a fix shape, unless a container is used. It also has the problem of water evaporation.

There is the possibility to visualize the temperature using a high-molecular-gel phantom, because becomes opaque when the phantom's temperature exceeds a given value (Miyakawa 1994).

9.2.2 Electric field measurement

In liquid phantoms, the SAR measurement usually is conducted by measurement of electric field. The SAR is calculated from the measured electric fields with Eq. (9.13). The main requirements for an electric field probe in SAR measurement are: (1) high sensitivity and linear response over a broad frequency range; (2) high spatial resolution; (3) isotropy in different media; (4) low interaction with the measured field; and (5) small size. Most present-day electric field probes are based on Schottky diode detectors. The measured signal at the probe output is a voltage proportional to the electric field E or the squared electric field E^2.

Since the SAR calculation requires all three components of the electric field, most electric field probes consist of three small dipoles, with detector diodes at their center gaps (Fig. 9.10). A triangular-beam structure is designed because of its smaller outline and the possibility of placing the surface detector in the center of the probe.

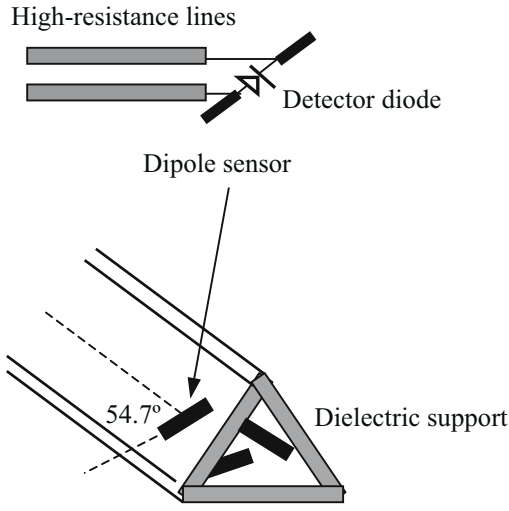

Fig. 9.10. A typical electric field probe with a triangular beam structure (Schmid et al. 1996).

The probe consists of three sensors, each sensor consists of (1) a short dipole antenna, (2) a diode detector at the dipole feed-gap, (3) a dielectric mechanical support, and (4) a highly resistive feed-line to extract the signal detected by the diode to measuring unit. A probe's tip length and tip diameter are on the order of ~20 mm and ~10 mm, respectively. This permits a high spatial resolution for scanning electric fields within a liquid phantom.

Such probes typically have (1) a frequency range from 10 MHz to 6 GHz, (2) a linearity of ±0.2 dB, (3) a dynamic range of 5 μW/g to 100 mW/g, and (4) a deviation from spherical isotropy of within ±0.4 dB. The directivity patterns of the three sensors in such probes are orthogonal, and the total electric field magnitude is proportional to the root-sum-square of the three orthogonal components. In the square-law region of the diode characteristic curve, the sensor output voltage is proportional to the mean square of the corresponding field component.

Probe calibration for SAR measurement in liquid phantoms will produce either an SAR or an electric field conversion factor. Because SAR is proportional to liquid conductivity, a direct calibration in terms of SAR is valid only for liquids with the exact same conductivity. The electric field sensitivity depends more on both the liquid permittivity and the liquid conductivity and therefore is less sensitive to the conductivity alone. Calibration in terms of electric field, rather than SAR, should have a broader range of validity.

Possible calibration means include both waveguide calibration and thermal calibration. In the waveguide calibration, the measured fields are compared with analytical solutions. In the thermal calibration, the SAR values are compared with the temperature rise. The former is preferable, because it gives higher precision.

9.2.3 Thermal measurement

For solid phantoms, such as TX-151 or glycerin phantoms, the SAR measurement is usually conducted via thermal measurement. Under the assumption of linear energy deposition over a fixed period of time, the SAR can be determined from

$$SAR = C_p \frac{dT}{dt} \approx C_p \frac{\Delta T}{\Delta t} \tag{9.19}$$

where C_p is the specific heat of phantom, ΔT is the temperature rise caused by the exposure, and Δt is the exposure time.

There are two methods for making the temperature measurement. One method is to use a flour-optic temperature probe. These probes utilize temperature-dependent fluorescent decay or interferometric micro shifts of cavity resonators. One can insert a flour-optic temperature probe into a phantom and measure the temperature over a period of time, meaning a history of temperature rise over time can be recorded.

The second method of measuring temperature is to use an infrared image camera. The solid phantom, before exposure, first is set close to the ambient temperature, and the corresponding infrared image is recorded. Immediately after the RF exposure, another infrared image is taken. Then the temperature rise at the phantom surface is obtained from the temperature difference, and the SAR is determined from Eq. (9.19).

For measuring the SAR inside the solid phantom using an infrared image camera, the phantom must be divided beforehand, e.g., at the phantom's midline. The images before and after the exposure are then taken at the center surface, and the temperature rise is obtained from their difference.

The exposure time for thermal measurement should be short enough to prevent heat transfer from phantom to air. (Development of a temperature difference will result in heat transfer). In order to keep a linear temperature rise over a short period of time, one requires use of a high-output power amplifier. For a rat-sized TX-151 phantom, at least 10 W is required to produce an efficient temperature rise of 1 –2 °C over several minutes. The thermal method therefore is a weak approach when measuring lower SAR values. Its precision also is poorer than that provided by an approach using an electric field probe.

9.3 *In vivo* Exposure Systems

9.3.1 Near-field exposure

Near-field exposure systems are developed mainly for investigating possible biological effects from mobile telephones. In an actual human exposure with a mobile telephone, the absorbed power is primarily in the human head. However, the whole-body averaged SAR is quite low, meaning thermal stress is not an issue. The ratio of the maximum SAR averaged over any ten grams of tissue to the SAR averaged over the whole head is as high as 20 – 40. This means that the exposure from mobile telephones is a highly localized exposure.

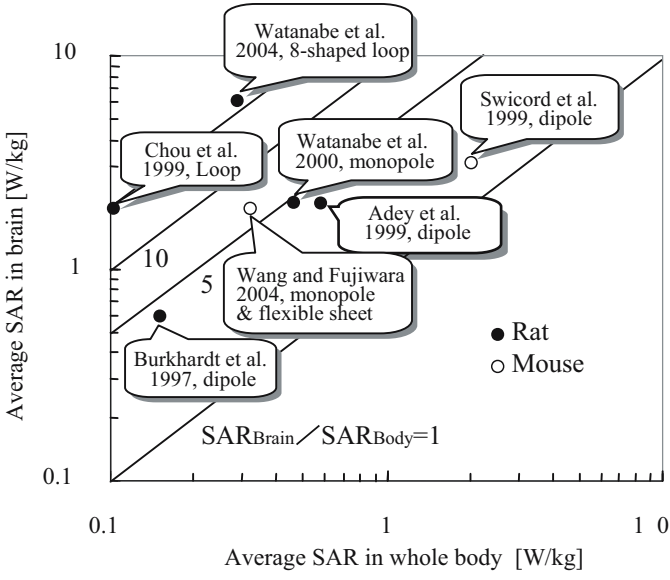

Fig. 9.11. Review of performance, in terms of ratio of specific absorbance rate in head and whole body, of seven localized animal exposure systems for mice or rats. Data were taken from Burkhardt et al. (1997), Adey et al. (1999), Chou et al. (1999), Swicord et al. (1999), Watanabe et al. (2000), Watanabe et al. (2004), and Wang and Fujiwara (2004).

Therefore, the basic requirement for a near-field exposure system for *in vivo* experiments is to produce a highly localized exposure to the heads of animals, such as rats and mice. The exposure should be as close as possible to mobile telephone exposure actually occurring in a human head.

Because various international standardization organizations, e.g., ICNIRP (1996), are limiting the localized SAR to no more than 2 W/kg, averaged over any ten grams of tissue, a typical exposure system design goal is to realize (1) an average SAR above 2 W/kg in the animal brain, and (2) an average SAR below 0.4 W/kg in the whole body, which is known to be unlikely to cause any thermal stress in small animals. In other words, for the exposed animals, the ratio of the average SAR in the brain to the average SAR in the whole body should be larger than 5.

Many *in vivo* exposure systems, most of them designed for rats or mice, have been developed for tests of the biological effect of mobile telephones. In early exposure systems a half-wavelength dipole or a quarter-wavelength monopole antenna usually was used. The ratios of the average SAR in the brain to the average SAR in the whole body were smaller than 5 for rats and 3 for mice, which indicates the difficulty in realizing a localized exposure using linear antennas (Fig. 9.11).

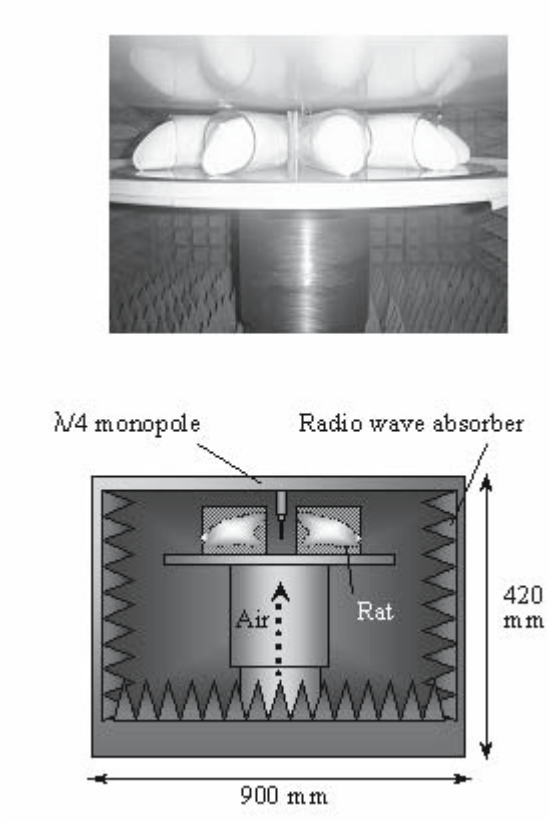

Fig. 9.12. An animal exposure system used in the study of mobile phone signals (Watanabe et al. 2000). A photograph of animas in the exposure system is given at the top, and the bottom provides a diagram of the interior of the exposure system.

9.3.1.1 Linear antennas

Watanabe et al. (2000) provided an example of such an exposure system (Fig. 9.12). The RF signal was fed to a quarter-wavelength monopole antenna at the center of the ceiling of each exposure box. The exposure box, which was made of metal, was $90 \times 90 \times 40$ cm. Except for its metal ceiling the inside was covered with pyramidal-shaped RF absorbers to simulate a free-space environment. Ten Fischer 344 rats, each of which was fixed in a plastic holder, were located in a carousel shape around the antenna. Fresh air was provided from the bottom and side of the exposure box to reduce thermal stress. The system provides a ratio of the brain-averaged SAR to the whole-body-averaged SAR of nearly 5.

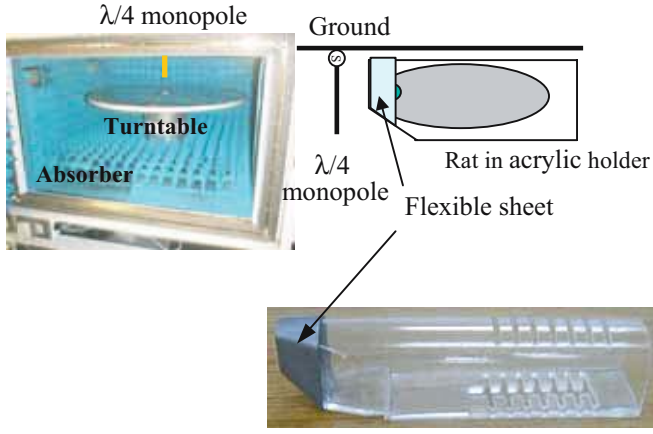

Fig. 9.13. A design for a near-field *in vivo* exposure system in which conventional acrylic rat holders are wound with a sheets of a flexible, high-permittivity material to produce enhanced exposure to the head of young rats or of mice (Wang and Fujiwara 2004).

9.3.1.2 Improvement with high-permittivity material

The above exposure system is difficult to apply to mice or to young rats, because they are smaller size than adult rats, it produces a higher whole-body-averaged SAR. For young rats, the ratio of brain-averaged SAR to the whole-body-averaged SAR decreases to 3 in the above exposure system. Wang and Fujiwara (2004) provided a technique to improve the ratio.

To maintain a fixed exposure level, rats usually are constrained within an acrylic holder to keep them in the vicinity of the antenna. If the head part of each acrylic holder is covered with a flexible sheet having high permittivity (Fig. 9.13), the electric flux would be concentrated along the sheet in a circle. This results in a strong magnetic field inside the holder at the region of the subject's head. According to the mechanism of electromagnetic absorption in tissue for near-field exposure, a strong magnetic field would induce a high SAR level. The electric flux density and magnetic field distributions inside the wrapped acrylic holder in the absence of rat can be calculated (Fig. 9.14). The complex relative permittivity of the flexible sheet is as high as 250-j10, and the complex relative permeability is 5.6-j6.3 at 1.5 GHz. A dramatic increase of the electric flux density along the flexible sheet, and also strong magnetic fields inside the holder in the rat's head region, can be observed clearly. As expected, in an infrared image (Fig. 9.15) taken after a 30-second exposure with an antenna input of 36 W at 1.439 GHz, the flexible sheet with a high permittivity indeed increases the localized SAR. As a result, the ratio of brain-averaged SAR to the whole-body-averaged SAR has been increased from 3 to 7 for young rats.

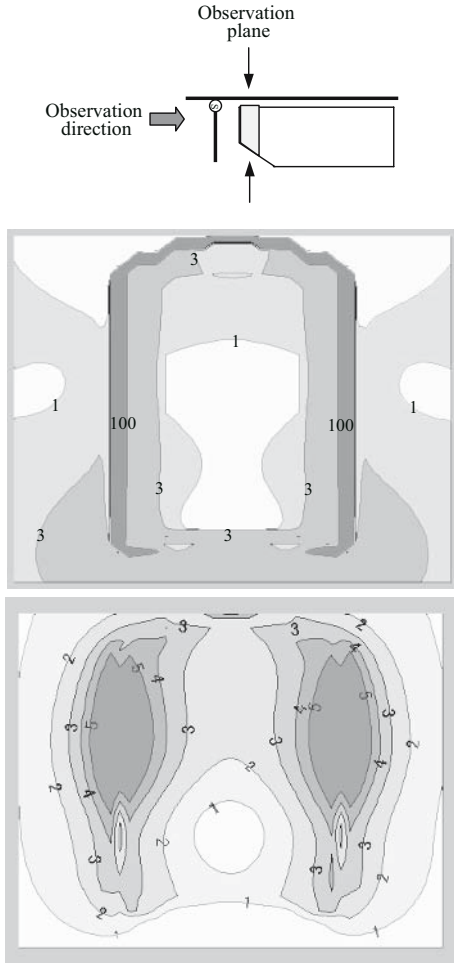

Fig. 9.14. Calculated electric flux density and magnetic field distributions with modified animal holder (Wang and Fujiwara 2004). The top shows electric flux density, in nC/m^2. The bottom shows magnetic field distribution, in A/m. Both distributions are observed at the observation plane in the absence of a rat.

9.3.1.3 Loop antennas

Another approach to producing a highly localized exposure is to use a small loop antenna with a size of several centimeters (Fig. 9.16). This low-impedance loop antenna can be matched to a 50 Ω source impedance through a section of capacitively coupled transmission line embedded in a Teflon rod (Chou et al. 1999). The loop can be tuned for frequency by adjusting the loop feed-line length and depth in the Teflon rod. The use of Teflon and resonance matching allows the loop to satisfy power requirements in large-scale *in vivo* experiments. Because of the strong magnetic fields around the

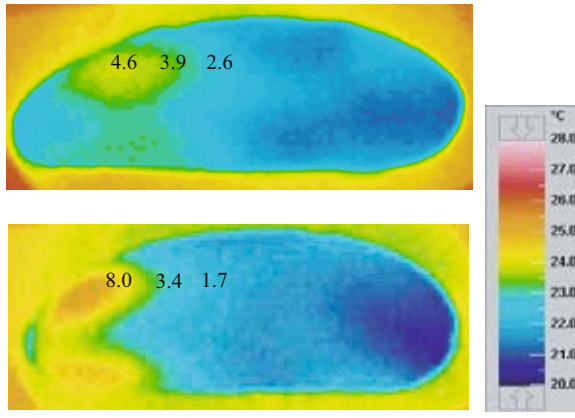

Fig. 9.15. An infrared image in a sagittal plane of a phantom of a young rat after a 30-second exposure with an antenna input of 36 W (Wang and Fujiwara 2004). The top shows temperature without the flexible, high-permittivity sheet, and the bottom shows the enhancement occurring when the flexible sheet is present. The numbers are SAR, in W/kg, as measured by three flour-optic temperature probes, normalized to an antenna input of 1 W.

loop antenna, a ratio of the brain-averaged SAR to the whole-body-averaged SAR as high as 20 can be realized, with a 5 cm spacing between the loop and a rat head.

Based on an 8-shaped coil used for magnetic stimulus of cranial nerves, an 8-shaped loop antenna has been developed for near-field exposure (Watanabe et al. 2004). It consists of two resonant loops (Fig. 9.17). The magnetic near-fields around it are strengthened at the center by the in-phase electric currents. The use of two resonant loops also contributes to reducing the mismatch between the antenna and the feed-line. There are no theoretical formulae for designing this type of antenna; the optimization of shape and size must be done by numerical simulation. Applying this 8-shaped loop antenna in Fig. 9.17 to a rat head exposure at 1.5 GHz has shown that the ratio of the brain-averaged SAR to the whole-body-averaged SAR is higher than 20.

9.3.2 Far-field exposure

Far-field exposure systems are used mainly to simulate a uniform RF exposure. Representative exposure systems include a waveguide structure and a TEM (Transverse Electromagnetic) cell structure.

9.3.2.1 Resonant waveguide structure

Balzano et al. (2000) used a wheel-like exposure system designed as resonant waveguide structure consisting of two parallel, circular-shaped metallic plates shorted with metallic bars along the outer edge and with an antenna at the center (Fig. 9.18). The

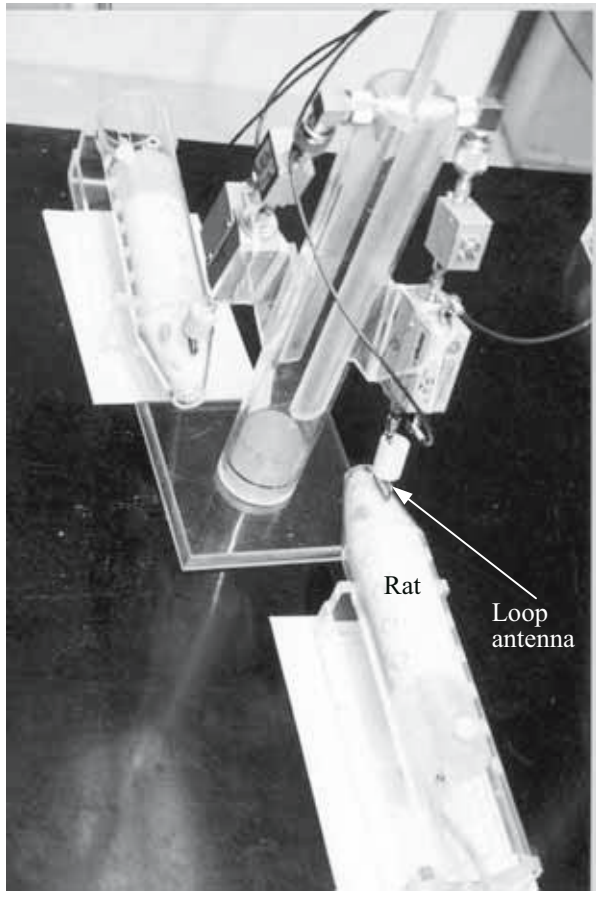

Fig. 9.16. A near-field *in vivo* exposure system using loop antenna (Chou et al., 1999).

antenna is a top-loaded monopole that is capacitively coupled with a passive counterpoise. This makes the current along the monopole, and the counterpoise, is fairly uniform. The antenna excites a cylindrical TEM wave that impinges on a carousel of symmetrically arranged small animals. The small animals are restrained in plastic holders, which are inserted through circular holes in the plates at a fixed distance from the antenna. The animal body axis is orientated parallel to the electric fields to maximize the absorption of RF energy. The symmetric arrangement provides uniform exposure to the small animals, while the whole-body TEM illumination induces fairly uniform RF absorption within each animal.

9.3.2.2 TEM cells

A TEM cell (Fig. 9.19) is known to generate uniform fields. It essentially is a broadened coaxial cable. Transitions in a taper shape convert the thin circular coaxial ca-

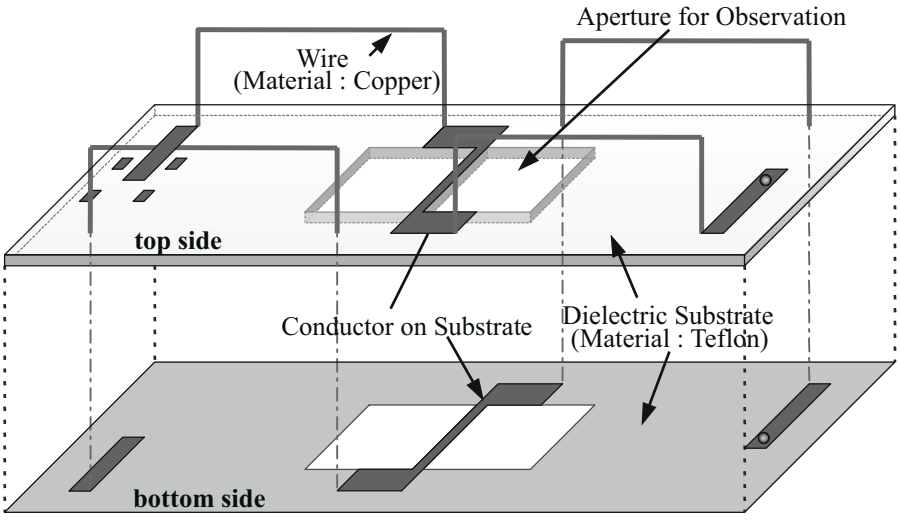

Fig. 9.17. An 8-shaped antenna for highly localized exposure to the brain of rat (Watanabe et al., 2004).

ble to the wider, rectangular cross-section of the TEM cell, while keeping a constant characteristic impedance of 50 Ω. At one side, the RF signal generator is connected as the input. The other side of the TEM cell is matched to a 50 Ω load. The basic field mode is TEM-mode, in which the electric fields direct from the center septum to the outside conductor.

The small animals to be exposed usually are placed above and/or below the septum. The presence of small animals, however, changes the linear polarization of the TEM-mode, changing the direction and amplifying the magnitude of the fields around the subjects. For this reason, the SAR inside a small animal, such as rat, can vary over a range of 6 dB.

9.4 *In vitro* Exposure Systems

In vitro exposure systems usually are designed to have a uniform electric field distribution inside the culture medium. The basic designs are based on (1) TEM cell, (2) waveguide, (3) radial transmission line, or (4) horn antenna. Performance usually is evaluated by the uniformity of the electric field or the SAR inside the culture medium, neglecting the presence of cells. (The influence of the cells on uniformity is not understood).

The culture medium usually is put in a circular Petri dish with horizontal dimensions considerably larger than its vertical dimensions but smaller than the wavelength. Based on the boundary conditions between the culture medium and air, the polarization of the electric field parallel to the culture plane should provide higher

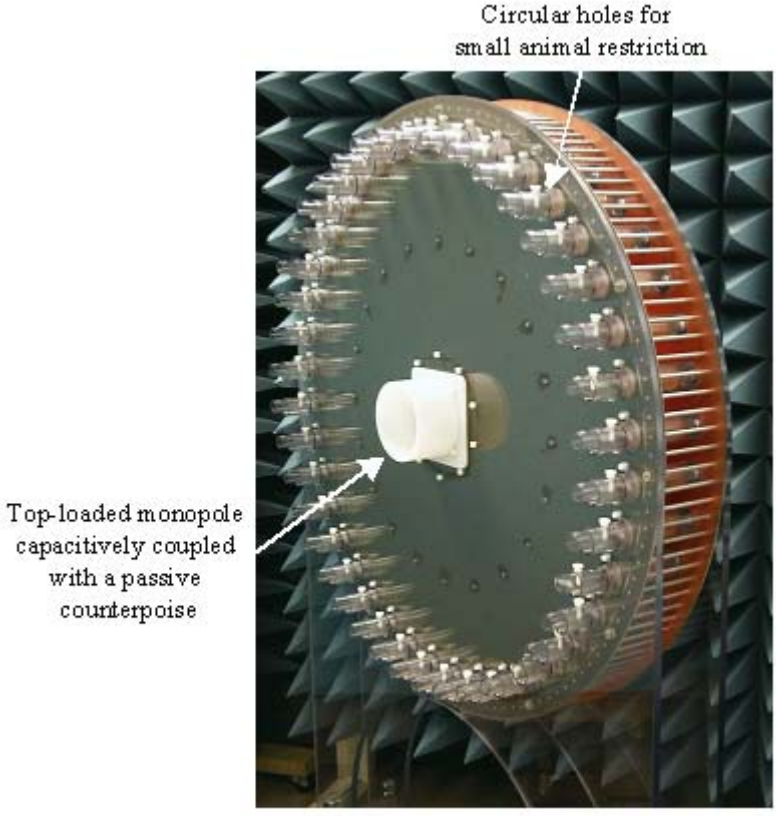

Fig. 9.18. A wheel-like, far-field exposure system designed as resonant waveguide structure (Balzano et al., 2000).

exposure efficiency, because of the continuity of the electric field. In contrast, polarization of the electric field perpendicular to the culture plane should provide lower exposure efficiency, because the relative permittivity is typically about 75 for the culture medium at RF band. The latter polarization, however, could provide an excellent uniformity of field distribution for a cell layer at the bottom of medium, i.e., a standard deviation < 20% for 60 mm Petri dishes.

From these considerations, most systems used for *in vitro* exposure are TEM cells, because they can easily be set to have the electric field perpendicular to the culture plane for obtaining a good uniformity of exposure inside the medium. Because the electric field decreases towards the walls of TEM cells, the Petri dishes should be placed as near the center as possible. TEM cells, therefore, are recommended only for small-scale *in vitro* studies.

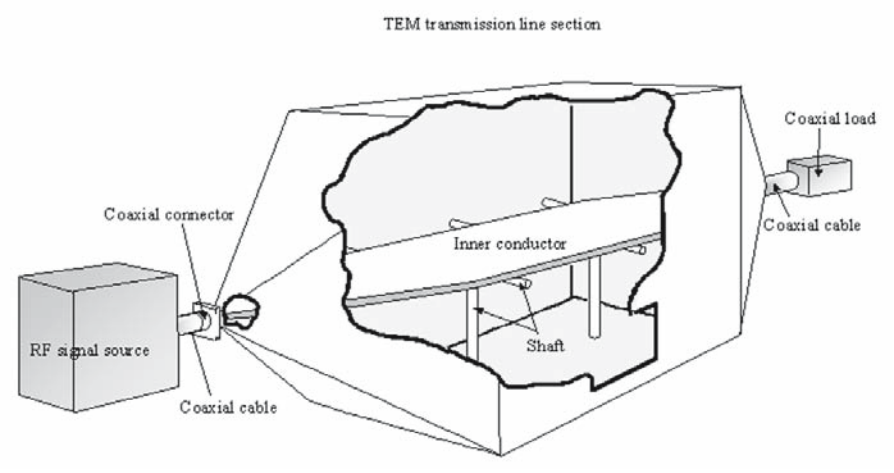

Fig. 9.19. A TEM cell structure for far-field exposure.

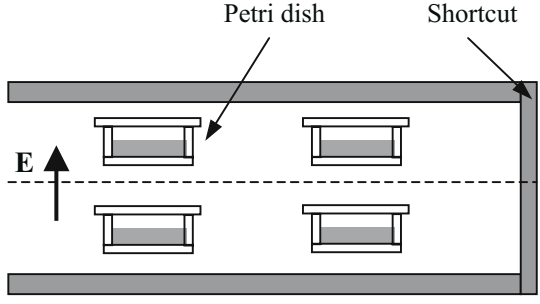

Fig. 9.20. Diagram of a rectangular waveguide structure used for *in vitro* exposure. The shortcut at one end is for increasing the efficiency.

A waveguide structure also is widely used as an *in vitro* exposure system, whose optimization is usually conducted via numerical techniques, such as the FDTD simulation. Figure 9.20 shows an overview of an optimized rectangular waveguide structure in which the basic TE_{10} mode is designed to be the only model propagating at the target frequency (Schonborn et al. 2001). Because the TE_{10} mode has the electric field perpendicular to the culture plane and consequently provides the higher degree of uniformity, but the lower exposure efficiency, the waveguide is shorted at one end to increase the efficiency.

Other choices for *in vitro* exposure include the radial transmission line structure (Moros et al. 1999). It consists of a circular parallel plate applicator, which is driven at its center by a conical antenna, and terminated radically by either mi-

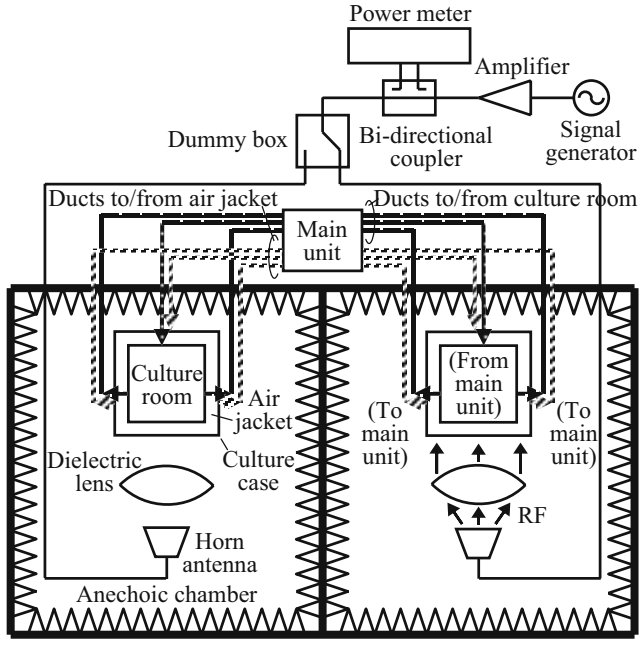

Fig. 9.21. An open-type in vitro exposure system coupling a horn antenna with a dielectric lens (Iyama et al., 2002).

crowave absorbers or a load. Theoretical analysis shows that the fundamental mode in a radial transmission line is the TEM mode. Although higher-order modes can be generated by irregularities and discontinuities, they can not propagate and will be evanescent (quickly decaying exponentially with distance below the cutoff frequency). This structure is appropriate for a large-scale exposure of cells. It also can provide exposure for a wider frequency band.

For a large-scale *in vitro* exposure, an open-type system coupling a horn antenna with a dielectric lens also is effective (Fig. 9.21). Such a system can provide a uniform electric field, within a standard derivation of ±16%, at an area of 30 cm × 30 cm (Iyama et al. 2004). This means that such a system can expose simultaneously 50 Petri dishes, each with a diameter of 4 cm.

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10

Radiofrequency Biology: *In vivo*

Masamichi Kato

When selecting papers to be summarized in this chapter, the goal was to provide a nearly comprehensive review of recent papers. The emphasis is on relatively recent (less than 10, or more typically, less than 5 years old) papers; older work is covered well in many published books and review articles.

10.1 Carcinogenesis

Carcinogenesis is a complex, multistage process. Normal cells develop into tumor cells as a result of genetic changes (initiation stage). Once a cell has been initiated, typically by the accumulation of genetic changes as a result of damage and faulty DNA repair, several kinds of chemicals and physical forces, such as ionizing radiation, can act as "promoter" (or co-promoter) to increase the rate of growth of tumor cells (promotion stage).

La Regina et al. (2003) carried out a study to determine whether chronic exposure to microwave fields from cellular phones increased incidence of spontaneous tumors in F344 rats. Eighty male and 80 female rats were randomly placed into one of three groups. The sham-exposed group received no irradiation; the Frequency Division Multiple Access (FDMA) group was exposed to 835.62 MHz, FDMA-modulated microwaves; and the Code Division Multiple Access (CDMA) group was exposed to 847.74 MHz, CDMA-modulated microwaves. Rats were exposed for 4 h/d, 5 d/w for over 2 yrs. The nominal, time-averaged brain SAR was 1.3 W/kg. There were no differences in final body weights or survival for either males or females in any group. No differences were found between treated and sham-exposed animals for any tumor in any organ. The authors conclude that chronic exposure for up to 2 years to 835.62 MHz (FDMA) or 847.74 MHz (CDMA) microwaves had no effect on the incidence of spontaneous tumors or the initiation stage of carcinogenesis.

Rat liver is the most commonly used experimental model for investigating multistage carcinogenesis in tissues. Imaida et al. (1998a) reported on a medium-term liver bioassay in which near-field exposure of F344 male rats to 900 MHz or to 1.5 GHz electromagnetic fields resulted in slightly decreased numbers and areas of

liver foci positive for glutathione S-transferase, which are pre-neoplastic liver lesions in rats. Imaida et al. (1998b) further completed an experiment in which a 929.2 MHz time division multiple access (TDMA) signal for Personal Digital Cellular (the Japanese cellular telephone standard) system was directed to rats through a quarter-wavelength monopole antenna. Maximum local SARs were 6.6 – 7.2 W/kg within the whole body and 1.7 – 2.0 W/kg within the liver, which was the target organ. Near-field exposure was for 90 min/day, 5 d/w, for 6 wks. The exposure apparatus was specially designed to allow exposure of the lateral, mid-section of the rat body to the electromagnetic field. Male F344 rats, 6 week-old, were "initiated" (at week 0) by a single dose of diethylnitrosamine (DEN). Two weeks later, exposure (n = 48) or sham-exposure (n = 48) was started. At week 3, all rats were subjected to a 2/3 partial hepatectomy, to stimulate growth of liver tissue. At week 8 (after 6 weeks exposure), the experiment was terminated. Carcinogenic potential was scored by comparing the numbers and areas of foci in the livers positive to the induced glutathione S-transferase placental form for the field-exposed and sham-exposed rats. Another group of 24 animals, given only DEN and partial hepatectomy, served as an additional control group. There were no differences between the exposed and sham-control groups. These findings showed that local body exposure has no significant promoting effect on rat liver carcinogenesis, under the experimental conditions tested.

In both experiments (Imaida et al. 1998ab), the daytime serum melatonin concentrations were increased in both 900 MHz and 1.5 GHz exposed groups as compared with sham-exposed control group values. Therefore, changes of serum melatonin levels might have modified the development of pre-neoplastic lesions in the livers of the microwave-exposed rats. Because melatonin is oncostatic, increased melatonin might have inhibited tumor development in microwave-exposed rats. (See section 2.2.2.1.3.)

In order to clarify this question, Imaida et al. (2000) analyzed the effects of different doses of melatonin in the same bioassay system employed for their previously reported EMF exposure studies. Six-week-old male F344 rats were given a single dose of DEN. Starting 2 wks later, they were treated for 6 wks with 0, 1, 5, 10 or 20 ppm melatonin in drinking water. Melatonin was provided only during the night (between 18:00 to 0:00) in order to maintain circadian rhythmicity. At wk 3, all rats were subjected to a two-thirds partial hepatectomy. At wk 8, the experiment was terminated. At this one time point, serum levels of melatonin, adrenocorticotropic hormone (ACTH), corticosterone, lutenizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were measured. Melatonin was elevated; LH and testosterone were reduced. Although clear dose-dependence was not apparent, both numbers and areas of foci positive for the induced glutathione S-transferase placental form in the liver were decreased in the 10 ppm melatonin group. These results suggest that an increase in serum melatonin levels is a possible reason for the associated tendency for decreased pre-neoplastic hepatocyte development in microwave-exposed rats.

Bartsch et al. (2002) studied whether a signal based on the Global System for Mobile communication (GSM) could stimulate development of mammary tumors,

induced by treatment with dimethylbenzanthracene, in female Sprague Dawley (SD) rats. Exposure was at 900 MHz, pulsed at 217 Hz (pulse width 577 μsec) with a relatively low power density (100 μW/cm^2) applied continuously to freely moving animals. The average whole body SARs were 17.5–70 mW/kg. The low-level microwave exposure did not appear to possess carcinogenic or cancer-promoting effects on mammary tumors induced with dimethylbenzanthracene.

The overall result of the three studies (Imaida et al. 1998ab, Bartsch et al. 2002) was that there was no effect of microwave exposure using cell-phone signals on tumor latency and cumulative tumor incidence. These results, obtained using the animal test procedures developed to assess carcinogenicity of chemicals, show that low-level microwave exposure like that associated with mobile or cell phones does not appear to possess carcinogenic or cancer-promoting effect. Performance of a few more such experiments remains warranted. If the results continue to be negative, the answer from animal experiments will be known. However, no matter what the results of the animal experiments, human epidemiology related to the question 'do cell phones cause cancer?' undoubtedly will continue for some time.

The results also suggest that microwave electromagnetic fields might have effects on melatonin. The limited initial data from these very ambitious melatonin studies indicates a beneficial, rather than an adverse effect. Clearly, additional research on microwave (and RF) fields and melatonin is warranted.

10.2 Central Nervous System

Given its critical importance for humans, the effect of exposure to RF energy on the central nervous system (CNS) has been studied extensively. The following sections review experiments assessing brain morphology, the blood-brain barrier (BBB), electroencephalogram (EEG), evoked potentials, behavior, the hippocampus, microwave field detection, and neurotransmitters.

10.2.1 Morphology

Very few investigators have assessed the cellular morphology of brains of animals that had been exposed to microwaves. Perhaps this is because it is a daunting challenge, and perhaps because there is little reason to expect any change when the microwave doses used do not produce excessive heating.

Albert et al. (1981b) studied the effects of either 2.45 GHz (SAR of 2.8 W/kg) or 100 MHz (SAR 2 W/kg) applied to young rats. Then the histological appearance of cerebellar Purkinje cells was assessed. Environmental stress, hypoxia, alcohol or fatigue can easily damage these cells, and glia cells subsequently replace the damaged neurons. Exposure to both frequencies had similar effects, producing irreversible decreases of Purkinje cells in rats irradiated either during fetal or fetal and early postnatal periods. Decreases in the relative number of Purkinje cells were apparent in animals exposed postnatally.

Tsurita et al. (2000) investigated the effects of exposure to 1.439 GHz TDMA (Time division Multiple Access) signals on Purkinje cells in cerebellum of rats. Mature male SD rats were divided into three groups of eight. The rats in the field-exposed group, which had their heads arrayed in a circle near the central antenna of an exposure system, were exposed to microwaves for 1h/day for either 2 or 4 wks. The rats in the sham-exposed group also were placed in the exposure system, but no microwave exposure was given. The cage-control group was neither placed in the system nor exposed. The SAR for the brain was 2 W/kg; the whole body SAR was 0.25 W/kg. No morphological changes were observed in any group. The different outcomes from Albert et al. (1981b) and from Tsurita et al. (2000) might be due to the very different ages of the rats used.

Albert et al. (1981a) exposed pregnant squirrel monkeys to 2.45 GHz microwaves (SAR 3.4 W/kg) for 3 h/d, 5 d/w; post-natal exposure continued until the offspring were 9.5 months of age. They then studied the effects of microwave exposure on the density of Purkinje cells in the cerebellar uvula. In contrast to the rat data (Albert et al. 1981b), there was no effect on the monkey's Purkinje cells. Albert et al. (1981a) point out that differences in the anatomical structures of the head and in the exposure conditions (free field vs. multipath) could have contributed to the difference in results. Also, 2.45 GHz might be closer to resonant frequency of the rat than the squirrel monkey, and the depth of penetration of RF energy can be important. Further the structure of the brain in each species presents a different exposure configuration. In the rat, the cerebellum is exposed in the dorsal and posterior aspect and is covered by a thin calvaria. In the squirrel monkey, the cerebellum is overlapped by the occipital lobes, a thicker calvaria, and thick muscles in the cervical region (Albert et al. 1981a).

Based on these three studies, it is impossible to know whether or not microwave exposure can affect the microscopic anatomy of regions of the brain. However, based on the relatively low energy levels and the presumed lack of tissue heating associated with them, it is unlikely that environmental exposures produce frank brain pathology. Presumably experiments associated with ongoing research programs intended to address the safety of cell phones will produce a few more studies relevant to this specific question.

10.2.2 Blood-brain barrier

The blood-brain-barrier (BBB) maintains the homeostatic environment of the brain by regulating the entry of vital substances and nutrients into brain and the expelling of carbon dioxide and metabolic waste products out of the brain. This barrier protects the brain from foreign toxic substances but allows passage of the molecules that are necessary for metabolism.

Brain capillaries are composed of endothelial cells, pericytes, that have smooth muscle-like properties and reside adjacent to capillaries, and of astroglial processes that ensheath more than 95% of the capillary surface (Fig 10.1). Among these structures, the endothelial cells are the principal anatomic site of the BBB. The endothelial

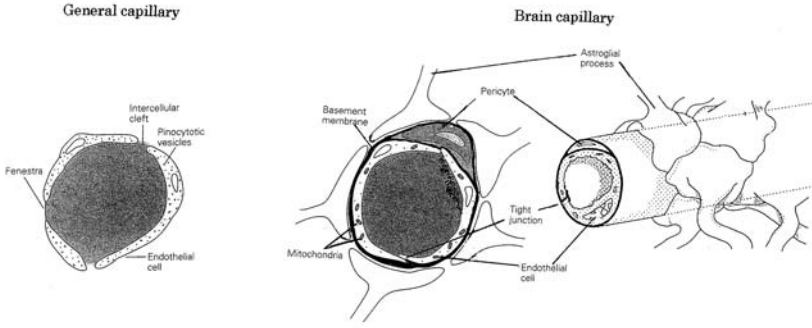

Fig. 10.1. Anatomical features of general capillaries and of brain capillaries. General capillaries have inter-endothelial clefts, fenestrae, and prominent pinocytotic vesicles. These features allow relatively non-selective diffusion across the capillary wall. Endothelial cells of the brain capillary contain an increased number of mitochondria to support energy-dependent transport systems and are inter-connected by complex, inter-endothelial "tight" junctions. These anatomical features, in conjunction with specific transport systems, result in highly selective transport of water-soluble compounds across the barrier endothelium. Astrocyte processes surround more than 95% of the brain capillaries. Functionally, this results in what is called the blood-brain barrier.

cells of the BBB are interconnected by complex arrays of "tight" junctions. These junctions block diffusion across the capillary wall.

In capillaries of other organs, and in the relatively few brain capillaries that do not form a barrier, blood-borne, polar molecules diffuse passively across vessels through "spaces" between endothelial cells, i.e., through specialized cytoplasmic fenestrations.

Normal brain function requires a large number of compounds that must be able to cross brain capillaries. Entry into the cerebrospinal fluid is achieved primarily in three ways: (1) by diffusion of lipid-soluble substances, (2) by energy-dependent, receptor-mediated transport of specific, water-soluble substances, and (3) by ion channels.

Most substances that must cross the BBB are not lipid soluble and therefore cross by specific carrier-mediated transport system. Glucose is transported through the Glut 1 system, which is not energy dependent. The net flux of glucose is driven by the relatively higher concentration of glucose in plasma. Amino acids are transported across barrier endothelial cells primarily by three distinct carrier systems: the L, and ASC systems. For further study, the readers are advised to consult with textbook of neuroscience (e.g., Kandel et al 2000)

In many diseases the BBB does not function effectively and substances that are normally excluded enter the brain. A number of causes – such as inflammation of

brain membrane (i.e., meningitis), edema, anoxia, hypertension, and ionizing radiation – have been shown to induce BBB changes, often increasing permeability of substances to the brain. Radiotracer, immunohistochemistry, or the classical staining method by Evans blue often are used to reveal permeability changes. With a functional BBB, Evans blue does not enter the brain. Thus, when areas of brain are stained blue, it indicates a breakdown of the BBB in those areas.

The existence of a microwave-induced BBB permeability increase was controversial for many years. Now most researchers believe that the permeability change is associated with an increase in temperature, and no permeability change is observed with athermal microwave exposure.

Ikeda et al. (1994) used 8 MHz RF exposure to investigate the distributions of temperature changes produced by interstitial hyperthermia in agar phantoms and in brains of dogs. The heating limits of normal dog brains were 42°C for 45 min or 43°C for 15 min; breakdown of the BBB, assessed using Evans blue, was observed with heating at 43°C for 60 min.

Ohmoto et al. (1996) used rats to investigate the temperature distribution, early histological changes, BBB disruption, and sequential changes in cerebral blood flow (CBF) following hyperthermia, ranging from 37 to 45°C, produced by RF-induced, localized cerebral hyperthermia. Histological changes and BBB disruption were observed in brain regions heated to 43°C and above.

Fritze et al. (1997) investigated the effects of GSM microwave exposure on the permeability of the BBB. Rats were restrained in a carousel of circularly arranged plastic tubes and sham-exposed or microwave irradiated at 900 MHz for 4 h at SARs of 0.3, 1.5 or 7.5 W/kg. The extravasation of protein was assessed, either at the end of exposure or 7 days later, by immunohistochemistry staining of serum albumin. An increase in serum albumin extravasation after microwave exposure was observed only in the group exposed to the highest SAR of 7.5 W/kg and the extravasation was present only immediately after exposure. Histological injury was not observed in any of the examined brains. The observed albumin extravasations were very modest and, moreover, were totally reversible.

Finnie et al. (2001) studied the effect of short-term exposure to GSM microwaves on vascular permeability in the brain. Mice (n = 30) were given a single, far-field, whole- body exposure at 898.4 MHz for 60 min at a SAR of 4 W/kg. Control mice were either sham-exposed (n = 10) or permitted free movement in a cage (n = 10) to exclude any stress-related effects. Vascular permeability changes were detected using albumin immunohistochemistry. No differences among groups were detected. Finnie et al. (2004) further studied the effect of long-term exposure to GSM fields at 900 MHz. Mice were given a 60 min, far-field, whole-body exposure 5 dy/wk for 104 wks at SARs of 0.25, 1.0, 2.0, or 4.0 W/kg. Albumin staining was used to detect increased permeability. The results suggest that prolonged exposure to mobile telephone-type radiation produced negligible disruption to BBB integrity under these experimental conditions.

Tsurita et al. (2000) investigated the effects of athermal exposure to 1.439 GHz modulated by the TDMA protocol on the permeability of the BBB. Adult male SD rats were assigned to one of three groups of eight rats. The rats in the microwave-

exposed group were exposed 1 hr/dy. The rats in the sham-exposed group were placed in the exposure system without microwave delivery. The animals in the cage control group were not placed in the exposure system. The exposure period was 2 or 4 wks. The SAR was 2 W/kg in the brain, and the whole body SAR was 0.25 W/kg. Core body temperature was measured; no increase in body temperature was detected. Changes in the BBB were sought using Evans blue injection and immunostaining of serum albumin. Microwave exposure had no effect on the BBB.

Contrary to the negative findings of these five papers, two other groups have reported that non-thermal microwave irradiation did cause BBB permeability changes. Salford et al. (1994) reported that weak, non-thermal level, pulsed microwaves give rise to a significant leakage of albumin through the BBB. Salford et al. (2003) further investigated whether a pathologic leakage across the BBB might be accompanied by neuronal damage. Three groups each of eight rats were exposed for 2 hr to a GSM microwave field at whole-body SARs of 2, 20, and 200 mW/kg, all well below the level expected to produce temperature elevation. The authors found a positive relationship between SAR and number of "dark" neurons, evidence for neuronal damage, in the cortex, hippocampus, and basal ganglia of exposed rats. The authors claim that they presented, for the first time, evidence for neuronal damage caused by a nonthermal microwave exposure.

Leszczynski et al. (2002) examined whether athermal exposures of cultures of a human endothelial cell line to 900 MHz GSM radiation could activate a cellular thermal stress response. Non-thermal ($37 \pm 0.3°C$), 1 hr microwave exposure caused a transient increase in phosphorylation of heat shock protein-27. The authors hypothesized that 900 MHz GSM radiation induced activation of heat shock protein-27 to cause an increase in BBB permeability through stabilization of endothelial cell stress fibers.

Efforts to reproduce the results of Salford et al. were reported by four groups at the 2005 annual meeting of the Bioelectromagnetics Society. McQuade et al. (2005) and Haro et al. (2005) examined albumin leakage and degenerative neurons, which were originally reported by Salford's group. Both parties found no differences between the microwave-exposed and sham-control groups. Two other groups also assessed other measures in addition to albumin leakage. Shirai et al. (2005) examined alteration of BBB-related genes, such as p-glycoprotein, aquaporin-4, and claudin-5, and Masuda et al. (2005) studied microcirculation. Neither group found any differences between the irradiated and the control animals.

Combined effects of microwave irradiation and other agent(s) have been reported by two groups.

Neilly and Lin (1986) studied the combined effects of ethanol and microwaves on the BBB in male Wister rats. Anesthetized rats, each with an implanted venous cannula, were infused with 0.1, 0.3, 0.5 or 0.7 gm/kg of absolute ethanol. A control group was given 0.7 g/kg of isotonic saline. The left hemisphere of each brain was irradiated by 3.15 GHz microwave energy at 3.0 W/cm^2(rms) for 15 min. The rectal temperatures remained at 37.0°C. Immediately after irradiation, Evans blue dye was injected through the cannula. The results showed that as the quantity of alcohol was increased, the degree of staining was decreased or eliminated. The temperature of the

irradiated area of the brain increased for the first 4 to 5 min of irradiation and then stabilized for the remainder of the irradiation period. The steady-state temperature was highest in animals receiving saline or the smallest dose of alcohol. As the quantity of alcohol was increased, the steady-state temperature was reduced. These results indicate that ethanol inhibits microwave-induced permeation of the BBB through reduced heating of the brain.

There is another report which investigated combined effect of microwave exposure and virus infection. The expression of Japanese Encephalitis Virus (JEV) lethality in mice requires entry of the virus into the CNS. This entry is presumably through the capillary endothelial cells, because entry between these cells is inhibited by bands of circumferential tight-junctions. A viremic stage occurs during the first 4 to 5 days after virus administration in mice. Lange and Sedmak (1991) assessed how both microwave radiation (2.45 GHz, continuous wave, 10 min exposure) and hypercarbia (CO_2 exposure) affected capillary endothelial cells permeability to JEV in adult Swiss-Cox mice. Exposure to microwaves at very high SARS of approximately 24–98 W/kg resulted in a dose-dependent increase in JEV-induced lethality. Similarly, hypercarbia produced by exposure to 5, 10, and 20% CO_2 was observed to produce a dose-dependent increase in virus-induced lethality. Both microwave radiation and hypercarbia are thought to promote pinocytosis within capillary endothelial cells of the CNS. This may be one mechanism by which they enhance JEV-induced lethality in adult Swiss-Cox mice.

Historically, a reasonably sized set of papers has addressed the possibility that microwave exposure can diminish the effectiveness of the BBB. The pattern of results is clear. If microwave exposure produces hyperthermia, BBB integrity is diminished. If the microwave exposures used do not produce hyperthermia in the brain, i.e., if they are athermal, there is no effect.

The Salford saga appears to be a recapitulation of a process that has occurred repeatedly over the past four decades. Some group claims an "athermal" effect. Because this would be important, if true, other investigators rapidly attempt to replicate the claim. Invariably, the alleged athermal effect is not substantiated.

10.2.3 Electroencephalogram

The human EEG changes according to a 24 h circadian rhythm of behavior in response to the 24 h astronomical cycle. Sleep states lasting approximately 8 h during night form the unconscious part of that cycle. A basic principle of sleep cycle control in human has been articulated by Borbely (2001) as a "Two Process Model," in which sleep-wake state transitions result from the combined effects of circadian factors and homeostatic factors. During sleep, a third regulator, the ultradian, REM-NREM oscillator comes into play. In terms of EEG, sleep comes in two forms, rapid eye movement (REM) – when dreaming occurs – and non-REM (NREM).

10.2.3.1 Animal studies

Johnson and Guy (1972) demonstrated thermographically that metal electrodes in a cat brain increased the local SAR by 50 times. Therefore the use of metallic elec-

trodes for EEG recordings made most early results questionable. Glass electrodes filled with Ringers solution (Johnson and Guy 1972) or carbon-loaded Teflon electrodes with conductivity close to that of tissue have been used to minimize field perturbation (Chou and Guy 1979). Even with minimally field perturbing electrodes, EEG electrodes can pick up RF fields and induce current into the head, making it difficult to differentiate between the direct effect of the RF field and an effect of the induced currents in acute experiments.

Takashima et al. (1979) reported on the effects of modulated RF fields (1–30 MHz, 15 or 60 Hz modulation) on the EEGs of male rabbits following acute (2–3 h) and chronic (2 h/d for 4–6 weeks) exposures. Although acute exposure up to 500 V/m did not cause effects, chronic exposure above 90 V/m enhanced the low frequency components of the EEG and decreased high frequency activities. The acute study showed that metal electrodes caused artifacts during recording. However, the effects of chronic exposure were not due to the presence of electrodes, because electrodes were not present during RF exposure.

The effect of 40 mW/cm^2, 2.4 GHz exposure for 1 min on EEG of rabbits was described by Chizhenkova (1988). Exposure of the head increased the number of slow waves and spindle-shaped firings in the EEG. It also changed the discharge frequency of neurons in the visual cortex, producing an enhancement of the evoked response of visual cortex neurons to a light stimulus.

Thuroczy et al. (1994) reported that the total power of EEG spectra increased in rats after whole-body 2.45 GHz, continuous wave microwave exposure (30 mW/cm^2) for 10 min; changes occurred at 10 mW/cm^2. The cerebral blood flow (CBF) increased after 10 mW/cm^2. The power of EEG δ waves (0.5 – 4 Hz) was increased by thermal level of brain localized 4 GHz (continuous wave) exposure at 42 mW/g with simultaneously increase of the CBF. Vorobyov et al. (1997) analyzed average EEG frequency spectra in eight unanesthetized adult rats with chronically implanted carbon electrodes in symmetrical somesthetic areas. Microwaves of 945 MHz, at 0.1 – 0.2 mW/cm^2, amplitude modulated at 4 Hz were applied for 1 min on and 1 min off during 10 min sessions. There were no differences, other than an elevation of EEG asymmetry in the 10 – 14 Hz range observed during the first 20 sec after onset of the exposure.

In summary, it appears that ELF-modulated microwave fields produced changes in EEG patterns by enhancing the low-frequency components and decreasing high-frequency activities.

10.2.3.2 Human studies during waking state

Hietanen et al. (2000) examined the possible influence of microwave radiation on human brain function by recording EEG activity of 19 volunteers. The sources of exposure were five different cellular phones (analogue and digital models) operating at a frequency of 900 MHz or 1.80 GHz. The EEG was recorded in an awake, closed-eyes situation. Six 30 min sessions, including 1 sham exposure, were completed for each subject. The duration of the real exposure phase was 20 minutes. Exposure to

the microwave fields emitted by cellular phones had no abnormal effects on human EEG.

Kramarenko and Tan (2003) recorded EEG changes during exposure of human head mobile phone emissions. The spatial distribution of the electromagnetic field was concentrated around the ipsilateral eye adjacent to the basal surface of the brain. Slow-wave activity (2.5–6.0 Hz) appeared in the contralateral frontal and temporal areas; the activity lasted for about 1 sec and reappeared every 15–20 sec. After turning off the mobile phone, the slow-wave activity disappeared 15–20 min later. The authors interpreted these results as indicating that cellular phones might reversibly influence human brain function by inducing abnormal slow waves in the EEG of awake persons.

Krause et al. (2000a: 2000b) studied the effects of the 902 MHz microwave field emitted by one model of a cell phone on the event-related desynchronization and synchronization of the 4–6 Hz, 6–8 Hz, 8–10 Hz, and 10–12 Hz EEG frequency bands of human subjects performing either (1) an auditory memory task (Krause et al. 2000a) or (2) a visual sequential letter task with three different working memory load conditions (Krause et al. 2000b). All subjects performed the memory task both with and without exposure to EMF in counter-balanced order. The microwave exposure increased EEG power in the 8–10 Hz frequency, only when examined as a function of memory load. However, in their own replication studies, which were conducted under a double-blind procedure, Krause et al. (2004) were not able to replicate the positive finding from their own earlier studies. They mentioned that microwave-exposure effects on the EEG and on the performance on memory tasks might be variable and not easily replicable, for reasons yet to be clarified.

10.2.3.3 Human studies during sleep

Studies of sleep EEG by Mann et al. (1998a) and Wagner et al. (2000) showed no effects were produced by irradiation with a 900 MHz microwave field, pulsed at 217 Hz and with an average power density of 0.02 mW/cm^2.

During an entire night-time sleep episode, Borbely et al. (1999) exposed subjects to 900 MHz, at a maximum SAR of 1 W/kg, using an intermittent schedule consisting of alternating 15 min on and 15 min off intervals. Spectral power of the EEG increased in the 10–11 Hz and 13.5–14 Hz bands during the initial part of NREM sleep and then subsided.

Huber et al. (2002) reported that pulse-modulated, 900 MHz electromagnetic field exposure increased relative CBF (rCBF) in the dorsolateral prefrontal cortex ipsilateral to exposure. This microwave exposure also enhanced EEG power in the alpha frequency range prior to sleep onset and in the spindle frequency range during stage-2 sleep, although the effects were subtle. Huber et al. (2003) extended the analysis. Unilateral exposure during waking induced a similar effect in both hemispheres. Microwave exposure during sleep reduced waking after sleep onset and affected heart rate variability. Huber et al. (2005) observed an increase in relative rCBF in the dorsolateral prefrontal cortex on the side of exposure (ipsilateral) after 30 min of unilateral head exposure to pulse-modulated, 900 MHz microwaves EMF. Two

types of EMF exposure were applied: a 'base-station-like' and a 'handset-like' signal (SAR of 1 W/kg for both conditions). The effect depended on the special power in the amplitude modulation of the RF carrier such that only 'handset-like' exposure, with its stronger low-frequency components, but not the 'base-station-like' exposure affected rCBF. The authors stated that pulse modulation of cell phone signals is necessary to induce changes in the EEG, both waking and sleeping.

Overall, outcomes of the various human studies have been inconsistent, and comparison between individual studies is difficult. Enhanced power in the alpha band was observed in both human and some animal studies. Effects reported on sleep EEG are more likely to involve NREM alpha waves, compared to other bands, as they do in these EEG experiments on young human subjects.

10.2.3.4 Preparatory potentials

Motor actions often are self-initiated without an outside cue. Almost 1 second before a self-initiated volitional movement begins, a characteristic negative shift in cortical potentials is seen in the EEG record of medial premotor areas, where the supplementary motor area is located. This slow negative potential, referred to as the preparatory potential, signals the planning that occurs before movement is executed. The region responsible for this negative potential was localized more precisely in a study comparing increase in rCBF during simple, complex, and imagined sequences of finger movements. Complex movement sequences require more planning than do simple repetitive movements. Imagining complex movements might require the same amount of planning as real movements (Krakauer and Ghez 2000).

Freude et al. (1998) searched for effects on preparatory potentials during exposure to cell phone emissions. In the first experiment, healthy male human subjects performed simple, self-paced finger movements to elicit a preparatory potential. In the second experiment, they performed a complex and cognitively demanding visual monitoring task. Both tasks were performed with and without microwave exposure, in counter-balanced order. Exposure produced a significant decrease of preparatory potentials at central and temporo-parieto-occipital brain regions while performing the visual monitoring task. No effect on the slow potentials was seen in the simple finger movement task.

Freude et al. (2000) studied 20 healthy male subjects who were exposed to modulated 916.2 MHz microwaves (217 Hz pulse frequency, pulse width 577 μsec; 2.8 W/kg SAR) emitted near the left ear. Two experiments were completed, about 6 months apart. In the first experiment, microwave exposure produced a decrease of preparatory potential while performing a complex visual monitoring task. This effect was replicated in the second experiment. Exposure effects on preparatory potentials were analyzed further with two, less demanding tasks: a simple finger-movement task and a two-stimulus task eliciting a contingent negative variation (CNV) response. CNV was explained in Chapter 2. In comparison to the complex visual monitoring task, no effects were found with the less demanding tasks. The results suggested a selective microwave-exposure effect on particular aspects of human information processing but did not indicate any influence on human performance.

10.2.3.5 Event-related magnetic fields

Hinrichs and Heinze (2004) investigated potential effects of GSM 1800 on verbal memory encoding by recording event-related magnetic fields from the brain during subsequent memory retrieval. After encoding words from a study list presented in the first phase, the 12 subjects had to discriminate old from new words mixed together in a test list. Subjects completed two experimental sessions, one with microwave exposure during the study phase, and one without. Field exposure produced changes an early (350–400 msec), task-specific component of the event-related magnetic field. The authors suggest field exposure interfered with item encoding, although behavioral measures were not affected.

10.2.3.6 Summary

The literature on possible microwave exposure effects on the EEG is not small, and it suggests effects can be measured. Unfortunately, EEG has proven to be of limited utility as a general neurotoxicology screening measure, and the problem of suitable electrodes for recording during RF exposure is an added special difficulty. The literature does suggest that a scattering of EEG changes can be measured as a consequence of microwave exposure. It would take a considerable amount of additional research to build this hint into useful knowledge.

Exposure can produce subtle effects on human EEG. However, there is little agreement on either what mental processes are affected or the meaning of the changes reported. Most studies test of cell phone signals and human cognitive performance used only one dose, had poor dosimetry, inadequate control groups, and poor traceability. Possible interference and reflection of microwaves should be assessed in exposure setups. Boredom and heat can induce sleep, and sleep-related changes in the EEG could account for positive findings. As a result no conclusions can be drawn from the presently available research on possible effects of microwave exposure and EEG.

10.2.4 Cognitive function

In both humans and animals, microwave fields are suspected of being able to affect cognitive functions. More specifically, several studies performed in rodents have suggested that spatial learning can be impaired by electromagnetic field exposure.

A major effect of exposure to RF frequencies above 100 kHz is heating. Microwave heating is known to affect memory and learning (Saunders et al. 1991). At 2 GHz, because of the shorter wavelength, exposure is mostly superficial; the RF energy is absorbed by skin rather than the deeper tissues, as it is with the lower frequencies (longer wave lengths) of 900 and 450 MHz (Adair et al. 1999: 2001a). These frequencies are used by current mobile communication systems. Threshold SARs were estimated to be 2.5 W/kg at 225 MHz (near body resonance, for the human) and 4–5 W/kg at the higher frequencies. However, colonic temperature rose in all cases by about 1°C. The lowest SAR threshold for learning effects was found

during exposure to more deeply penetrating fields at lower frequencies, such as 225 MHz (Saunders et al. 1991). Resonant frequencies for humans and animals differ, because of their different body sizes.

10.2.4.1 Cognitive studies with animals

Referring to body temperature changes, Yamaguchi et al. (2003) studied two behavioral tasks, using a T-maze for the assessment of memory: Subjects first performed a spatial discrimination task, being rewarded on one side in the training session; they then performed on a reversal task, being rewarded on the other side in the test session. Reversal discrimination is described by a 3-stage model. In the first stage, after the reward location has been reversed, the undoing of old habits is required. The second stage is the period when the animal responds by chance. In the third stage, the new habit is acquired. Working memory is required in the first stage, where the rats have to remember which of the two baited arms contains the food reward, whereas reference memory is involved in the following stage. SD rats were exposed daily for 1 h for either 4 days or 4 weeks. Exposure consisted of a pulsed (TDMA) 1439 MHz microwave field in a carousel-type exposure system; two different SARs were used. In one group, the SAR at the brain was 7.7 W/kg, and the whole-body SAR was 1.7 W/kg, which did not cause an increase in core body (intraperitoneal) temperature. Other subjects were exposed at the brain average SAR of 25 W/kg and the whole body average SAR of 5.7 W/kg for 45 min daily for 4 days. In this group intraperitoneal temperature began to rise soon after the beginning of exposure and rose by about 2°C within 60 minutes. The rats with a brain SAR of 25 W/kg for 4 days showed decreases in the transition in number of correct choices in the reversal task, compared to cage-control or sham-exposed subjects. However, rats exposed with a brain SAR of 7.5 W/kg, for either 4 days or for 4 weeks, showed no impairment of T-maze performance. These results suggest that the exposure to a TDMA microwave field at levels about 4-fold stronger than emitted by cellular phones does not affect the learning and memory processes when there are no thermal effects.

Wang and Lai (2000) reported that the exposure to pulsed 2.45 GHz with a whole body SAR of 1.2 W/kg for 1 h daily for 6 days caused deficits in spatial reference memory in rats using a Morris water-maze. Microwave-exposed rats were slower than sham-exposed and cage-control rats in initial learning to locate the platform. However, there was no difference in swim speed among the three groups of animals, indicating that the difference in learning was not due to a change in motor functions or motivation. During the probe trial, microwave-exposed animals spent less time in the quadrant that had contained the platform. Also, their swim patterns were different from those of the sham-exposed and cage-control animals. These observations indicated that microwave-exposed rats used a different strategy in learning the new location of the platform. The authors indicate that acute exposure to pulsed microwaves caused a deficit in spatial "reference" memory in the rat.

Lai (2004) investigated the effect of a temporally incoherent magnetic field ('noise') on microwave-induced spatial learning deficit in the rat. Rats were trained

in six sessions to locate a submerged platform in a circular water-maze. Four treatment groups of rats were studied: (1) microwave-exposure (2.45 GHz, continuous wave; whole-body SAR 1.2 W/kg), (2) magnetic field 'noise' exposure (60 mG), (3) microwave + magnetic field exposure, and (4) sham exposure. Animals were exposed to these conditions for 1 h immediately before each training session. One hour after the last training session, animals were tested in a 2 min probe trial in the maze, during which the platform was removed. The time spent the quadrant of the maze in which the platform had been located was scored. Results show that microwave-exposed rats (1) had a deficit in learning to locate the submerged platform, when compared with performance of the sham-exposed animals. Exposure to magnetic field noise alone (2) did not affect the performance of the animals. However, simultaneous exposure to magnetic field noise (3) attenuated the microwave-induced spatial learning deficit. Thus, simultaneous exposure to a temporally incoherent magnetic field attenuated microwave-induced spatial learning and memory deficits in the rat.

However, there are several reports which found no effects of microwave exposure on learning and memory tasks.

Sienkiewicz et al. (2000) studied the effect of repeated, acute exposures to a low-intensity 900 MHz microwave field pulsed at 217 Hz on a spatial learning and working memory task. Adult male C57BL/6J mice were exposed under far-field conditions in a GTEM cell for 45 min each day for 10 days at a SAR of 0.05 W/kg. Their performance in an 8-arm radial maze was compared to that of sham-exposed control animals. Animals were tested in the maze immediately following exposure or after delays of 15 or 30 min. No field-dependent effects on performance were observed in choice accuracy or in total times to complete the task.

Dubreuil et al. (2002) used a head-only exposure system emitting 900-MHz GSM microwaves, pulsed at 217 Hz, to expose rats for 45 min with SARs of either 1.0 or 3.5 W/kg. Two behavioral tasks were employed to demonstrate performance deficits in spatial learning after microwave exposure: an 8-arm radial maze and a spatial navigation task in an open-field arena, i.e., another version of the Morris water-maze. There were no differences in performance on the two spatial learning tasks among microwave-exposed, sham-exposed, and cage-control rats. Dubreuil et al. (2003) extended their study by using a more complex spatial learning task and a non-spatial object recognition task. Altogether, this set of experiments provides no evidence indicating that spatial or non-spatial memory can be affected by a 45 min, head-only exposure to a 900 MHz (GSM) microwave field.

Cobb et al. (2004) examined the possibility of changes in 'working memory' (Baddeley 1986) of rats following whole body exposure to microwave radiation. During each of 10 days, rats were exposed within circularly polarized waveguides for 45 min to pulsed (2 μsec pulses, 500 pps) 2.45 GHz fields at whole body SAR of 0.6 W/kg, followed by testing in a 12-arm radial maze. Rats received a pre-exposure injection of one of three psychoactive compounds or saline, to determine whether the drugs would interact with microwave exposure to affect maze performance. There was no evidence that exposure to pulsed 2.45 GHz microwaves caused decrements in the ability of rats to learn the spatial memory task.

Cosquer et al. (2005) studied whether whole-body exposure to 2.45 GHz electromagnetic fields affected anxiety responses of rats in a plus maze Rats were exposed for 45 min to pulsed 2.45 GHz microwaves (2 μsec pulse width, 500 pps) with a whole-body SAR of 0.6 W/kg and brain SAR of 0.9 W/kg. Exposure failed to induce any effects on anxiety responses.

D'Andrea et al. (1989) studied the effect of very high peak-power microwave pulses in the absence of whole-body heating on a time-related behavioral task. Five rhesus monkeys, *Macaca mulatta*, were exposed to peak-power densities of 131.8 W/cm^2(rms) while performing a time-related behavioral task. The task was composed of a multiple schedule of reinforcement consisting of three distinct behavioral components: inter-response time, time discrimination, and fixed interval. Trained monkeys performed the task during exposure to 1.3 GHz pulses at low pulse-repetition rates (2–32 Hz). No changes in performance were observed during field-exposure compared to sham-exposure sessions. These might be species difference between rodents and monkeys.

It is difficult to make broad generalizations about the presence or absence of microwave-exposure effects on learning and memory in animals. There are many studies reporting positive effects, but there are just as many reporting absence of effects. When attempting to compare and contrast experiments, the many differences in task, subject size, microwave exposure conditions, etc. prevent closure and produce frustration. Science would be better served if investigators stopped independently shot gunning experiments, (looking for an effect, any effect) and focused on key questions, comparing hypotheses with single experiments and conducting replicative experiments across laboratories.

10.2.4.2 Cognitive studies with humans

Koivisto et al. (2000a: 2000b) examined possible influences of a 902 MHz microwaves on cognitive function of healthy human subjects. Microwave exposure shortened response times in simple reaction time and vigilance tasks, and time to complete a mental arithmetic task was decreased (Koivisto et al. 2000a). Also, microwave exposure speeded up response times when the memory load was three items or more but had no effects with lower loads (Koivisto et al. 2000b). These results indicate that microwave exposure with a signal like that of a cell phone actually can improve human cognitive performance. However, in their own replication study, which was conducted with improved methodology including multi-center testing and a double-blind design, researchers from the same laboratory (Haarala et al. 2004) could not replicate their previous results. The authors argue that the inability to replicate previous findings could have been caused either (1) by the lack of actual effects, or (2) the magnitude of effects being at the sensitivity threshold the tests used.

Maier et al. (2004) examined effects of exposure to pulsed electromagnetic fields (GSM standard) on cognitive performance of humans by using a psychophysiological test paradigm. Eleven subjects performed an auditory discrimination task. Following a first test cycle, the volunteers relaxed for 50 min while being either field-exposed or sham-exposed. Subsequently, the test was repeated. Data acquired

before and after the resting phase were compared for both experimental conditions. Nine of the 11 test participants (81.8%) showed worse auditory discrimination performance after microwave exposure, as compared with the sham-control condition.

10.2.4.3 Human attention

Two recent papers reported facilitating effects on human attention from exposure to the microwave fields used by mobile phones.

Edelstyn and Oldershaw (2002) investigated the effects of acute mobile phone exposure on a range of tasks assessing capacity and processing speed of the attentional system. Cognitive performance was assessed at three points (prior to mobile phone exposure, and at 15 and 30 min post-exposure) using six cognitive neuropsychological tests (digit span and spatial span forwards and backwards, serial subtraction and verbal fluency). Thirty-eight subjects were assigned randomly to either an experimental group, which was exposed for 30 min to the microwaves emitted by a 900 MHz mobile phone, or to a control group in which the mobile phone was switched off. Subjects remained blind to mobile phone status throughout duration of study. Differences between the two groups were evident after 5 min on two tests of capacity (digit span forwards and spatial span backwards) and one of processing speed (serial subtraction). In all three instances, performance was facilitated following mobile phone exposure. No deficits were evident.

Lee et al. (2003) studied the relationship between the facilitating effect and the duration of exposure. Seventy-eight university students were assigned randomly to either an experimental or a control group, and then performance on the administered attention tasks was compared. Participants in the experimental group performed better on one of the two measures of attention only after they had been exposed to the electromagnetic field emitted.

In summary, the results of recent studies of cell phone effect on human cognitive performance suggest that attentional functions can be enhanced by exposure to the low-intensity microwaves emitted by mobile phones. The noting of any bioeffect is of interest, given the small amount of energy involved. The discovery of improved performance is an ironic development in a research program that is focused on hazard assessment. Note also that other research suggests cell phone use while driving is adverse, apparently because it distracts drivers from the driving task. These laboratory tasks do not duplicate all aspects of the real world situation.

10.2.5 Hippocampal slice preparation

In order to obtain some clues to help elucidate the mechanisms of microwave irradiation on the CNS, two groups have carried out experiments on hippocampal slices. The hippocampal slice preparation, in which a portion of the hippocampus is placed *in vitro* and an input pathway is stimulated and activity in an output pathway is recorded has been used extensively to study the electrophysiology and neuropharmacology of processes related to learning and memory. One classic approach is to

study long-term potentiation (LTP). The amplitude of the response gets bigger as after some initial input stimulation is applied, and the change persists over time.

Tattersall et al. (2001) reported the possibility that low-intensity microwave fields can have effects on hippocampal tissues *in vitro*. Slices of rat hippocampus were exposed to 700 MHz, continuous wave (25.2–71.0 V/m, 5–15 min exposure) microwaves in a stripline waveguide. At low field intensities, the predominant effect on the electrically evoked field potential in CA1 was a potentiation of the amplitude of the population spike by up to 20%. However, higher intensity fields could produce either increases (up to 120%) or decrease (up to 80%). To eliminate the possibility of microwave-induced artifacts associated with the use of a metal stimulating electrode, the effect of microwave exposure on spontaneous epileptiform activity induced in CA3 by 4-aminopyridine (50–100 μM) also was investigated (Tattersall et al. 2001). Exposure to a microwave field (50 V/m) reduced or abolished epileptiform bursting in 36% of the slices tested. The maximum field intensity used in these experiments, 71.0 V/m, was calculated to produce a SAR of between 0.0016 and 0.0044 W/kg in the slices. Measurements with a Luxtron fiberoptic probe confirmed that there was no detectable temperature change (+/−0.1°C) during a 15 min exposure to this field intensity. Furthermore, imposed temperature changes of up to 1°C failed to mimic the effects of RF exposure. The authors claim that low- intensity microwaves can modulate the excitability of hippocampal tissues *in vitro* in the absence of gross thermal effects.

Pakhomov et al. (2003) studied effects of short, extremely high-power microwave pulses on neuronal network function using electro-physiological techniques in the rat hippocampal slice model. Population spikes in the CA1 area were evoked by repeated stimulation (1 per 30 sec) of the Schaffer collateral pathway. Brief tetanic stimulation (2 sec at 5 Hz) was used to induce LTP of synaptic transmission. In three different series of experiments with a total of 160 brain slices, the extremely high-power microwave pulse irradiation was performed before, during or after the tetanus. The carrier frequency was 9.3 GHz, the pulse width was from 0.5 to 2 μsec, and the repetition rate was from 0.5 to 1 Hz. The peak SAR in brain slices reached up to 500 MW/kg. (The instantaneous power is incredible.) Microwave heating of the preparation ranged from 0.5°C (at 0.3 kW/kg) to 6°C (at 3.6 kW/kg). The experiments demonstrated that the only effect caused by extremely high peak power microwave exposure, within the studied range of parameters, was a transient and fully reversible decrease in the population spike amplitude. Recovery took no more than a few minutes after the cessation of exposure and return to the initial temperature. The effect's features were characteristic of an ordinary thermal response. Also, the effect was proportional to the temperature rise but not to any specific parameter of microwave exposure. The decrease in the amplitude of the population spike also could be induced by a continuous wave irradiation or by conventional heating. Irradiation did not affect the ability of neurons to develop LTP in response to tetanus or to retain the potentiated state that was induced before irradiation. No lasting and delayed effects were observed. The results are consistent with a thermal mechanism action for extremely high peak power microwaves, and no indication of other mechanisms on neuronal function is suggested.

In comparing Pakhomov et al. (2003) with Tatersall et al. (2001), Pakhomov et al. pointed out that of the over 400 brain slices studied to date, no positive effect could be found without a temperature increase of the preparation of at least 0.5°C, whereas Tattersall et al. elicited variable effects, comparatively, with no measurable increase in preparation temperature. Pakhomov et al. also indicated that "if the reported nonthermal effects are unambiguously replicated under artifact-free conditions, this finding could have a major impact on modern microwave biology and RF exposure safety guidelines". Further study is warranted in this area.

10.2.6 Detection of RF electromagnetic fields

10.2.6.1 Perception

Koivisto et al. (2001) studied whether healthy humans sense the presence of microwave fields from digital GSM mobile phones (902 MHz, 217 Hz pulse modulation). The subjects were assigned in two single-blind experiments. The duration of the microwave exposure was about 60 min in Experiment 1 and 30 min in Experiment 2. Each subject rated symptoms (headache, dizziness, fatigue, itching or tingling of the skin, redness on the skin, and sensations of warmth on the skin) or sensations in the beginning of the experimental session and at the end of both the exposure and the non-exposure conditions. The results did not reveal any differences between exposure and non-exposure conditions, suggesting that a 30–60 min exposure to the present microwave field for a cell phone does not produce subjective symptoms in healthy humans.

10.2.6.2 Electromagnetic hypersensitivity

Many terms are used to name hypersensitivity to electromagnetic fields; such as electromagnetic hypersensitivity, electro-hypersensitivity, hypersensitivity to electricity, hypersensitivity to electric and magnetic fields, and electrical hypersensitivity (EHS). These terms are used to describe people who claim to be sensitive to electric and magnetic fields from a variety of sources, such as power lines, mobile telephones, mobile phone base stations, household appliances, visual display monitors, and light sources (COMAR 2002). There is no independent measure for verifying this condition; currently it is totally dependent on verbal report. The COMAR report mentioned skin problems associated with visual display units in Norway (Linden and Rolfsen 1981); later reports have come from other Nordic countries, elsewhere in Europe, and North America.

Individuals claiming EHS generally report a prevalence of symptoms that are related to the nervous system, such as fatigue, stress, and sleep disturbances. The second most prevalent are skin symptoms, which include facial pricking, burning sensations, and rashes. These are followed in importance by various body symptoms (body ache and pain), eye symptoms (burning sensations), and less commonly ear, nose, throat, and digestive symptoms (COMAR, 2002). Generally, the field that elicit

EHS are reported to be very weak, well below what is known to affect normal individuals, and far below currently accepted safety standards. Nevertheless EHS is a real phenomenon which is an annoying problem for the affected persons.

The prevalence rate of self-reported EHS among the general population was studied recently in Sweden and California.

Hillert et al. (2002) analyzed a cross-sectional questionnaire completed in 1997 by 15,000 men and women between 19 and 80 years in Stockholm County. The response rate was 73%, and 1.5% of the respondents reported hypersensitivity to electric or magnetic fields. Prevalence was highest among women in the 60- to 69 year age group.

Levallois et al. (2002) completed a study based on questions regarding electromagnetic fields and chemical sensitivities that were added to the 1998 California Adult Tobacco Survey. Self-reported electric and magnetic field sensitivity was defined as "allergic or very sensitive to getting near electrical appliances, computers or power lines". Self-reported chemical sensitivity was defined as considering oneself "allergic or unusually sensitive to everyday chemicals". Among a sample of 2,072 Californians, 68 subjects (3.2%) reported to be "allergic or very sensitive" to being near electrical devices as well as to chemicals. Among the 68 subjects, 27 subjects (1.3%) reported sensitivity to electrical devices but not to chemicals.

Three sets of researchers have conducted provocation studies in which individuals claiming EHS were exposed to electric or magnetic fields in an attempt to probe any possible links between reported symptoms and exposure conditions. Researchers have tried to find objective changes, because a major problem in studying EHS is the lack of established pathophysiological markers. Several studies were performed to evaluate EHS under controlled laboratory conditions, and one provocation study was carried out under everyday conditions.

Andersson et al. (1996) tested if psychological treatment of EHS patients was effective. Seventeen patients were assigned randomly to a treatment group or to a waiting-list control group in a retest-posttest control group design. The patients also participated in double-blind provocation tests before and after the treatment. The provocation test showed that this group of EHS patients did not react to the electromagnetic fields. A 'treatment package' of cognitive-behavioral methods were used. First, information about a model for understanding of how somatic symptoms could interact with the person's interpretation of the symptoms was provided, using a 'vicious circle' model. Second, with the help of a homework assignment, each patient registered symptoms, the situations in which they occurred, and the interpretations made. With this psychological perspective, the patients could more realistically "test" the evidence. The patients in the experimental group reduced their evaluations of the disability more than the control group did, indicating that psychological approaches can be of value for these patients. Also, blood samples were analyzed for prolactin, cortisol, dehydroepiandrosterone, and cholesterol: no differences were found.

Using double-blind provocation experiments, Hietanen et al. (2002) tested the hypothesis that EHS persons can reliably report subjective symptoms from exposure to the microwaves emitted by handheld mobile phones (cellular phones). They also tested whether sensitive subjects were able to determine whether a phone was on or

off by sensing microwaves. The study group consisted of 20 volunteers (13 women and 7 men) who reported themselves as being sensitive to cellular phones. The exposure sources were one analog NMT phone (900 MHz) and two digital GSM phones (900 MHz and 1.80 GHz). The duration of a test session was 30 min, and three or four sessions were performed in random order for each subject during a single day. The subjects were asked to report symptoms or sensations as soon as they perceived any abnormal feelings. Various symptoms were reported; most of them appeared in the head region. However, the number of reported symptoms was higher during sham-exposure than during real-exposure conditions. Furthermore, none of the subject could distinguish real exposure from sham exposure. Also, blood pressure, heart rate, and respiration rate were monitored every 5 min, without finding any changes. From these results, the authors concluded that adverse subjective symptoms, though unquestionably perceived by the EHS subjects were not produced by cellular phones.

Flodin et al. (2000) carried out a provocation study in the homes or workplaces of the EHS patients; 24 hours after the test sessions, the subjects reported their symptoms and judgments as to on-off status. Fifteen subjects selected as having 'fast and distinct reactions' from electric equipment were provoked on four occasions: usually two true and two sham challenges were completed. The intervals between exposures were a few or more days, in order to provide the subjects with an opportunity to recover before the next provocation. A control group of subjects verified that the provocations were performed in a blind manner, i.e., they could not discriminate real and sham exposures. The EHS patients were no better than the control group in deciding whether or not they were exposed to electric and magnetic fields. From these data the authors concluded that exposure to electric and magnetic fields *per se* does not seem to be the cause of the symptoms experienced by this patient group.

Several groups have tried to find out indicators that might explain the complaints of EHS persons.

Sandstrom et al. (1997) hypothesized that there are other factors in the office environment that can affect the autonomic nervous system and/or CNS, resulting in the symptoms reported. Flickering light is one such factor, and it was therefore chosen as the exposure parameter in this study. Ten patients complaining of EHS and the same number of control subjects were exposed to amplitude-modulated light. The sensitivity of the brain to this type of visual stimulation was tested by means of two objective electrophysiological methods, electroretinography and visual evoked potential. A higher amplitude of brain cortical responses at all frequencies of stimulation was found when comparing EHS patients with controls. No differences in retinal responses were revealed.

Lonne-Rahm et al. (2000) assigned 24 EHS patients to one of two groups and tested them in a double-blind provocation study. These patients, who reported increased skin symptoms when exposed to electromagnetic fields, were compared with 12 controls. Both groups were exposed to 30 min periods of high- or low-stress situations, with and without simultaneous exposure to the electric and magnetic fields from a visual display unit (VDU). The matched controls were tested twice as were the patients, but the fields were turned off both times for the controls. Stress was induced by requiring the participants to act in accordance with a random sequence of flash-

ing lights while simultaneously solving complicated mathematical problems. Blood samples were analyzed for concentrations of melatonin, prolactin, ACTH, neuropeptide Y, and growth hormones, and the expression of different peptides, cellular markers, and cytokines (somatostatin, CD1, factor XIIIa, and TNF-α). Skin biopsies also were analyzed for the occurrence of mast cells. Stress provocation resulted in feelings of more intense mental stress and elevated heart rate. The EHS patients reported increased skin symptoms when they knew or believed that the VDU's "electromagnetic field" was turned on. With the blind conditions there were no differences between 'on' or 'off'. Inflammatory mediators and mast cells in the skin were not affected by the stress exposure or by exposure to electromagnetic fields. Hormones and cytokines, etc. all showed no differences among groups or conditions. The authors concluded that the patients did not react to the electric and magnetic fields associated with a VDU device.

Hillert et al. (2001) investigated the nature and possible etiology of fatigue in people claiming EHS. The aim was to test the hypotheses that perceived fatigue was due to alterations in cholinesterase activity. The rationale of the study is that fatigue has reportedly been associated with cholinesterase inhibition due to exposure to organophosphates (Markowitz 1992). Symptoms have been suggested to appear even when cholinesterase activity is near or within the range of normal or very slightly depressed (< 20%). The study group consisted of 14 people who reported a hypersensitivity to electricity, including disabling fatigue. Cholinesterase activity was assessed three times: twice based on current symptoms reported by the subjects. and once at a randomly selected time. No significant reduction in acetylcholinesterase was identified in any subject. The results do not support the hypothesis that a change in cholinesterase activity mediates fatigue in people reporting hypersensitivity to electricity.

Although not in microwave frequencies, but at 60 Hz, Lyskov et al. (2001a) performed a neurophysiological study of EHS patients by comparing visual functions, blood pressure, heart rate, electrodermal activity, respiration, EEG and visual evoked potentials. The authors found that the patients had a trend to hyper-sympathetic tone, hyper-responsiveness to sensory stimulation, and heightened arousal.

Lyskov et al. (2001b) further investigated possible neurophysiological effects of intermittent (15 sec on/off), 60 Hz, 10 μT magnetic field exposure on patients with EHS and on control subjects during rest and performance of a mental arithmetic task. EEG, visual evoked potentials, electrodermal activity, ECG, and blood pressure were recorded from 20 EHS patients and 20 control volunteers. The total duration of the test was 40 min, divided into two 10 min rest periods and two 10 min periods of mathematical performance. Magnetic field and sham exposures were presented randomly during these periods, resulting in four different conditions: Field-Rest, Sham-Rest, Field-Math, and Sham-Math. The data showed main effects of the Group factor (EHS vs. control subjects) on heart rate ($P < 0.01$), heart rate spectrum ratio, i.e., heart rate variability ($P = 0.04$); these measures were higher in the EHS group. Also, electrodermal activity was shorter in latency and higher in amplitude in the EHS group. On the other hand, EEG characteristics did not differ between groups. The Condition factor (math task vs. relaxed) showed main effects for heart rate ($P < 0.01$), heart

rate spectrum ratio ($P = 0.06$), electrodermal activity ($P < 0.01$), and alpha and theta spectrum bands of EEG. However, the Field factor (real or sham magnetic field exposure) did not affect either the autonomous functions mentioned above or the EEG variables of either group. These data do not indicate that EHS patients or controls were affected by low-level 60 Hz magnetic field exposure. However, persons reporting EHS differed from the control subjects in baseline values of several physiological characteristics.

In summary, these studies all suggest that (1) the EHS individual cannot detect electric and magnetic fields, and (2) that the symptoms reported by EHS individuals are not related to electric or magnetic field exposures. Given this, perhaps it is no surprise that, so far, researchers have not found any hormonal, immunological, or neurochemical 'markers' which are associated with EHS. However, as suggested by studies of autonomic nervous functions, such as heart rate and electrodermal activity (Lyskov et al. 2001b), or study of brain cortical responses (Sandstrom et al. 1997), it might be possible to demonstrate that EHS patients might have a physiological predisposition to hyper-sensitive responding to physical and psychological environmental stressors. EHS patients might falsely attribute their real symptoms.

10.2.7 Neurotransmitters

Because neurotransmitters are critical messenger of information within the nervous system, many researchers have focused their attention on the issue of whether microwave radiation can affect transmitter actions. (An outline of neurotransmitters is provided in section 2.1.4.)

10.2.7.1 Microwave exposure alone

Gandhi and Ross (1987) exposed rats to 700 MHz at 15 mW/cm^2, that raised the core temperature by 2.5°C. Of six brain regions investigated, only the hypothalamus showed significant changes in receptor states, confirming its pivotal role in thermoregulation. Adrenergic receptors showed a 36% decrease in binding following radiation after a 2.5°C increase in body temperature, suggesting a mechanism to facilitate noradrenaline release, which maintains thermal homeostasis by activating heat dissipation. Muscarinic cholinergic receptors showed a 65% increase in binding at the onset of radiation, which might be attributed to the release of acetylcholine (ACh) in the hypothalamus in response to heat accumulation.

Inaba et al. (1992) exposed rats to 2.45 GHz at an ambient temperature of 21–23°C. Microwave exposure at power densities of 5 and 10 mW/cm^2 increased the rectal temperature by 2.3°C and 3.4°C, respectively. The noradrenaline content in the hypothalamus was significantly reduced after microwave exposure at a power density of 10 mW/cm^2. There were no differences in the dopamine contents of any region of the brain between microwave-exposed rats and control rats. The dihydroxyphenyl acetic acid content, which is the main metabolite of dopamine was increased in the pons and medulla oblongata at a power density of 10 mW/cm^2. The dopamine turnover rates and the ratio of metabolite to dopamine in the striatum and cerebral

cortex were increased at a power density of 10 mW/cm^2. The serotonin content in all regions of the brain did not change. The 5-hydroxyindoleacetic acid content in the cerebral cortex was increased at power densities of 5 and 10 mW/cm^2. Clearly microwave exposures producing a modest rise in core body temperature produce a variety of changes in CNS neurotransmitters. The mechanisms for, and the significance of, the observed changes remains to be determined.

Mausset et al. (2001) developed a protocol of neurotransmitter detection based on immunohistochemistry and image analysis. Gamma-vinyl-GABA, an inhibitor of GABA-transaminase, was injected in rats to increase GABA concentration in the CNS. The cellular GABA contents then were revealed by immunohistochemistry and semi-quantified by image analysis using three parameters: optical density, staining area, and number of positive cells. The increase in GABA content induced by gamma-vinyl-GABA in the molecular and the granular layers of cerebellum was reflected by these three parameters. This protocol was used to investigate the effects of exposure to 900 MHz. Both pulsed microwave exposure with a SAR of 4 W/kg and continuous wave exposure with a high SAR of 32 W/kg were tested. A selective diminution of the stained processes in the Purkinje cell layer after exposure to pulsed microwaves, in addition, a decrease in optical density in the three cell layers were apparent after continuous wave exposure. Whether this effect is, at least partly, due to a local heating of the tissues is not known. Overall, it appears that microwave exposure with a relatively high SAR of 32 W/kg, one that might produce hyperthermia, induces a diminution in cellular GABA content in the cerebellum.

Testylier et al. (2002) studied ACh release in the brain of freely moving rats exposed to a 2.45 GHz continuous wave microwave field (2 or 4 mW/cm^2) or to a 800 MHz field amplitude-modulated at 32 Hz. The rats were exposed for 1 hr during the day or exposed for either 1 h or 14 h during the night. Serial neurotransmitter measurements were performed by microdialysis using a membrane implanted through the upper CA1 region of the hippocampus. After irradiation with the 2.45 GHz microwaves, at 2 mW/cm^2, rats did not show a significant modification of ACh release, whereas those exposed at 4 mW/cm^2 showed a 40% decrease in ACh release from hippocampus. This decrease was maximal at 5 h post exposure. Exposure to the 800 MHz microwaves for 1 hr do not cause any effects, but exposure for 14 hrs induced a 43% decrease in ACh release during the period 11 p.m. – 4 a.m. compared to control rats. This work indicates that neurochemical modification of the hippocampal cholinergic system can be observed during and after an exposure to a low-intensity microwave field.

Pakhomov et al. (2003) investigated the effects of high-power microwave (9.3 GHz) pulses (pulse widths from 0.5 to 2 μsec at a rate of 0.5 or 1.0 Hz) on synaptic transmission and LTP in rat hippocampal slices. (See Pakhomov et al. (2003) in section 10.2.5.1.) Microwave heating of the preparation ranged from 0.5°C (at a SAR of 0.3 kW/kg) to 6°C (at 3.6 kW/kg). The only effect produced by the high-power pulsed microwave exposure was a transient and fully reversible decrease in the population spike amplitude. Irradiation did not affect the ability of neurons to develop LTP. These results are consistent with the view that the only mechanism of action for extremely high power microwave pulses is thermal.

The available literature clearly indicates that, if the SAR is high enough, microwave exposure can alter neurotransmitter function in the CNS.

10.2.7.2 Microwaves and drug effects

Psychoactive agents affect neuronal function by modifying neurotransmitter activities, resulting in some sort of behavioral changes or altered physiological parameters, depending on the experimental conditions. If microwave irradiation caused interaction with the known effects of psychoactive agents, one could investigate, as the next step, the affected transmitter substances with established physiological and pharmacological experimental techniques.

Lai et al. (1983) studied the effects of various psychoactive drugs in rats exposed for 45 min to a circularly polarized, pulsed (2 μsec pulses, 500 pps) microwave field (2.45 GHz) at a SAR 0.6 W/kg. Apomorphine-induced hypothermia and stereotypy were enhanced by irradiation. Amphetamine-induced hyperthermia was attenuated, but stereotypy was unaffected. Morphine-induced catalepsy and lethality were enhanced by irradiation, at certain dosages of the drug. These results suggest that microwave exposure interact with certain types of psychoactive agents.

Lai et al. (1987) measured sodium-dependent, high-affinity choline uptake in various regions of the brain, including frontal cortex, hippocampus, hypothalamus, inferior colliculus and striatum, of rats irradiated for 45 min with either pulsed or continuous wave, low-level microwaves (2.45 GHz; power density, 1 mW/cm^2; average whole-body SAR 0.6 W/kg). Pulsed microwave irradiation (2 μsec pulses, 500 pps) decreased choline uptake in the hippocampus and frontal cortex. Pretreatment with a narcotic antagonist (naloxone or naltrexone; 1 mg/kg) blocked the effect of pulsed microwaves on hippocampal choline uptake but did not alter the effect on the frontal cortex. Continuous wave exposure decreased the uptake in the frontal cortex. This is the first report that microwave irradiation reduced sodium-dependent, high-affinity choline uptake, an index of cholinergic activity.

Lai et al. (1992a) studied the effects of single (45 min) and repeated (ten daily 45 min sessions) pulsed (500 pps, pulse width 2 μsec) 2.45 GHz exposures (SAR of 0.6 W/kg) on the concentration and affinity of benzodiazepine receptors in the cerebral cortex, hippocampus, and cerebellum of the rat. A receptor-binding assay, with 3H-flunitrazepam as ligand, was used. Benzodiazepine receptors in the brain are responsive to anxiety and stress. Immediately after a single exposure, an increase in the concentration of receptor was observed in the cerebral cortex. However, in rats subjected to repeated exposures, no change in receptor concentration was found in the cerebral cortex immediately after the last exposure, which might indicate an adaptation to repeated exposures.

Lai et al. (1992b) performed experiments to investigate subtypes of opioid receptors in the brain involved in the effect of acute (45 min) pulsed (2 μsec pulses, 500 pps) microwave exposure (2.45 GHz; SAR 0.6 W/kg) on cholinergic activity in the rat brain. Rats were pretreated by microinjection of specific antagonists of μ, δ, and κ opioid-receptors into the lateral cerebroventricle before microwave exposure. The

data showed that all three subtypes of opioid receptors are the intermediate step in the microwave-induced decrease in cholinergic activity in the hippocampus.

Lai et al. (1994) studied effects of microwave irradiation on radial-arm-maze performance of rats. After 45 min of exposure to pulsed 2.45 GHz microwaves (2 μsec pulses, 500 pps; SAR 0.6 W/kg), rats showed retarded learning, indicating a deficit in spatial 'working memory' function. This behavioral deficit was reversed by pretreatment before exposure with the cholinergic agonist physostigmine or the opiate antagonist naltrexone. However, pretreatment with the peripheral opiate antagonist naloxone methiodide showed no reversal of the effect. These data indicate that both cholinergic and endogenous opioid neurotransmitter systems in the brain are involved in the microwave-induced spatial memory deficit.

Lai et al. (1996) reported that intra-septal microinjection of β-funaltrexamine blocked a microwave-induced decrease of hippocampal cholinergic activity in the rat after 45 min exposure to pulsed 2.45 GHz microwaves (2 μsec pulses, 500 pps; SAR 0.6 W/kg). These data indicate that μ-opioid receptors in the septum mediate a microwave-induced decrease in cholinergic activity in the hippocampus and support the hypothesis that microwaves at a whole body SAR of 0.6 W/kg can activate endogenous opioids in the brain.

10.2.7.3 Summary

Work from several investigators shows that microwave exposure can modulate brain neurotransmitter activity, especially that involve with thermoregulation. Additonally, an interesting series of papers from one laboratory clearly suggests that psychoactive agents can modulate the effects of microwave exposure on neurotransmitters (and behavior). These results indicate that (1) endogenous opioids play an important role in some of the neurological effects of microwaves, and (2) parameters of microwave exposure are important determinants of the outcome of the microwave effects. Further research in this area should be a fruitful activity, contributing to an understanding of the mechanisms connecting microwave exposure to behavior.

10.3 Peripheral Nervous System

10.3.1 Intact nerves

Yamaura and Matsumoto (1972: 1973) studied the effect of 2.45 GHz microwave irradiation on crayfish peripheral nerve. Response characteristics of discharging impulses of the slowly adapting stretch receptor were obtained by calculating the cross-correlation function between input and output of the receptor neurons. The M-sequence signal of the 2.45 GHz microwave was applied as the input. Temperature change of the receptor was measured by a thermistor. Results showed a strong correlation between the impulse frequency and temperature change. The authors concluded that the impulse frequency change was caused by the thermal change produced by the microwave irradiation.

Courtney et al. (1975) and Chou and Guy (1978) used a temperature-controlled waveguide to expose frog sciatic nerves, cat saphenous nerves, rabbit vagus nerves, and rabbit superior cervical ganglia, and rat diaphragm muscles to 2.45 GHz in either continuous wave or pulsed modes. No specific microwave effects were observed. These results show that under a constant ambient temperature, microwave effects on the selected electrically excitable tissues could not be detected. However, it was shown that a temperature rise in the solution as small as 0.2°C could induce observable effects on action potential (AP) latency or muscle contraction (Chou and Guy, 1978).

McRee and Wachtel (1986) reported that exposure to 2.45 GHz, continuous wave microwave fields (at a SAR of 10 W/kg) would consistently lower the survival time of isolated frog sciatic nerves stimulated at high repetition rates (50 pulse-pairs per sec). To assess the role that these microwaves might have on active transport of K and Na ions, McRee and Wachtel also performed a series of experiments in which the active Na-K pump was substantially blocked by ouabain prior to microwave exposure. Paired nerves were soaked for 5 min in a high concentration (10^{-3} g/liter) of ouabain to rapidly produce blockage of the Na-K pump. With stimulation at 50 pulses/sec, the 'rundown time course' was, as expected, accelerated in all ouabain-treated nerves. However, the microwave-exposed nerves showed no additional shortening of survival time. The experiments were repeated at a slower stimulation rate (5 pulses/sec) so that the survival time of the nerves more closely approximated that of nerves not treated with ouabain (1 to 2 h vs. 30 min or less for ouabain-treated nerves). Results at the lower stimulation rate also showed that there was no difference in the survival times of ouabain-treated exposed and ouabain-treated, unexposed-control nerves. These results lend support to the view that the relative loss of excitability in microwave-exposed nerves is related to an interference with or counteraction of the Na-K pump.

Pakhomov et al. (1997) studied effects of an acute, continuous wave exposure to millimeter waves (40–52 GHz) on compound action potential (CAP) conduction in an isolated frog sciatic nerve preparation. CAPs were evoked by either low-rate (4 paired pulses/sec) or high-rate (20 paired pulses/sec) electrical stimulation of the nerve. The low-rate stimulation did not alter the functional state of the nerve, and the amplitude, latency, and peak latency of CAPs could stay stable for hours. Microwave irradiation for 10–60 min at 0.24–1.5 mW/cm^2, either at various constant frequencies or with a stepwise frequency change (0.1 or 0.01 GHz/min), did not cause any detectable changes in CAP conduction or nerve refractoriness. At a higher field intensity of 2–3 mW/cm^2, a subtle and transient reduction of CAP latency and peak latency along with a rise of the test CAP amplitude were observed.

Rogers et al. (2004) used a classical electrophysiology method and preparation to assess the strength-duration (S-D) curve for the frog sciatic nerve and gastrocnemius muscle preparation. With a pulse of 1 nsec, the voltage threshold for elicitation of a muscular twitch was 4.5 kV and 35 A. The authors expressed the S-D curve in terms of all relevant units, including tissue current density, tissue electric field, calculated temperature increase in tissue, tissue SAR, external power density, and external SAR.

The computations suggest that a single 1 nsec stimulus of 35 A in tissue is athermal; the estimated temperature rise was 1.9×10^{-3}°C.

From the experimental results presented in these five papers, it certainly appears that the effects of microwave exposure on the nerves are caused primarily by tissue heating.

10.3.2 Regenerating nerves

Kolosova et al. (1998) studied the effects of exposure to 53.57 GHz at power density 4 mW/cm^2 on the recovery of function in damaged rat sciatic nerve. Lesions were produced by nerve section followed by microsuturing. Irradiation was applied to the skin of the thigh in the area of suturing. Total number of APs recorded from the nerve was used to study the functional properties of regenerated fibers 5 mon after damage. Microwave exposure had a stimulatory effect on regeneration processes: conduction velocity increased by 25–30% with increase in total AP as compared with the sham-operated control group. It is not known whether this effect is due to local temperature changes or some other factors, such as increased local blood flow, because there is no description of temperature measurement.

The limited data available suggests RF or microwave exposure can modulate functional properties of nerves. It seems most likely that the effects are related solely to heating. Classic electrophysiology techniques applied to nerves would appear to be an excellent preparation to be used for studying microwave and RF effects and mechanisms.

10.4 Endocrinology

In many ways, the endocrine system is a key regulatory system, using diffuse chemical messages (hormones) placed into the general circulation for dispersal throughout the body. (See section 2.2 for background.)

10.4.1 Pituitary gland and its axes

The pituitary gland is the primary interface between the brain and the endocrine glands. The pituitary has been recognized as the ‘master gland’ for many decades. The primary targets of the hormones produce by the pituitary gland are other endocrine organs, such as adrenal or thyroid glands. A linked system, such as brain-pituitary-adrenal is called an axis.

10.4.1.1 Corticosteroids in animals

Endocrine responses can be an adaptive response to environmental stimuli; such responses are not necessarily adverse, analogous to a febrile response when there is a need for host immune defense. The adrenal is a “dual gland”. ACTH acts on the cortex, i.e., the outer layer, of the adrenal gland and stimulates the secretion of steroid

hormones. Catecholamines are synthesized in the medulla, i.e., core, of the adrenal which release adrenalin and noradrenalin. Noradrenalin is a transmitter substance of sympathetic fibers, as well.

To characterize the response of the pituitary-adrenal axis to microwave exposure, Lotz and Michaelson (1978) measured plasma corticosterone and colonic temperature in unanesthetized male rats exposed to continuous wave 2.45 GHz. The rats were exposed in the far field of a horn antenna for 30 or 60 min at power densities of 0, 13, 20, 30, 40, 50 or 60 mW/cm^2. Other exposures were conducted for 120 min at 0, 13, 20, 30 or 40 mW/cm^2. The average energy absorption rate of the rats was 0.16 W/kg absorbed per mW/cm^2. Thus, for example, at 60 mW per cm^2, the SAR was about 9.6 W/kg. Colonic temperature was significantly elevated after exposures to power densities of 13 mW/cm^2 or greater, with progressively larger increase after high intensity exposures. Plasma corticosterone concentration was elevated above that of sham-exposed controls only after exposures at 50 or 60 mW/cm^2 for exposures of either 30 or 60 min. With 120 min exposures, plasma corticosterone concentration was increased at 20, 30, and 40 mW/cm^2. The relationship between the increased levels of circulating corticosterone and colonic temperature suggested that the increases in corticosterone levels reflected the level of physiological response to the body temperature elevations caused by microwave exposure. Lotz and Michaelson (1979) further studied this effect by examining the effects of acute microwave exposure of normal, hypophysectomized, or sham-hypophysectomized rats. (Hypophysectomized means the pituitary has been removed.) Plasma corticosterone levels in acutely hypophysectomized rats exposed to 60 mW/cm^2 for 60 min were below control levels, indicating that the microwave- induced corticosterone response observed in normal, intact rats is dependent on ACTH secretion by the pituitary. In other groups of rats pretreated with dexamethasone before being exposed microwaves for 60 min, the corticosterone response to a 50 mW/cm^2 exposure was completely suppressed by doses equal to greater than 3.2 μg dexamethasone/100 g body weight. However, the corticosterone response to a 70 mW/cm^2 exposure was only partially suppressed by prior administration of 3.2 or 5.6 micrograms dexamethasone/100g body weight. The evidence obtained in these experiments, combined with the results of previous report (Lotz and Michaelson, 1978), indicate that the stimulation of the adrenal axis in the microwave-exposed rat is a systemic, integrative process resulting from to general hyperthermia.

Lu et al. (1985) studied changes in thyroid stimulating hormone (TSH) concentration in unanesthetized rats exposed to 2.45 GHz, amplitude-modulated (120 Hz) microwaves in the far field for 2 or 4 h, using power densities between 0 and 55 mW/cm^2. Individual rats were exposed from 1 to 10 times to seek any possible accumulation, acclimation, or sensitization. Colonic temperature was measured using a thermistor. TSH concentration decreased in rats after microwave exposure. The influence of microwave exposure on serum TSH concentration was independent of the number of exposures, indicating absence of accumulation, acclimation, or sensitization. Decreased TSH concentration usually was accompanied by increased colonic temperature. For 4 h exposure, the lowest irradiance was 20 mW/cm^2, which produced a 0.3°C increase in colonic temperature that was independent of the number of

exposures. For 2 h exposure, the lowest irradiance was 30 mW/cm^2,which produced or a 1.1°C increase in colonic temperature, regardless of the number of exposures. The rats exposed at 10 mW/cm^2 for 2 hr had a lower TSH concentration than did sham-exposed rats. These results suggest that the microwave effect of serum TSH could be dependent on duration of exposure. None of the rats exposed at an irradiance lower than 10 mW/cm^2 had any change in TSH concentration. The effect of microwave exposure on TSH concentration was not persistent after exposure. The relation between TSH concentration and colonic temperature was curvilinear (exponential).

Imaida et al. (1998a) measured ACTH, corticosterone, and melatonin after 6 weeks of near-field exposure of F344 rats to a 929.2 MHz microwave field like that used by cellular phones. Increases occurred for all three hormones in the exposed group compared with the sham-exposed control group. Experimental conditions are described in section 10.1.1.

These examples make it clear that microwave exposure can alter hormones, when body temperature is elevated. These hormonal changes are part of the normal physiological response to hyperthermia.

10.4.1.2 Studies with humans

de Seze et al. (1998) conducted an experiment to evaluate the effect of an 800 MHz signal emitted by a GSM cell phone (217 Hz impulses, one-eight duty cycle, 2 W peak power) on endocrine function of 20 male volunteers, aged from 19 to 40. End points were serum adrenocorticotropin, thyrotropin, growth hormone, prolactin, LH, and FSH concentrations. Each subject was exposed to microwaves through the use of a cellular phone 2 h/d, 5 d/w, for 1 mon. The hormone levels were determined in weekly blood samples, obtained starting 3 weeks before the commencement of the exposure and ending 3 weeks after exposure ended. (All but one blood sample was drawn 48 h after each weekly session. The seventh drawing was performed in the morning after the last weekly exposure.) Within each individual, the pre-exposure hormone concentration was used as the control value. There was a change only in thyrotropin concentration, and it occurred only on one occasion; on the seventh sampling, a 21% decrease was found. This change recovered fully during the post-exposure period. Therefore, cell phone exposure did not induce a long-lasting effect on the hormone-secretion rate of the anterior pituitary gland in humans.

Mann et al. (1998b) investigated the influence of 900 MHz microwaves (pulsed at 217 Hz, average power density 0.02 mW/cm^2) from a circularly polarized antenna on the endocrine system in healthy humans. Nocturnal hormone profiles of growth hormone, cortisol, LH, and melatonin were determined under polysomnographic control. Only one alteration in activity of the hypothalamo-pituitary-adrenal axis was found: a slight, transient elevation in the cortisol serum level immediately after onset of field exposure, which persisted for 1 hour. For growth hormone, LH and melatonin, no effects were found on either total hormone production during the entire night or the dynamic characteristics of the secretion pattern.

Radon et al. (2001) tested 8 healthy male students to see whether or not the microwave fields used by the GSM standard have any noticeable effects on salivary melatonin, cortisol, neopterin, and immunoglobulin A levels during and several hours after exposure. In a shielded experimental chamber, the circularly polarized electromagnetic field was transmitted by an antenna position 10 cm behind the head of sitting subjects. The carrier frequency of 900 MHz was pulsed with 217 Hz, and the average power flux density was 1 W/m^2. In double blind trials, each subject received a total of 20, randomly allotted, 4 hour periods of exposure and sham exposure, equally distributed among day and night. The salivary concentrations of melatonin, cortisol, neopterin and immunoglobulin A did not differ between exposure and sham exposure.

Based on the experiments conducted with animals and humans, it appears that microwave exposure at the athermal levels produced by cell phones causes no important changes in hormones. Experiments with animals in which higher doses can be applied, resulting in increased body temperature. Under this circumstance, microwave exposure can alter hormones. These hormonal changes are part of the normal physiological response to hyperthermia.

10.4.2 Pineal gland and melatonin

There is ample experimental evidence that changes of earth-strength static magnetic fields and ELF magnetic fields can depress the nocturnally enhanced melatonin synthesis in the pineal gland of mammals, including humans. Thus, it is reasonable to ask if the electromagnetic fields associated with cell phones might also influence secretion of melatonin.

10.4.2.1 Experiments with animals

Vollrath et al. (1997) reported that exposure to 900 MHz microwaves, both continuous or pulsed at 217 Hz, for 15 min to 6 hr, at day or night, had no notable, short-term effect on pineal melatonin synthesis in male or female SD rats and Djungarian hamsters. The SARs were approximately 0.06 to 0.36 W/kg in rats and 0.04 W/kg in hamsters. Pineal synaptic ribbon profile numbers, which were studied in rats only, likewise were not affected. With such low whole-body SARs, thermal effects are very unlikely. However, brain SARs presumably were somewhat higher, and the possibility of local hot spots can not be excluded without additional study. It should be noted that much of the brain is highly vascularized, meaning high blood flow rates will dissipate heat into the large heat sink provided by the rest of the body; thus the brain is protected against local heating.

Hata et al. (2005) investigated the effect of 4 hour exposure during the dark to a 1.44 GHz TDMA signal on serotonin and melatonin synthesis in a total of 208 male and female SD rats. The brain SAR was 7.5 W/kg; the whole body SARs were 1.9 W/kg for the somewhat heavier males and 2.0 W/kg for the somewhat lighter females. No differences in melatonin or serotonin levels were observed among the microwave-exposed, sham-exposed, and cage-control groups.

Bakos et al. (2003) found no changes in the 6-sulfatoxymelatonin excretion of exposed Wistar rats (n = 18) compared to a sham-exposed or control group (n = 18) after exposure to 900 MHz (100 μW/cm^2) and 1.80 GHz (20 μW/cm^2), GSM-like radiation. The animals were exposed daily for 2 h, between 8:00 am and noon, during a 14 day exposure period. The exposure occurred during the morning, when melatonin production is low. One wonders what would have been found if exposure had been scheduled during the night, particularly in the earlier portion of the night when nocturnal melatonin production is rising.

Imaida et al. (1998a) mentioned increased daytime melatonin concentration after 6 weeks of near-field exposure to 929.2 MHz microwaves. (Exposure conditions were described in section 9.1.1.) It should be noted the elevation of melatonin was observed during daytime; blood samples were acquired between 9:30 a.m. and noon). A similar daytime increase after 6 weeks of exposure to a 50 Hz circularly polarized rotating magnetic fields was observed by Kato et al. (1993). (See section 3.4 for a complete review of the ELF literature.)

Stark et al. (1997) investigated the influence of 3–30 MHz RF fields on salivary melatonin concentration in dairy cattle. Two commercial dairy herds at two farms were compared. The exposed group was located at a distance of 500 m from the transmitter: the average nightly field strength was 1.59 mA/m. The control group was located at a distance of 4,000 m from the antenna: the average nightly field strength was 0.076 mA/m. At each farm, salivary melatonin concentration in five cows was monitored over a period of 10 consecutive days. Saliva samples were collected at 2 hour intervals during the dark phase of the night. As an additional intervention, the short-wave transmitter was switched off during three of the ten days (off phase). The salivary melatonin values of the two initial nights did not show a difference between exposed and unexposed cows. However, on the first night of re-exposure after the transmitter had been off for 3 days, the difference between groups (3.89 pg/ml) in salivary melatonin concentration of the two groups differed.

There are three reports which observed no effect following short-term microwave exposure (15 min, 6 hr, and 14 day) on rodents, but there is one positive result from a 6 week study. In addition, one environmental study of RF suggests a subtle effect occurred in dairy cows. In summary, the picture is suggestive, but not convincing, that microwave signals also can reduce nocturnal melatonin production. Clearly, additional research is required, particularly with longer exposure intervals.

10.4.2.2 Experiments with humans

de Seze et al. (1999) examined whether microwave fields from cell phones would alter melatonin levels in the human. Volunteers were two groups totaling 37 men, 20–32 yr old. Exposures were to commercially available cellular telephones of the GSM 900 type (900 MHz) or to the DCS type (Digital Communication System, 1.80 GHz), for 2 hr/day, 5 days/week, for 4 wks. The phones were at their maximum power. Blood samples were collected hourly during the night and every 3 hr in the daytime. Four sampling sessions were performed at 15 day intervals: before the beginning of the exposure period, at the middle and the end of the exposure period, and 15 days

later to evaluate the persistence or late appearance of potential effects. The melatonin circadian profile was not affected.

Bortkiewicz et al. (2002) evaluated the effect of cell phone on 6-hydroxymelatonin sulfate (6-OHMS) excretion of nine healthy males, aged 19–29 years. The experiment was performed under controlled lighting conditions: light intensity was 50 lx until midnight and 0 lx during the remainder of the night. Each person was sampled twice: once on a day without exposure, and once on a day with cellular phone exposure for 60 min, from 7 to 8 p.m. The phone used operated at 900 MHz, pulsed with 217 Hz (pulse width 576 μsec), and the SAR was 1.23 W/kg. The subjects did not know sham-exposed or field-exposure days. From 8 p.m. until midnight, the subjects listened to music, and then they slept till 7 a.m. Urine samples were collected at 7 p.m., at midnight, and at 7 a.m. The 6-OHMS concentration in both phone-type experiments did not differ between exposed and control sessions at any of the three time points. Circadian variation of 6-OHMS level was detected in all subjects. The results demonstrated that EMF emitted by cellular phones has no distinct influence on the melatonin level.

Jarupat et al. (2003) studied the effects of 1.9 GHz microwave fields emitted from a cellular phone on nocturnal melatonin secretion in saliva under carefully designed experimental protocol. The subjects were eight females (mean age; 27, range 16–36 years), all in the follicular phase of the menstrual cycle. The subjects had not used a cellular phone for at least one week before the experiment. A cell phone was attached to the left ear for 30 min every hour, from 19:00h to 01:00h: on one day it was on, and on the other day was off. In the within-subjects comparison, melatonin concentration on the field-exposed day was significantly ($P < 0.05$ by paired test) lower than it was on the sham-exposure day.

Santini et al. (2003) studied the effects of more than one month of exposure to VDUs (KHz frequency range) on nocturnal urinary excretion of 6-OHMS by 13 women. Six women, who worked at least 4 h/d, 5d/w, in front of a VDU, constituted the exposed group. Seven women served as the unexposed control group. The 6-OHMS concentration in urine was 54% less ($P < 0.01$) in the field-exposed group as compared with the control group.

Burch et al. (2002) evaluated the relationship between cellular telephone use and excretion of the melatonin metabolite 6-OHMS in two populations of male electric utility workers (Study 1, $n = 149$; Study 2, $n = 77$). Participants collected urine samples and recorded cellular telephone use over three consecutive workdays. Personal 60-Hz magnetic field and ambient light exposures were characterized on the same days using EMDEX II meters. No change in 6-OHMS excretion was observed among those with daily cellular telephone use >25 min in Study 1 (5 worker-days). However, in Study 2, workers with >25 min cellular telephone use per day (13 worker-days) had lower creatinine-adjusted mean nocturnal urinary 6 OHMS concentrations and lower overnight total 6-OHMS excretion, compared to subjects not using cellular telephones. There also were linear trends of decreasing nocturnal 6-OHMS/creatinine concentrations ($P = 0.02$) and total overnight 6-OHMS excretion ($p = 0.08$) across categories of increasing cellular telephone use. A combined effect of cellular telephone use and occupational 60 Hz magnetic field exposure also was

observed in Study 2. From these results, the authors concluded that prolonged use of cellular telephones can lead to reduced melatonin production. Furthermore, elevated 60-Hz magnetic field exposure might potentiate the effect.

10.4.2.3 Summary

Three of the four studies of microwave exposure and melatonin in animals are negative. One possibility is that the exposure durations examined were too short to detect an effect. In studies with humans, two short-term (1 hour and 2 hour) laboratory experiments with cell phone exposure during the light phase revealed no influence on melatonin rhythm in young men. Two experiments using women as subjects reported melatonin reduction. In a laboratory experiment, acute (3 hr) cell phone use was examined. In an occupation study, subacute (1 month) VDU use was examined. An additional occupational exposure study suggests melatonin reduction in men with greater cell phone use. In addition, an additive effect of 60 Hz magnetic field exposure and cell phone use was noted. Although the initial database is small, it does suggest that microwaves, like ELF electric and/or magnetic fields, can depress melatonin production.

Certainly additional studies are required. The physiological importance of melatonin and the economic importance of mobile telephony make mandatory acquisition of additional scientific knowledge.

10.5 Cardiovascular System

10.5.1 Experiments with animals

Jauchem et al. (1999) investigated the thermal distribution and cardiovascular effects produced by sustained exposure of rats to 94 GHz electromagnetic radiation. Sixteen anesthetized SD rats were exposed individually at a power density of 75 mW/cm^2 under far-field conditions in the "E" orientation. Irradiation began when colonic temperature was 37°C and continued until death. Large, immediate increases in subcutaneous temperature on the irradiated side were accompanied by more moderate, delayed increases in colonic temperature. During irradiation, arterial blood pressure initially increased and then precipitously decreased until death. The heart rate increased throughout the exposure period. The patterns of body temperature, heart-rate and blood pressure changes that occurred before death were similar at 94 GHz and 35 GHz. Because of the very short wavelength, exposure to 94 GHz produces extreme peripheral heating without similar levels of core heating, and this pattern of heat deposition can be sufficient to produce circulatory failure and subsequent death. Jauchem et al. (2000) exposed male SD rats (n = 58) individually to one of three conditions: (1) 1 GHz, (2) 10 GHz, or (3) combined 1 GHz and 10 GHz, all at an equivalent whole-body SAR of 12 W/kg. Microwave exposure was started when colonic temperature was 37.5°C, and it was continued until lethal temperatures were attained. During exposure, arterial blood pressure initially increased, but it then

decreased until death. Heart rate increased throughout the exposure period in all groups, indicating no unusual physiological responses occur during multi-frequency microwave exposure, compared with single-frequency microwave exposure. In general, the cardiovascular system is influenced by body temperature changes in the same manner, no matter how the heating is produced.

10.5.2 Experiments with humans

Mann et al. (1998a) investigated the influence of pulsed, high-frequency electromagnetic fields emitted by digital mobile radio telephones on heart rate during slow-wave as well as REM sleep in healthy humans. No significant effects were observed on heart rate and heart rate variability. The authors conclude that autonomic control of heart rate was not affected by weak, pulsed, high-frequency electromagnetic fields.

Braune et al. (2002) found no non-thermal influence of the fields emitted by mobile phones on the vascular autonomic nervous system of healthy humans. The exposure was implemented using a GSM-like signal (900 MHz, pulsed at 217 Hz; 2 W) using a mobile phone mounted on the right-hand side of the heart in a typical telephoning position. Each period of sham-exposure and of field-exposure consisted of 20 min of supine rest, 10 min of 70 degrees upright tilt on a tilt table, and another 20 min of supine rest. (Assessment cardiovascular performance with a tilt table is a standard technique in cardiovascular physiology.) Blood pressure, heart rate and cutaneous capillary perfusion were measured continuously. In addition, serum levels of norepinephrine, epinephrine, cortisol and endothelin were analyzed in venous blood samples taken every 10 min. All parameters measured showed no changes under a nonthermal electromagnetic exposure.

Tahvanainen et al. (2004) evaluated cardiovascular responses in terms of blood pressure and heart rate during controlled breathing, spontaneous breathing, head-up tilt table test, Valsalva maneuver, and deep breathing test in a randomized, double-blind, placebo-controlled cross-over trial in which 32 healthy subjects were submitted to 900 MHz (2 W), to 1.80 GHz (1 W) cellular phone exposure and to sham exposure, in separate sessions. Arterial blood pressure (arm cuff method) and heart rate were measured during and after the 3 min microwave and sham-exposure sessions. Compared to sham exposure, arterial blood pressure and heart rate did not change significantly during or after the 35 min microwave exposure at 900 MHz or 1.80 GHz

Szmigielski et al. (1998) studied the autonomic nervous system's regulation of cardiovascular function by assessing the time course of diurnal rhythms of blood pressure and heart rate in a group of workers (61 healthy workers; 30–50 years) exposed to various intensities of RF fields (0.738 – 1.50 MHz). They found a reduction in the amplitudes of blood pressure and heart rate rhythms and a shift of the acrophase to an earlier time. These changes were more pronounced among workers exposed to higher intensities of RF electromagnetic fields.

Summarizing these results from animal and humans, two primary conclusions about the data seem apparent. First, when there is no heating, there are no cardiovascular effects. Second, in animal experiments where exposure sufficient to produce

lethality from hyperthermia can be conducted, numerous, expected cardiovascular effects become manifest. These few studies are preceded by decades of work establishing the same points. Thus, one can conclude that there are no important effects on the cardiovascular system in the usual environments.

As a secondary point, it must be noted that the "engineering" aspects of these human studies often are not very good. Well-trained medical personnel who are experts in the biology of the dependent variables need to understand they must obtain engineering support to deal with independent variable issues relating to exposure, dosimetry, etc.

10.6 Ocular Responses

Studies on ocular effects from microwave exposure started with Daily et al. (1950), and many papers have been published since then, particularly in the 1970s and 1980s. Some researchers have radiated the whole body of the experimental subjects, and others have limited exposure to the eyes. Short-term (e.g., 30 minutes), repeated or chronic (e.g., several weeks or months) exposures have been used.

It has been reported that microwaves can cause a variety of ocular effects, most often cataract in the lens. However, effects on the retina, cornea, and other ocular systems also have been reported.

Cataract formation has been observed in some experimental animals when one eye was exposed to a localized, very-intense microwave field and the other eye was the sham-exposed control. Daily et al. (1950) used the dog as the experimental animal. However, the most frequently used animal has been the albino rabbit; in recent years, non-human primates also have been used. Microwaves at 2.45 GHz have been used in many recent studies, although other frequencies, such as 1.25 GHz or 94 GHz, also are used. Experimental results obtained using rabbits will be presented first; monkey and human data follow.

10.6.1 Experiments with rabbits

It is known that, under conditions of partial-body exposure to intense microwaves, significant thermal damage can occur in vulnerable tissues. Kramar et al. (1975) investigated microwave-induced (2.45 GHz) cataract formation in the rabbit eye. There was a direct relationship between maximal energy absorption and maximal temperature in the vitreous body at a point midway between the posterior surface of the lens and the retinal surface. The locus of peak energy absorption and peak temperature correlated well with cataract formation in the posterior cortical lens, after a latent period of a few days, when the microwave exposure was at or above threshold levels, e.g., 150 W/kg for 30 min and retrolental temperature above 41°C. Kramar et al. (1987) tried to explain cataractogenesis by introducing hot water into the ocular cavity of rabbit. Cataract was consistently produced by retrolental temperatures between 43°C and 45°C. The authors claim that these findings support the assumption

that microwave cataractogenesis is due to the local production of elevated temperatures.

Hirsch et al. (1977) studied effects of repeated microwave irradiation to the albino rabbit eye. They used 3.0 GHz microwaves irradiated to the eye of albino rabbits for 15 min/day for a month. Clinical examination was carried out for a period up to 1 year. No change occurred below a power density of 300 mW/cm^2. However, at and above this value, posterior subcapsular iridescence and posterior cortical cataracts were produced.

Chou et al. (1983) investigated the effects of exposure of rabbits to 0.5 and 5 mW/cm^2, 2.45 GHz continuous wave radiation for 90 days. Sixteen male New Zealand rabbits were divided into two groups: eight of them were exposed 7 hr/dy, 5 dy/wk for 13 wks; the other eight rabbits were sham exposed. Eyes were examined for cataract formation. The only difference identified was decrease of food consumption during the 5 mW/cm^2 exposure. There were no ocular differences between microwave-exposed and sham-exposed groups.

Foster et al. (1986) reported that a single, 30 min exposure to 2.45 GHz radiation, with 5.75 W being absorbed, produced a cataract in half of the exposed eyes of New Zealand white rabbits.

Saito et al. (1998) investigated the effects of acute microwave exposure at 2.45 GHz on the eye of unanesthetized rabbits, with the contralateral eye serving as the control. Nine restrained, adult Japanese white rabbits were irradiated unilaterally by 2.45 GHz continuous wave microwaves for 160 min to 240 min. Using phantom material, the estimated SAR was 26.5 W/kg. The average increment in corneal surface temperature increment was 3.0°C for 15 min. Miosis occurred in all rabbits within 15 min. Post-exposure ophthalmologic signs included 1) miosis and papillary congestion; 2) keratoleucoma and corneal edema; 3) endothelial cell detachment and floating in aqua oculi, 4) fibrogenesis in the anterior chamber, and 5) conjunctiva edema. These signs disappeared within 1 week after exposure. There was no cataract formation.

More sophisticated experimental equipment and methods have been employed in recent investigations. Kojima et al. (2004) investigated the effect of systemic anesthesia on ocular effects and temperature in rabbit eyes exposed to microwaves. One eye of male pigmented rabbits was exposed at 2.45 GHz for 20–60 min (300 mW/cm^2; 108 W/kg), either under anesthesia (ketamine hydrochloride + xylazine) or without anesthesia. Temperatures within the eye were measured during microwave exposure by a Fluoroptic thermometer. Changes in the anterior segment were evaluated by image analysis utilizing a Scheimpflug camera, specular microscopy, and a laser flare cell meter. The exposed eyes showed miosis, conjunctival congestion, corneal edema, and an increase in the light scattering of the anterior shallow cortex in the papillary area of the lens. The group under systemic anesthesia showed much stronger symptoms than those without anesthesia. The highest temperature during exposure was in the vitreous, followed by the anterior chamber, and the retrobulbar cavity of the orbit. The ocular temperatures of the rabbits under systemic anesthesia were 2 – 9°C higher than those without anesthesia. Body temperature showed an increase of 1°C during the exposure. The more pronounced ocular effects in the anesthetized rab-

bits were associated with the significantly higher ocular temperatures. The authors stressed that the influence of systemic anesthesia on ocular changes should be considered when examining microwave-induced cataract formation.

Considerable research has been done on cataractogensis in rabbit eyes exposed to microwaves. Cataracts result from excessive heating. Safe and dangerous exposure levels can be predicted.

10.6.2 Experiments with monkeys

Kramar et al. (1978) investigated possible species differences relating to cataract formation following microwave exposure. The authors irradiated both rabbits and monkeys (*Macaca mulatta*) in the near field of continuous wave 2.45 GHz to determine the cataractogenic threshold. Rabbits developed cataracts and transient changes such as miosis, dilated vessels etc. at "apparent" incident power densities of 180 mW/cm^2 or greater. Monkeys sustained facial burns, but no lens damage, even at "apparent" incident power densities of 500 mW/cm^2.

These rabbit and monkey experiments showed clearly that the same incident power density microwave exposure did not produce similar effects on the face and eye of these two experimental animals (Kramar et al. 1978). The dissimilar effects reflect the different patterns of 2.45 GHz energy absorption in the monkey and rabbit heads due to their different facial structures. Rabbit eyes protrude in comparison to monkey eyes, which are more embedded within the eye sockets. The SAR threshold for cataracts in the monkey eye might be the same as the SAR threshold for cataracts in rabbit eyes. These results showed that cataractogenic power density levels in rabbit and dogs should not be directly extrapolated to primates, including human beings.

Kues et al. (1999) examined ocular effects associated with exposure to 60 GHz waves on rabbits and monkeys (*Macaca mulatta*). An antenna that produced a uniform energy distribution was used at an incident power density of 10 mW/cm^2. Acute exposure of both rabbits and monkeys consisted of a single, 8 h exposure; the repeated exposure protocol consisted of five separate 4 h exposures on consecutive days. One eye was exposed, and the contralateral eye served as the sham-exposed control. After post-exposure diagnostic examinations, ocular tissue was examined by both light and transmission electron microscopy. No ocular changes were found in either rabbits or monkeys that could be attributed to millimeter-wave exposure at 10 mW/cm^2. As mentioned above, Kramar et al. (1978) also saw no cataracts in microwave-exposed monkeys.

McAfee et al. (1979) trained unrestrained monkeys (*Macaca mulatta*) to expose their own face and eyes to pulsed microwave radiation at a frequency of 9.31 GHz and an average power density of 150 mW/cm^2. Twelve monkeys were individually irradiated during for periods of 30 to 40 days, for 294 to 665 minutes each day. The subjects then were observed for a period of one year. No deleterious effects, such as cataracts, were been observed. McAfee et al. (1983) further reported the outcome of microwave irradiation of rhesus monkeys' eyes at 9.31 GHz over 34 months and 2.45 GHz for 4 months at average power density of 150 mW/cm^2. Irradiation of 17 monkeys (*Macaca mulatta*) was accomplished without restraint or anesthesia by training

the monkeys to irradiate themselves. No cataracts, no effects on cornea, aqueous and vitreous humors or retina, and no loss of visual capability were found.

Lu et al. (2000) studied the effects of 1.25 GHz, high-peak power microwaves on retina in rhesus monkeys. Pre-exposure fundus photographs, retinal angiograms, and electroretinograms (ERG) were obtained to screen for normal ocular structure and function. Seventeen monkeys were randomly assigned to receive either sham or pulsed microwave exposure. The pulse characteristics were 1.04 MW (approximately 1.30 MW/kg, temporal peak retinal SAR), 5.59 μsec pulse length at pulses repetition rates of 0, 0.59, 1.18, and 2.79 Hz. (These are extremely high power values, but the pulses are very short.) Microwaves were delivered to the face with retinal SARs of 0, 4.3, 8.4, or 20.2 W/kg. Nine exposures were given at 4 hr/dy, 3 dy/wk for 3 weeks. Pre-exposure and post-exposure fundus pictures and angiograms all were within normal limits. The response of cone photoreceptors to light flash was enhanced in monkeys exposed at 8.4 or 20.2 W/kg, but not in monkeys exposed at 4.3 W/kg. Enhanced cone photoreceptor b-wave responses were observed in a SAR-dependent manner; these could be an early indicator of mild injury. However, no evidence of degenerative change or ERG depression was seen. From these results, retinal injury is very unlikely at 4 W/kg delivered to the retina. Functional changes probably were reversible, because no evidence of histopathologic correlation with ERG changes was observed.

Chalfin et al. (2002) evaluated anterior segment bioeffects of pulsed, 35 GHz and 94 GHz microwave exposure in the nonhuman primate eye. Five juvenile rhesus monkeys (*Macaca mulatta*) underwent baseline anterior segment ocular assessment consisting of slit lamp examination, corneal topography, specular microscopy, and pachymetry. These studies were repeated after exposure of one eye to pulsed 35 GHz or 94 GHz microwaves at varied fluences, with the other eye serving as a control. The fluence required to produce a threshold corneal lesion, i.e., faint epithelial edema and fluorescein staining, was 7.5 J/cm^2 at 35 GHz and 5.0 J/cm^2 at 94 GHz. Transient changes in corneal topography and pachymetry were noted at these fluences. Endothelial cell counts remained unchanged. Threshold corneal injury from 35 GHz and 94 GHz microwave exposure is produced at fluences below those previously reported for CO_2 laser radiation. These data might help elucidate the mechanism of thermal injury to the cornea.

10.6.3 Magnetic resonance imaging

Magnetic resonance imaging (MRI) has been widely used worldwide since 1980. High-field-strength/high-frequency MRI systems can cause tissue heating. The eye is particularly susceptible to temperature elevations because of its relatively poor blood supply. Shellock and Crues (1988a) and Shellock and Schatz (1992) measured corneal temperatures in 33 patients immediately before and after MRI performed with a 1.5 T, 64 MHz imager and a transmit/receive head coil; estimated peak SARs ranged from 2.54 to 3.05 W/kg. There was an increase in the average corneal temperature: 32.7 ± 0.7°C before imaging and 33.2 ± 0.5°C after. The changes in corneal

temperature ranged from 0.0°C to 1.8°C (mean 0.5°C), and the highest corneal temperature measured after imaging was 34.4°C. In animal models, the eye temperature threshold for microwave-induced cataractogenesis is between 41°C and 55°C. From these results, it was concluded that clinical MRI with use of a head coil delivering microwaves at the SARs studied causes relatively minor increases in corneal temperature that do not appear to pose any thermal hazard to ocular tissue.

From the results described above, one can conclude that microwave-induced cataracts are caused by an elevation in corneal temperature. Whole body (or far-field) exposure studies show that cataracts do not form in rabbit eyes unless intense microwave fields, at or near lethal levels, are applied.

Because microwave-induced cataracts and other ocular changes have been reported in experimental animals, especially rabbits, several researchers have been concerned with human eyes. Dozens of papers, referring to communication facilities and equipment, were published during 1960s and 1980s. However, cataracts caused by microwave exposure have not been reported in humans. Accidental exposures have produced either sub-clinical changes to the lens or no ocular effects (Elder 2003).

In summary, experimentally induced cataracts have been reported in rabbits, but not monkeys following microwave exposure. The presumed mechanism for cataract initiation is hyperthermia. At frequencies of about 100 kHz or greater, the dominant interaction mechanism in biological tissues is heating. At lower frequencies, the dominant interaction mechanism in biological tissues is induced current. The photon energy of microwave radiation is far too low to affect chemical bonding directly. The electric fields induced in tissues by RF radiation result in energy absorption due to the polarization of electrically charged structures and the flow of ions. It is assumed that the increase in linear and rotational energy is rapidly dissipated by molecular collision, resulting in generalized heating.

10.7 Auditory Responses

Investigations on auditory responses to microwaves can be classified into two categories. One is perception, i.e., hearing of microwaves, and the other is any possible effects on the auditory pathway.

10.7.1 Sensation and perception

Audible frequency ranges for sound waves are 10 Hz to 20,000 Hz in humans; 15 Hz to 50,000 Hz in dogs; and 1 kHz to 120 kHz in bats. The receptor cells for hearing are the inner and outer hair cells in the organ of Corti of the cochlea within the inner ear. Both hair cells and the auditory nerve fibers are tonotopically organized; at any position, they are most responsive to a particular frequency. The tectorial membrane arises from the organ of Corti. The longest stereocilia of the outer hair cells are tightly attached to the lower surface of the tectorial membrane. When the basilar

membrane vibrates in response to a sound, the organ of Corti and the overlying tectorial membrane are carried mechanically with it. Because the basilar and tectorial membranes pivot about different lines of insertion, their oscillating displacements are accompanied by back-and-forth shearing motions between the upper surface of the organ of Corti and the lower surface of the tectorial membrane. The mechanical deflection of the hair cell bundle is the proximate stimulus that excites each hair cells of the cochlea. This deflection is transduced into a receptor potential. The receptor potentials trigger APs that eventually are transmitted to the cortical auditory areas, which are responsible for perception of sound.

The localization of sound sources sets stringent limits on the speed of direct mechano-electrical transduction of hair cells. If a sound source lies directly to one side of an animal, an emitted sound will reach the nearer ear somewhat sooner than the farther ear. For a human, this delay is at most 700 μsec. Both humans and owls can locate sound sources on the basis of much smaller temporal delays, about 10 μsec. For this to occur, hair cells must be capable of detecting acoustical waveforms with microsecond-level resolution (Hudspeth 2000). At 1 MHz, the wave duration is 1 μsec. Any frequencies higher than 1 MHz are therefore "out of limit" for activation of hair cells in the organ of Corti. Indeed, there are no known reports of continuous wave signals causing microwave-induced sound in humans or microwave-induced auditory responses in experimental animals (Elder and Chou 2003).

10.7.2 RF hearing

10.7.2.1 Basic phenomenon

All the reports on 'hearing' of radiofrequency deal with auditory response to pulsed (27 to around 1,000 Hz) microwaves, which commonly is called radiofrequency hearing. The 'sound was something like that of a bee buzzing on a window, but with, perhaps, more high frequencies' (Ingalls 1967). It also has been reported to be similar to other common sounds, such as a click, hiss, knock ,or chirp. A quiet environment is required for the MF hearing, because the normal noise levels in outdoor or laboratory settings mask the hearing of microwave sounds. The necessary condition for hearing the radiofrequency-induced sound is the ability to hear audio frequencies above approximately 5 kHz and bone conduction hearing at lower acoustic frequencies.

10.7.2.2 Mechanisms for RF hearing

Cochlear microphonics are the alternating potential changes which follow the stimulus frequency. Microphonics are recorded from near the cochlea and are mainly composed of receptor potentials from outer hair cells. Chou *et al*. (1975) recorded cochlear microphonics that were similar to those evoked by acoustic stimuli, from the round window of guinea pigs during irradiation by pulsed 918 MHz (repletion rate 100 Hz) microwaves. Recording of cochlear microphonics demonstrated that the microwave auditory effect was due to mechanical distortion of the cilia of cochlear hair

cells. These experiments indicate that the primary site of transduction of microwave energy is outside or at the cochlea.

Chou and Galambos (1979) recorded the brain-stem evoked response from guinea pigs stimulated at various intensities by acoustic pulses. Either blocking of the external ear or destruction of the middle-ear produced little change in the brain-stem evoked responses elicited by microwave pulses. From these results, it was suggested that conduction of pressure waves through the bones appear to be the mechanism responsible in perception of pulsed microwaves.

Using conventional glass microelectrodes, Seaman and Lebovitz (1989) recorded extracellular APs of neurons in cat dorsal and postventral cochlear nuclei while the head of the cat was exposed to microwave pulses at 915 MHz. Response thresholds to acoustic tones, acoustic clicks, and microwave pulses were determined for auditory units with characteristic frequencies from 278 Hz to 39.2 kHz. The midline brainstem SAR threshold was as small as 11.1 mW/g/pulse, and specific absorption threshold was as small as 0.6 μJ/g/pulse. Microwave thresholds were generally lower for characteristic frequency less than 9 kHz, as were most acoustic thresholds. These results show that microwave pulses directly stimulated cochlea, then the evoked APs propagated along the normal auditory system, i.e., eighth cranial nerve, medial geniculate nucleus, and primary auditory cortex.

Foster and Finch (1974) proposed thermoelastic expansion as a mechanism for RF hearing. They conducted experiments in water and in KCl solution exposed to microwave pulses similar to those that produce sounds in humans. They showed that pressure changes would result from the absorption of microwave pulses that could produce significant acoustic energy in the solution. Thus, they concluded that audible sounds were produced, via bone conduction, by rapid thermal expansion caused by absorption of the energy in the microwave pulses.

10.7.3 Effects on auditory pathway

10.7.3.1 Experimental data

Arai et al. (2003) investigated whether the pulsed microwaves emitted by a mobile phone have short-term, adverse effects on the human central auditory system. Using 15 volunteers with normal hearing, the auditory brainstem response (ABR), the ABR recovery function, and the middle latency response were recorded before and after using a mobile phone for 30 min. None of the three measures were affected by exposure to the field for 30 min.

Bak et al. (2003) reported no effects of microwave exposure at 450 MHz, 935 MHz, or 1.80 GHz on ABR during and after exposure for 20 min. The subjects were 45 young, healthy volunteers of both sexes. The ABR evaluation was performed before, during, and immediately after the exposure, and the latencies of waves I, III, and V, and inter-waves I-V were analyzed. The authors concluded that brief mobile phone use does not affect propagation of electrical stimuli along the auditory nerve to auditory brainstem centers.

Ozturan et al. (2002) studied the effects of mobile phone use for 10 minutes on human hearing. Using 30 volunteers with normal hearing, evoked otoacoustic emissions (OAEs) were measured before and after cell phone exposure. No measurable changes in evoked OAEs were detected, and none of the subjects reported a deterioration in hearing level. The authors concluded that a 10 min exposure to the microwaves emitted by a mobile telephone had no effect on hearing, a least at outer ear, middle ear, and cochlea. Then the same group (Kizilay et al. 2003) studied the effects of chronic exposure to the microwaves emitted by a mobile phone on the inner ear of adult and developing rats using distortion-product OAEs. Seven of 14 adult rats and four newborn rats were exposed 1 hr/day for 30 days; the other seven adult rats were assigned to control group. No measurable difference in distortion-product OAEs were found between exposed and control groups, and no changes were found in developing rats. It was concluded that 30 days of exposure at 1 hr/dy did not cause any hearing deterioration in either adult or developing rats.

The RF hearing effect occurs because the ELF-modulated pulses stimulate the cochlea, which then responds in the normal manner. Apparently the cochlea is stimulated via bone conduction caused by rapid thermal expansion resulting from the absorption of the energy in the microwave pulses.

These available data from animals and humans suggest that the microwave fields associated with cell phones have no effect on the auditory pathway. However, the data base is small, and the durations of experimental exposure are short, especially in humans. Because so many people already make extensive use of their cell phones, finding unexposed (or not recently exposed) subject will become increasingly difficult. Perhaps laboratory tests show no differences, because the auditory system has been affected before the subject arrives at the laboratory.

10.8 Thermoregulatory Responses

It is essentially important for the existence of animals or humans to maintain body temperature within a certain limited range, while living environments of diverse and varying temperatures. Said the most basic level, the maintenance of body temperature is achieved by balancing heat production within the body and heat loss to the surroundings.

10.8.1 Regulation of body temperature

Change of body temperature is detected, especially externally at the skin and internally by a specialized region of the brain. The information is integrated in the CNS, and regulation is achieved by autonomic and behavioral thermoregulatory reactions (Fig 10.2). These reactions act to overcome the thermal load for maintaining the normal body temperature.

The balance of body temperature is expressed by the following equation;

$$M \pm W = E \pm R \pm C \pm D \pm S$$

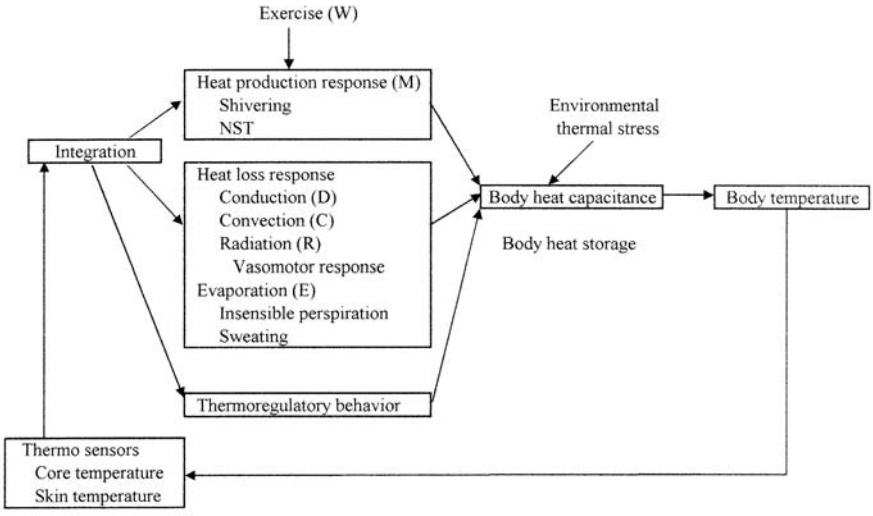

Fig. 10.2. Block diagram of thermoregulation. The system has two groups of sensors, cutaneous and internal detectors.

where M is the rate at which thermal energy is produced through metabolic processes; W is the rate at which work is produced; E is the rate of heat exchange with the surroundings via evaporation; R is the rate of heat exchange with the surroundings via radiation; C is the rate of heat exchange with the environment via convection; D is the rate of heat exchange with the surroundings via conduction; and S is the rate of body heat storage.

Heat production is achieved by non-shivering thermogenesis (NST) and shivering heat production. The basal metabolic rate, a part of NST, is the heat production of a resting human being in a thermo-neutral condition (20 – 27°C) at a time exceeding 12 hours from the last meal. NST consists of both basic NST, which is related to energy demand of the body, and thermoregulatory NST, which takes place during cold exposure. Body tissues that produce thermogenesis are skeletal muscles, thoracic and abdominal organs (such as heart, liver, and alimentary canal), brain, and brown adipose tissues. NST is humorally controlled by β-adrenergic activity.

Shivering is non-voluntary contraction of skeletal muscles that occurs simultaneously on both flexor and extensor muscles at 9–11 Hz. The produced heat is conducted by blood flow throughout the body.

Heat exchange between body and the surroundings is achieved by heat loss responses and evaporation.

Thermosensors are located under the skin and deep inside the body. The deep sensors are mainly located in medial preoptic/anterior hypothalamic region. However, some additional thermosensors are found in medulla oblongata, spinal cord, and deep abdominal organs. Among these receptors, the skin sensors are the most impor-

tant, but medial preoptic/anterior hypothalamic receptors also contribute greatly to production of thermoregulatory responses.

Integration of temperature information from these sensors takes place in hypothalamus. The thermoregulatory system can be described as a "closed loop, with negative feedback", as are other biological regulatory systems, like endocrine system.

Exposure to RF at frequencies above 100 kHz, i.e., exposure to microwaves, generates heat in body tissues. The sensation of tissue warming is necessary to initiate appropriate behavioral action, although physiological responses such as sweating and peripheral circulation changes also can be initiated automatically and autonomously by thermal stimuli. Therefore, any microwave experiments should be planned and interpreted vis-à-vis thermoregulatory mechanisms.

10.8.2 Experiments with animals

Spiers et al. (1989) reported neonatal rats (6–7 days of age) showed a 1.7°C increase in the colonic temperature at the end of a 60 min continuous wave exposure to 2.45 GHz microwaves (5 mW/cm^2, SAR = 3 W/kg) at cold ambient temperature of 25°C, without any change in metabolic heat production. Colonic temperature was increased by 3.4°C after exposure to 20 mW/cm^2 for 60 min. The results indicate that the hypothermic rat pup can be effectively warmed by low-level microwave irradiation. Furthermore, the pup is capable of altering metabolism in response to such heating.

Adair et al. (1985) studied changes in thermoregulatory physiological responses and behaviors in squirrel monkeys chronically exposed to continuous wave, 2.45 GHz microwaves, for 40 hr/wk for 15 wk at power densities of 1 or 5 mW/cm^2. The whole body SAR was 0.16 W/kg per mW/cm^2. Three different, controlled environmental temperatures, 25, 30 or 35°C, were used. Most previous studies paid no attention to the factor of environmental temperature. Physiological responses were measured three times: (1) during a pre-exposure phase of 8–12 weeks, (2) during a 15-week exposure period, and (3) during a post-exposure period of 4–8 weeks. Variables measured were body mass, blood properties, metabolic heat production, sweating, skin temperature, deep body temperature and behavioral responses by which the monkeys selected a preferred environmental temperature. Results showed no reliable alteration of metabolic rate, internal body temperature, blood indices, or thermoregulatory behavior. An increase in sweating rate occurred in the 35°C environment. Skin temperature, reflecting vasomotor state, was reliably influenced by both ambient temperature and microwaves. The most robust consequence of microwave exposure was a reduction in body mass.

Adair et al. (1992) studied whether exposure to microwave fields at the resonant frequency generated heat deep within the body. Adult male squirrel monkeys, held in the far field of an antenna within an anechoic chamber, were exposed (10 min or 90 min) to either resonant 450 MHz or supra-resonant 2.45 MHz continuous wave fields (E polarization) in cool environments. Whole-body SARs ranged from 0–6 W/kg (450 MHz) and 0–9 W/kg (2,450 MHz). Colonic and several skin temperatures, metabolic heat production, and evaporative heat loss were monitored continuously. During brief microwave exposures in the cold, the reduction of metabolic heat

production was directly proportional to the SAR, but 2,450 MHz energy was a more efficient stimulus than was the resonant frequency (450 MHz). Detailed analyses of the data indicate that temperature changes in the skin were the primary source of the neural signal for a change in physiological interaction processes during microwave exposure in the cold.

The essential message from these experiment is that microwave irradiation is just another source of heat, which the body can deal with by the normal processes of the thermoregulatory system. Microwaves are not an extraordinary, highly-dangerous stimulus that is beyond the body's coping mechanisms. Microwaves are just a heat source, like a heat lamp or hot air. However, too much heat, like a very high SAR or a forest fire, is a serious threat to existence.

10.8.3 Experiments with humans

Adair et al. (1998) measured thermoregulatory responses of heat production and heat loss in seven adult volunteers (4 women and 3 men, aged 21–57 years) during 45 min dorsal exposures of the whole body to continuous wave, 450 MHz microwaves. Two exposure levels, SARs of 5.76 or 7.68 W/kg, were tested in each of three ambient temperatures (24, 28 and 31°C). No changes in metabolic heat production occurred under any of the exposure conditions. Vigorous increases in sweating rate on back and chest, directly related to both ambient temperature and power densities, cooled the skin and ensured efficient regulation of the deep body (esophageal) temperature to within 0.1°C of the normal level. These results indicate that dorsal exposures of humans to microwaves at the supra-resonant frequency of 450 MHz, at local peak SAR up to 7.68 W/kg, are mildly thermogenic and are counteracted efficiently by normal thermophysiologic heat loss mechanisms, principally sweating. Adair et al. (1999) further measured thermoregulatory responses of heat production and heat loss in two different groups of seven adult volunteers (males and females) during 45 min dorsal exposures of the whole body to continuous wave 450 MHz or 2.45 GHz microwave fields. At each frequency, two power densities were tested at each of three ambient temperatures [T(a)] = 24, 28 and 31°C) plus T(a) controls (no RF). The normalized peak SAR was the same for comparable power densities at both frequencies, i.e. peak surface SAR = 6.0 and 7.7 W/kg. No change in metabolic heat production occurred under any exposure conditions at either frequency. The magnitude of increase in those skin temperatures under direct irradiation was directly related to frequency, but local sweating rates on back and chest were related more to T(a) and SAR. Both efficient sweating and increased local blood flow contributed to the regulation of the deep body (esophageal) temperature to within 0.1°C of the baseline level, which agreed with the previous study.

Many reports present data showing that continuous wave and pulsed microwave fields, at the same frequency and average power density, produce similar responses in the exposed organism. During whole-body exposure of squirrel monkeys at 2.45 GHz using either continuous wave or pulsed fields, heat production and heat loss responses were nearly identical. To explore this question in humans, Adair et al.

(2001a) exposed two different groups of volunteers to 2.45 GHz using either continuous wave (two females, five males) and pulsed (65 μsec pulse width; three females, three males) microwave fields. Thermophysiological responses of heat production and heat loss were measured under a standardized protocol (30 min pre-exposure baseline, 45 min field or sham exposure, 10 min post-exposure baseline), conducted in three T(a) levels: 24, 28, and 31°C. At each T(a), the SARs were 0, 5.94 and 7.7 W/kg. Data for each group showed minimal changes in core temperature and metabolic heat production for all test conditions. Local skin temperatures showed similar patterns for continuous wave vs. pulsed exposure; skin temperature depended only on SAR. However, there was one reliable difference between continuous wave and pulsed exposure. Only the skin temperature of the upper back (i.e., the area facing the antenna) showed a greater increase during pulsed exposure than during continuous exposure. For all other measurements, no clear evidence for a differential response to continuous vs. pulsed microwave fields was found.

Adair et al. (2001b) studied thermoregulatory responses of human beings following partial body exposure to 2.45 GHz continuous wave microwaves at higher peak power densities of 50 and 70 mW/cm^2. Seven volunteers (four males and three females) were tested at each power density at three T(a) values: 24, 28 and 31°C. The lab's standard protocol of 30 min baseline, 45 min exposure, and 10 min baseline was used. Esophageal and six skin temperatures, metabolic heat production, local sweating rate, and local skin blood flow were measured. No change in esophageal temperature or metabolic heat production was recorded at any power density at any T(a). At peak density of 70 mW/cm^2, skin temperature on the upper back (irradiated directly) increased 4.0°C with T(a) at 24°C; 2.6°C with T(a) at 28°C, and 1.8°C with T(a) at 31°C. These differences were due primarily to increases in local sweating rate, which was greatest in ambient temperature at 31°C. Also at peak power density at 70 mW/cm^2, local skin blood flow on the back increased 65% over baseline level with T(a) at 31°C; the increase was only 40% at a T(a) of 24°C. Vigorous heat loss responses of blood flow and sweating maintain thermal homeostasis efficiently.

Adair et al. (2003) measured thermophysiological responses of heat production and heat loss in seven adult volunteers (six males and one female, aged 31–74 years) during 45 min dorsal exposures of the whole body to a 100 MHz (continuous wave) microwave field. SARs of 0.27, 0.41 and 0.54 W/kg were tested in each of three T(a) (24, 28, and 31°C), along with sham exposure. A standardized protocol consisting of 30 min pre-exposure baseline, 45 min microwave or sham exposure, and 10 min post-exposure baseline was used. Measured responses included esophageal and skin temperatures at seven locations, metabolic heat production, local sweating rate, and local skin blood flow. No changes in metabolic heat production occurred under any test condition. Unlike published results of similar exposure at 450 MHz and 2.45 GHz, local skin temperatures, even those on the back that were irradiated directly, changed little or not at all during 100 MHz exposures. During the 45 min microwave exposure, esophageal temperature showed modest changes (range = −0.15 to 0.13°C) and never exceeded 37.2°C. Thermoregulation principally was controlled by appropriate increases in evaporative heat loss (sweating) and, to a lesser extent, by changes in skin blood flow. Because of the relatively deep penetration of microwave

energy at this frequency, effectively bypassing the skin, these changes must have been stimulated by thermal receptors deep in the body, rather than by those located in the skin. From these results, the authors argued that continuous microwave radiation with an intensity less than 10 mW/cm^2 is unlikely to affect physiology significantly through athermal mechanisms.

Walters et al. (2004) studied the role of baseline skin blood flow on the rate of cutaneous heating induced by 94 GHz microwave energy in humans (3 female, 3 male). Exposure intensities were high power, 1 W/cm^2 for 4 sec, and low power, 175 mW/cm^2 for 180 sec. At the time of exposure, skin blood flow was (a) normal, (b) eliminated using a blood pressure cuff to occlude forearm blood flow, or (c) elevated by heating the skin prior to irradiation. Results showed that only a two-fold elevation in baseline skin blood flow had a profound impact on the subsequent rate of heating, resulting in a substantially lower rate of heating. Occlusion to block increased blood flow to the skin reversed this lower rate of heating. These results demonstrate that relatively small changes in skin blood flow can produce substantial alterations in the rate of skin heating during prolonged 94 GHz exposure.

In summary, results from humans tell the same story as experiments with animals. Microwaves produce tissue heating, and the body uses its thermoregulatory system in the usual manner to deal with the added heat load. The strong implication is that microwaves and RF are not hazardous, so long as they do not exceed the thermoregulatory capacity of the body.

10.8.4 Magnetic resonance imaging

For clinical investigation of patients, MRI devices are used widely. The primary concern about this technology is the possibility of tissue heating caused by microwave exposure. Shellock and Crues (1988b) measured body and skin temperatures in 35 patients immediately before and after clinical MRI. MRI was performed with a 1.5 T system using a 28 cm, open-bore microwave transmit/receive head coil specifically designed for examination of the brain. Body temperature did not change. However, forehead skin temperature and outer canthus skin temperature increased by 0.2°C and 0.6°C, respectively. Shellock et al. (1994) further studied physiological responses to an MRI procedure performed at a SAR of 6.0 W/kg at 1.5 T, 64 MHz. Assessment was made before, during a 16 min MRI procedure, and immediately after MRI. Statistically significant ($P < 0.005$) increases in temperature of the tympanic membrane, the skin of the chest, abdomen, upper arm, hand and thigh, plus increases in heart rate and cutaneous blood flow, were associated with exposure to high SAR. However, these small changes all are well within the limits that can be tolerated by persons with normal thermoregulatory function.

10.8.5 Conclusions

Mammals have evolved elaborate systems for regulating optimal body temperature in hot or cold environments. Experiments with animals show that microwaves are just another heat source, like hot air or an infrared heat lamp. If heat load produced by

microwave (or RF) exposure is small, the thermoregulatory system adjusts and there are no adverse effects. However, if the heat load applied is too great, lethality can result. Experiments with humans, including those with MRI, are directly in agreement with the viewpoint based on animal experiments. However, with humans, only low SARs can be used. Many details enter the story, such as differences in penetration depth of different wavelengths (i.e., frequencies), but the big picture is clear.

Biological systems are fundamentally noisy – on the molecular scale as a consequence of thermal agitation – and macroscopically – as a consequence of physiological functions and animal behavior. If electromagnetic fields are to significantly affect physiology, their direct physical effect must be greater than that from the ubiquitous, endogenous noise. Thermal noise is an essential attribute of living systems, and the lack of thermal noise means death.

One of the classic problems in bioelectromagnetics has been the 'needle in the haystack' problem. How can an imposed stimulus producing tiny energy effects have any influence under conditions where the noise is many orders of magnitude bigger than the signal? Scientists have offered theoretical formulations by which the signal/noise ratio could be overcome, but it has been very difficult to illustrate the workings of such theoretical mechanisms in data from real biological systems.

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11

Radiofrequency Biology: *In vitro*

Junji Miyakoshi

11.1 Cell Growth

Bioeffects have paid less attention to cell growth as a dependent variable than have investigators interested in ELF bioeffects. Presumably that primarily is a matter of history. The ELF question is older, and the techniques in use when it was "hot" were simpler. The impact of molecular biology has been substantial in recent years; today investigators first impulse is run a gel, not to count cells, as had been done earlier.

Among the papers reviewed in subsequent sections, only four touch upon cell growth as an important part of their results. Maes et al. (1997) found no effect. Garaj-Vrovac et al. (1991) found a reduction in cell growth with RF exposure. Maes et al. (1993) reported no effect on number of cell divisions. Tian et al. (2002) reported that at SARs greater than 20 W/kg cell survival rates were reduced.

11.2 Genotoxic Effects

Given contemporary emphasis on genes, searching for effects of RF exposure on genotoxicity is an active area of research. Genes are the coding, the program, for the life of cells, and if an environmental stimulus were to affect the code, it would have obvious adverse implications. As noted in Chapters 2 and 4, the genotoxic effects routinely assessed are (1) chromosomal aberration, (2) DNA strand breaks, (3) micronucleus formation, and (4) mutation.

Typically the signals used in recent experiments are based on one of the many signal patterns used in mobile telephony. Given the rapidly developing technology and expanding market penetration of mobile telephony, many different signal modulation schemes, such as CDMA, GSM, TDMA, etc. are being used. Biologists typically base their independent variables on these schemes. They also try to cover a range of SAR values, with the upper limit being exposures producing overt hyperthermia. For about a century, it has been recognized that if cells are heated beyond about 38°C, adverse changes start to occur.

11.2.1 Chromosomal aberration

In this section, three positive and six negative studies are reviewed.

11.2.1.1 Studies reporting negative results

Several studies indicate that exposure of cells to RF did not cause chromosomal aberration or sister chromatid exchange (SCE). Vijayalaximi and colleagues exposed human peripheral blood-derived lymphocytes to 835.62 MHz, continuous wave (frequency division multiple access: FDMA) at either 4.4 or 5.0 W/kg of SAR for 24 h. They investigated mitotic index, chromosomal aberration, percentage of binucleate cells, and formation of micronuclei (MN). In both RF fields, no difference was found from the sham-exposed group (Vijayalaxmi et al. 2001a). They also conducted another study with 847.74 MHz, continuous wave (code division multiple access: CDMA) at a SAR of either 4.9 or 5.5 W/kg for 24 h. They measured mitotic index and the frequency of chromosomal aberration, finding no differences from the sham group (Vijayalaxmi et al. 2001b).

Human blood-derived lymphocytes were exposed to 900 MHz (continuous wave, pseudo-random signal, and dummy burst signal) for 2 h at 0 to 10 W/kg of SAR and the frequency of chromosomal aberration was investigated. In addition, interactive effects with X-rays (1 Gy) and mitomycin C (MMC, at 0.1 $mg{\cdot}ml^{-1}\mu g$) were investigated. With either continuous wave or pseudo-random signals, RF exposure alone induced no changes in the frequency of chromosomal aberration, and interactive effects with X-rays were not found. As for interactive effects with MMC, in the 2 W/kg group, half of cells showed significant differences but the other half did not (Maes et al. 2001). Chinese hamster ovary (CHO)-K1 cells were exposed to pulsed 2450 MHz for 2 h at a SAR of 33.8 W/kg; cells were RF exposed and treated concurrently with MMC or adriamycin to investigate effects of combined exposure. RF exposure alone and with MMC had no effect. With RF exposure combined with adriamycin, the number of changed cells per 100 cells was than that of the control at 37°C. However, it was found that this increase was not induced by RF but by increased temperature which was suggested by results of the temperature-controlled group. There were no differences in mitotic index between the RF-exposed and temperature-controlled groups (Kerbacher et al. 1990). Human blood-derived lymphocytes were exposed to 2450 MHz RF (continuous wave) for 90 min at a SAR of 12.46 W/kg and the frequency of chromosomal aberration and mitotic index were investigated. No changes were found in the frequency of chromosomal aberration or of MN formation, and mitotic index was not affected (Vijayalaxmi et al. 1997).

Human blood-derived lymphocytes were exposed to 935.2 MHz (GSM modulation) for 2 h at SARs of 0.3 to 0.4 W/kg. No differences were found in the frequency of chromosomal aberration (Maes et al. 1997). Maes et al. (1997) also conducted another study in which human lymphocytes were exposed to 954 MHz (GSM modulation) for 2 h at 1.5 W/kg of SAR; cells were treated with MMC after exposure. SCE and the number of cell divisions were counted. RF treatment alone had no effect on

SCE. After RF exposure, cells were treated with MMC. SCE was increased slightly; However, the number of cell division were not changed (Maes et al. 1996).

In a recent study, human blood-derived lymphocytes were exposed to 900 MHz (GSM modulation) for 2 h at 0.3 or 1.0 W/kg of SAR; mitotic index and frequency of SCE were determined. In both 0.3 and 1 W/kg groups, none of the dependent variables were changed (Zeni et al. 2005).

11.2.1.2 Studies reporting positive results

Some studies have shown that exposure of cells to an RF field produces increased chromosomal aberration. Chinese hamster V79 cells were exposed to 7.7 GHz for 10 to 60 min at 0.5 to 30 W/cm^2, and the frequency of chromosomal aberration was investigated. Compared to sham controls, the frequency of chromosomal aberration was increased with exposure time (Garaj-Vrhovac et al. 1991). In this study, cell survival rate (colony-forming ability) was decreased, which suggested that this exposure condition had considerable toxicity on cells.

Human blood-derived lymphocytes were exposed to 830 MHz (continuous wave) for 72 h at SARs in the range of 0.0 to 8.8 W/kg; chromosome 17 aneuploidy and frequency of abnormal DNA replication were determined (Mashevich et al. 2003). Under some circumstances, the temperature in the exposure device could be increased during irradiation; thus, effects of increased temperature also were investigated. No effects were observed at 38.5°C or less. In this study, the temperature was less than 38°C at 8.8 W/kg; therefore, any biological effects observed here not the result of increased temperature. At SARs of 2.6 to 8.8 W/kg, chromosome 17 aneuploidy and frequency of asynchronous replication were increased (Mashevich et al. 2003).

Human blood-derived lymphocytes were exposed 2450 MHz, with a 50 Hz (1/3 loaded) pulse, for 30 or 120 min at the very high SAR of 75 W/kg, and the frequency of chromosomal aberration and the number of cell division were counted (Maes et al. 1993). In the group exposed for 120 min, the frequency of chromosomal aberration was increased; however, no changes were found in SCE and the number of cell divisions. Human blood-derived lymphocytes were exposed to 7.7 GHz electromagnetic radiation for 10 to 60 min at 0.5 to 30 mW/cm^2. The frequency of chromosomal aberration was increased in the groups exposed at 10 mW/cm^2 for 30 min and at 30 mW/cm^2 for 10 min or longer (Garaj-Vrhovac et al. 1992).

11.2.1.3 Summary

Some studies have reported that RF electromagnetic field exposure *in vitro* can result in increased chromosomal aberration. However, most of the studies have shown negative results. It generally is believed that RF electromagnetic fields do not induce chromosomal aberration at SARs which are so low as to be incapable of producing tissue heating.

11.2.2 DNA strand breaks

Six negative and three positive studies are reviewed. In all of these studies, the Comet assay (see Fig. 2.6.) is the primary tool for assessing presence or absence of DNA strand breaks.

11.2.2.1 Studies reporting negative results

To evaluate effects of an RF electromagnetic field on DNA damage, the Comet assay has been used in many studies. A paper from the Roti-Roti lab provides an example of such a study.

The chronological effects (in cells cultured after 2, 4, 24 and 2+4 h exposures) of 835.62 MHz frequency-modulated, continuous wave or to 847.74 MHz code-division, multiple-access (CDMA) signals were investigated using alkaline Comet assay in U87MG and C3H10T1/2 cells in the logarithmic growth phase and C3H10-T1/2 cells at confluent state (see 2.3.2.2). In these conditions, no increase in temperature was observed; the mean among all exposed conditions was 37 ± 0.3°C. The positive control was gamma ray irradiation. In all RF-exposed groups, no differences from the sham group were found (Malyapa et al. 1997a).

Similarly, chronological effects of 2.45 GHz exposure of cells in the logarithmic growth phase at 0.7 or 1.9 W/kg were investigated using alkaline Comet assay. In all RF-treatment groups, no differences were found from the sham group (Mayapa et al. 1997b). Effects of exposure for 2 h to a pulse-modulated 1.9 GHz electromagnetic field at SARs between 0 and 10 W/kg were assessed. DNA damage of human lymphocytes was investigated using Comet assay and MN formation. Under these conditions, no effects of the RF exposure were found (McNamee et al. 2002b).

Also using the Comet assay (Fig. 4.1), Miyakoshi and colleagues have investigated the effects of RF exposure on cultured cells human brain tumor-derived MO54 cells. The temperature during exposure at 2.45 reached 38.9°C, at the exposure of 100 W/kg. No effect from RF exposure was found from 2 hour exposures at SARs of 13 and 100 W/kg (Input power: 13W) (Miyakoshi et al. 2001). Figure 11.1. shows results of mean tail moment.

Four kinds of RF field were used: (1) 837 MHz (phonetic modulation, analog RF field) with TDMA (time division multiple access), (2) 837 MHz with CDMA (code division multiple access), (3) 837 MHz (non-phonetic modulation), and (4) 1909.8 MHz (phonetic modulation with GSM-PCS {global system of mobile communication-type personal communication systems}). Exposures were for either 3 or 24 h at SARs of 1 to 10 W/kg. Results of Comet assay analysis showed no effect of exposure (Tice et al. 2002). In this study, the frequency of MN formation was increased in one group, that was exposed at 10 W/kg of SAR for 24 h.

Lagroye et al. (2001) used C3H10T1/2 cells to assess effects of exposure to 2450 MHz on DNA damage, DNA-protein cross-linking, and DNA-DNA cross-linking. DNA damage was evaluated using Comet assay, and DNA-protein cross-linking was detected using proteinase K. As a positive control for cross-linking, cisplatinum also

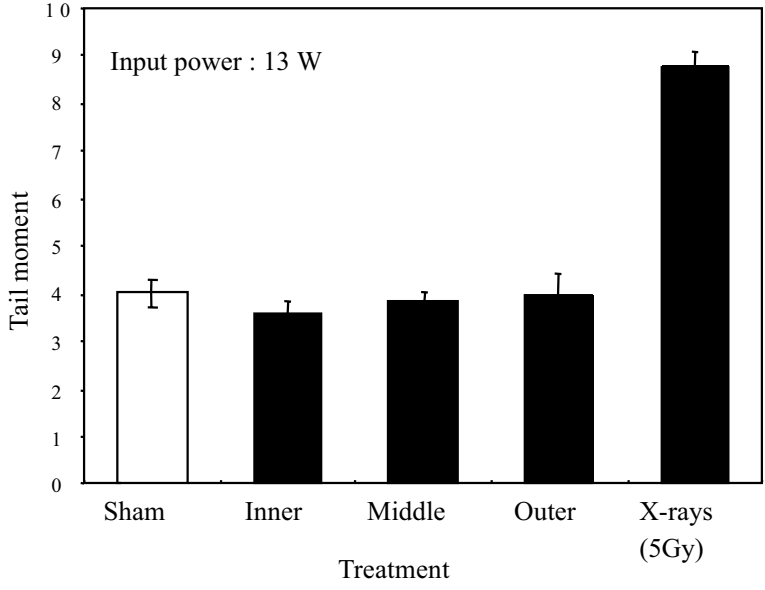

Fig. 11.1. Average tail moment at 13 W, 2 h (average SAR In middle ring: CW 100 W/kg)

was used. For the exposure condition at 1.9 W/kg for 2 h, no effects were found in the above three examinations.

To assess effects with an *in vivo* exposure, the same group exposed Sprague Dawley rats to an RF field (2.45 GHz at 1.2 W/kg) for 2 h. After 4 h, brain cells were isolated and evaluated using two kinds of Comet assay (Singh and Olive methods), with and without addition of proteinase K. No exposure effects were found by either Comet method, with or without proteinase K (Lagroye et al. 2004).

11.2.2.2 Studies reporting positive results

Mei-Bian et al. (2002) used the Comet assay to evaluate human lymphocytes treated with one of three kinds of exposure: (1) 2450 MHz RF field (5 mW/cm^2) for 2 h, (2) 24 h MMC treatment only, and (3) 24 h MMC treatment after 2 h of RF. RF alone had no effect. However, cells exposed to RF followed by treatment with MMC (at a high concentration of 0.025 to 0.1 μg/mL) had increased Comet length, as compared with MMC alone. MN formation also was assessed. A similar increasing trend was found.

Phillips *et al*. (1998) used MOLT-4 lymphoblast cells to examine effects of (1) iDEN signal (813.5625 MHz; SAR = 2.4 and 24 mW/kg) and (2) TDMA signal (836.55 MHz; SAR = 2.6 and 26 mW/kg) on single-strand DNA breaks. They used the alkaline Comet assay. DNA damage was decreased in two groups: 2.4-μW/g iDEN signal for 21 h, and 2.6-μW/g TDMA signal for 21 h. In the two respective groups with SARs that were 10-fold greater, DNA damage was increased with iDEN

signal and decreased with TDMA signal. The authors interpret this pattern of mixed results as indicating that RF fields can have an effect on DNA directly and DNA repair mechanisms.

In the 1980's, studies using plasmid (PUC8.c2) were conducted (Sagripanti and Swicord 1986). Plasmid DNA solution was exposed to an RF field (2.55 GHz; 2 and 8.5 W/kg, or 21 and 85 W/kg; 20 min; 20°C), and effects of exposure were evaluated with plasmid form conversion. SAR-dependent decreases in Form I, plus SAR-dependent increases in Forms II and III, were observed. The maximum temperature increase during RF exposure was 0.8°C. When the effect of temperature on plasmid form distribution was assessed form conversion was not found, even when temperature was increased by 8°C. Therefore, it was suggested that this RF effect on plasmids, i.e., effect on DNA strands, was caused directly by RF exposure in the absence of appreciable temperature rise.

11.2.2.3 Summary

Despite the existence of some positive papers, the weight of the evidence supports the general consensus that RF exposures do no break DNA bonds. Scientists appropriately are conservative and will persist in supporting well established fundamental findings until contrary evidence becomes overwhelming. From the perspective of biophysics, there simply is not enough energy in RF to break DNA bonds. For example, boiling water kills cells, but 100°C does not break chemical bonds.

11.2.3 Micronucleus formation

11.2.3.1 Experimental data

C3H10T1/2 cells in either the resting or the proliferative phases were exposed to 835.62 MHz (FDMA) and 847.74 MHz (CDMA) RF fields for 3 to 24 h at 3.2 or 5.1 W/kg of SAR (FDMA) and at 3.2 or 4.8 W/kg of SAR (CDMA). When the frequency of MN formation was measured, no differences between exposed and control frequencies were found in any condition (Bisht et al. 2002). Human blood-derived lymphocytes were exposed to 1.9 GHz (continuous wave) for 2 h at 0 to 10 W/kg of SAR, and MN formation was measured (McNamee et al. 2002a). The exposure device kept the temperature at 37±0.5°C, even at SAR of 10 W/kg. Increased frequency of MN formation was not observed.

Using MN formation as the dependent variable, Miyakoshi and colleagues have investigated the interactive effects of RF with chemicals using a wide range of SARs, including the extremely high value of 100 W/kg (Koyama et al. 2003). CHO-K1 cells were exposed to 2450 MHz for 18 h at 13 to 100 W/kg , and effects of combined exposure with bleomycin also were investigated. No differences were found between the RF exposed and sham groups with SARs of up to 50 W/kg. MN formation was increased with higher SAR, both in the RF-alone and RF+bleomycin groups. Also in the 39°C treated group used as a temperature control, MN were increased, compared to sham exposure.

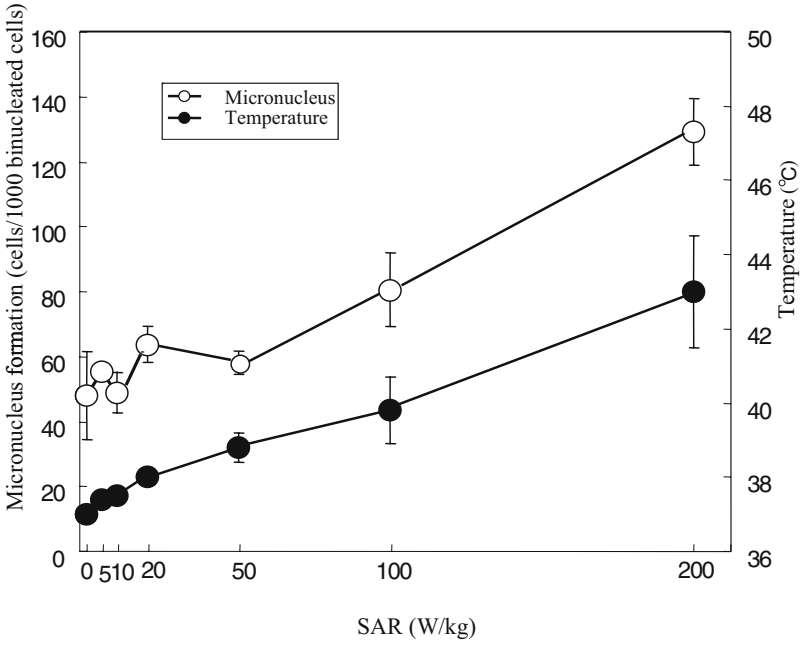

Fig. 11.2. The relationship between the effect of exposure to HFEMF and temperature. Columns indicate the means of MN frequency and the line graph indicates the change in temperature generated by exposure to HFEMF. The bars represent standard deviations from three experiments. There were no temperature changes on sham exposure or HFEMF exposure at SARs of 5, 10, 20 and 50 W/kg. An asterisk indicates a statistically significant difference between sham exposure and HFEMF exposure ($P < 0.01$). R represents the correlation coefficient between the frequency of MN formation and temperature.

Koyama et al. (2004) exposed CHO-K1 cells to a 2450 MHz electromagnetic field for 2 h, using SARs of 5 to 200 W/kg; effects of combined exposure with bleomycin also were investigated. No differences were found between the RF-alone up to 50 W/kg of SAR. However, at 100 W/kg and higher, MN formation was increased (Figure 11.2). As for combined MF + bleomycin exposure, an increase was observed only at 200 W/kg. Also in the 39°C and higher-treated groups as temperature control, MN were increased. However, in combined exposure with heating and bleomycin, increased MN formation was observed only in the group exposed to 42°C. From these results, it was suggested that RF fields at SAR intensities less that those associated with the normal environments encountered daily have no effect on MN formation. However, MN formation was observed at extremely high SARs (more than 50 W/kg) with associated increased temperature.

11.2.3.2 Summary

In general, cells exposed to RF electromagnetic fields do not show an increased incidence of MN formation. However, at relatively high SAR, such as 50 W/kg and greater, increased MN formation has been reported.

11.2.4 Mutation

At present, RF electromagnetic fields have not been found to induce mutations in cells. Meltz et al. (1990) investigated mutation of the thymidine kinase gene locus induced by RF fields alone and with concomitant chemical treatment. Mouse leukemia cells (L5178Y) were exposed for 4 h to pulsed 2.45 GHz RF at SARs of up to 30 W/kg. No differences in the frequency of mutation were found between RF-exposed and sham-exposed groups. Furthermore, compared with MMC-alone treated groups (positive control), no differences were found in (1) the RF and MMC groups, and (2) the temperature and MMC groups. Similarly, no increase on frequency of induced mutation was observed under a different exposure condition (up to 40 W/kg of SAR; for 4 h), and no modifying effect of RF on the response to Proflavin (positive control) was found. In summary, this series of experiments, which were conducted in the same laboratory, and suggested that RF fields, even at extremely high SAR, have no effect on induced mutation.

11.3 Gene Expression

11.3.1 Heat shock proteins

At present, searching for effects of RF exposure on gene expression is an active research area. The most attractive issue at the cell level is effects of heat shock protein (hsp), whose expression is induced by various stresses, on gene expression. As described above, when RF energy is absorbed, heating can occur at the higher SARs. Therefore, it should be analyzed carefully whether hsp expression is induced by (1) heating associated with RF electromagnetic field or (2) is a response initiated as non-thermal but RF-associated response. Such experiments must be conducted using RF exposure systems that accurately control and record temperature. Studies that have been reported up to the present are summarized below. However, many relevant international studies are in progress, and new results will be published.

A Finnish research group (Leszczynski et al. 2002) exposed human endothelial-derived EA.hy926 cells to 900 MHz (GSM)) at 2 W/kg of SAR for 1 h. Then protein expression volume and its phosphorylation were investigated using 2-dimensional cataphoresis with the radioactive isotope ^{32}P. In addition, expression volumes of hsp27 and phosphorylated hsp27 were determined using the Western blot method. Transiently increased ^{32}P protein and phosphorylated hsp27 were found in the RF exposed group as compared to a sham-exposed control group. The expression volumes of hsp27 and p38 mitogen-activated protein kinase (MAPK) were increased

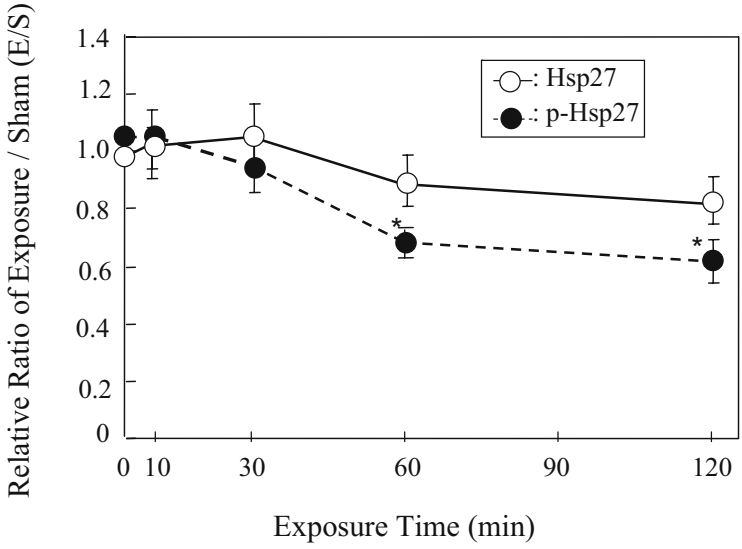

Fig. 11.3. Changes in Hsp27 and p-Hsp27 expression after exposure to RF field at SAR of 10 W/kg for 10 – 120 min in MO54 cells. (a) Representative Western blots of Hsp27 and p-Hsp27; (b) the curves of the relative ratio. The data point represents the mean value of these experiments. The experiment was performed six times ($^*P < 0.05$)

transiently by exposure to RF field. These results suggested the possibility that RF exposure had some effects on signaling, especially on the stress-responding mechanisms of hsp27 and p38 mitogen-activated protein kinase.

Miyakoshi and colleagues also have investigated effects of RF on hsp expression. Using an exposure dish with three sections, human brain tumor-derived MO54 cells were exposed to 2.45 GHz at SARs of 5, 20, 50, or 100 W/kg, and cell survival rates and hsp70 expression were determined using the Western blot method. At 5 W/kg, no effect on hsp70 expression was observed. However, at 20 W/kg and higher, hsp70 expression was increased in a manner dependent on SAR and on exposure time (Tian et al. 2002).

Similarly, MO54 cells were exposed to 1950 MHz at 1 to 10 W/kg, and expression volumes of hsp27, hsp70 and phosphorylated hsp27 (serine 78) were determined. Compared with the sham group, no differences in expression volumes of hsp27 and hsp70 were found. However, expression volumes of phosphorylated hsp27 were decreased by 1 and 2 h exposure (Figure 11.3) (Miyakoshi et al. 2005).

Developing embryonic stem (ES) cells deficient in p53 were exposed to a GSM-217 RF field of 1.71 GHz at a SAR of 2 W/kg. The amount of mRNA of hsp70 was amplified and transient, slight increases were found simultaneously in c-jun, c-myc and p21. However, such changes were not found in wild-type ES cells with the normal p53 gene, suggesting that effects of RF on hsp70 are dependent on the genetic background of cells, including the p53 gene (Czyz et al. 2004).

11.3.2 Oncogenes

Rat pheochromocytoma (PC-12) cells treated with nerve growth factor (NGF) were exposed to 836.55 MHz (TDMA) for 20 to 60 min at 0.09 to 9 W/kg, and expression levels of c-jun and c-fos were determined using Northern blot analysis (Ivaschuk et al. 1997). The mRNA level for c-fos was not changed. However, expression of c-jun in cells that were exposed at for 20 min at 9 mW/cm^2 was lower than that of the sham group. Additionally, in cells that were exposed for 40 to 60 min, the expression of c-jun did not differ from sham-exposure, perhaps implying recovery. The authors indicate that these results suggest that RF has a transitory inhibitory effect on c-jun expression.

In (1) the logarithmic growth phase, (2) the phase transiting to the plateau phase, and (3) the plateau phase, mouse-derived C3H10T1/2 cells were exposed to two kinds of RF field (835.62 MHz, MCW) or 847.74 MHz (DMA) for 4 days at SAR of 0.6 W/kg. In all RNA that was isolated from cells, mRNAs of c-fos, c-jun and c-myc were synthesized using the RT-PCR method and verified using gel electrophoresis. No differences from the sham-exposed group were found. In addition, there was no difference in DNA binding capacity of the AP1, AP2, and NF-κB transcription factors. However, in the FMCW-exposed group in both (1) the phase transiting to plateau level and (2) the plateau phase, mRNA of c-fos was increased about 2-fold. A similar increase (approximately 1.4 fold) mRNA of c-fos also was observed following CDMA RF exposure (Goswami et al. 1999).

11.4 Signal Transduction

Interestingly, contemporary experiments on RF bioeffects have not placed the emphasis on signal transduction that occurred in the area of ELF bioeffects. In ELF, energy considerations made it obvious that chemical bonds would not be broken. Thus, genotoxicity was not a "hot" topic. If ELF were to do something biologically important, it might do it at the cell membrane, where much of signal transduction occurs. Thus, for research on possible ELF bioeffects, signal transduction received considerable attention. One might speculate that in the area of RF bioeffects, as the issue of genotoxicity is laid to rest, increasing emphasis will be placed on processes of signal transduction.

11.5 Cell Transformation

In 1967, Russian scientists (Stodolnik-Baranska 1967) published in *Nature* the first report that exposure to RF electromagnetic fields can increase frequency of cell transformation. Human lymphocytes were exposed to pulsed microwave RF at 7 and 20 mW/cm^2, for 4 h per day for 3 to 5 days. The frequency of cellular transformation was increased in a manner apparently dependent on exposure duration. In addition,

the percent of cells in the mitotic phase was increased, also in a manner related to increasing exposure duration.

However, most recent studies, which have used focus formation as the dependent variable; about effects of exposure to RF fields alone on transformation have provided negative results. For example, C3H10T1/2 cells were exposed to 835.62 MHz, frequency modulated continuous wave (FMCW) or to 847.74 MHz CDMA at 0.6 W/kg for 7 days, and results were tested using the focus-formation method. No transformation induced by exposure to RF electromagnetic field was found (Roti-Roti et al. 2001). When RF was combined with TPA a carcinogenic promoter, or with X-rays (4.2 Gy), transformation was not enhanced by a 42 day exposure.

Similarly, C3H10T1/2 cells exposed to an RF field (836.55 MHz, TDMA) combined with TPA treatment showed no enhanced transformation when examined using the focus formation test under any of the exposure and treatment conditions (Cain et al. 1997). SARs of 0.15, 1.5, and 15 mW/kg were used, along with TPA concentrations of 10, 30, and 50 ng/mL.

Some positive effects of RF field exposure combined with external factors were reported by a single research team during the 1980s. Balcer-Kubiczek and Harrison (1985) conducted a study in which C3H10T1/2 cell were exposed to RF (2.45 GHz, 4.4 W/kg) for 24 h with either benzopyrene or X-rays. Dose-dependence of benzopyrene and X-rays were evaluated with and without of TPA treatment after exposure. After correction for survival rate of each treatment, the frequency of transformation was increased by combined exposure to X-rays, RF and TPA. To the authors, this suggested that RF had co-promotional effect on oncogenesis.

Similarly, C3H10T1/2 cells were exposed to pulsed 2.45 GHz RF at 4.4 ± 0.8 W/kg for 24 h. Results were evaluated using the focus test method. No effect of RF alone was observed. However, combined exposure to RF, TPA(0.1 μg/mL) and X-rays (1.5 Gy) increased the frequency of transformation (Balcer-Kubiczek and Harrison 1989).

Finally, Balcer-Kubiczek and Harrison (1991) conducted another study in which C3H10T1/2 cells were exposed to 2.45 GHz, modulated at 120 Hz, for 24 h at 0.1, 1 or 4.4 W/kg. RF treatment was combined with X-rays; and after exposure, the additional effects of both the presence and the absence of TPA were evaluated. Results were evaluated using the focus test. The frequency of transformation was not induced by RF alone. However, combined exposure with TPA treatment increased the frequency of transformation.

From these results, Balcer-Kubiczek and Harrison concluded that exposure to RF alone had no effect on the frequency of transformation. However, some studies indicated the possibility that the frequency of transformation was increased by combined exposure to RF with initiators for the transformation-inducing process and or with other external factors promoting transformation. At present, similar studies are in progress in several institutes of several countries, and they soon collectively will provide comprehensive results concerning the interactions of RF with other transformational agents.

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12

Summary and Conclusion

Masamichi Kato

Biological effects from extremely low frequency (ELF) electric and magnetic fields and from radio frequency (RF) electromagnetic fields have been demonstrated in several areas. However, bioeffects from ELF magnetic fields are caused by field intensities greater than those encountered in everyday life, which rarely are greater than 10 μT. Similarly, bioeffects can occur from exposure to RF, especially to microwaves. Bioeffects from RF electromagnetic fields are caused by field exposures, i.e., by specific absorbance rates (SARs) greater than 4 W/kg; these are much stronger than those encountered in everyday life.

The field of bioelectromagnetics continues to face a serious problem: observed phenomena, either *in vivo* or *in vitro*, are not always confirmed by independent researchers. However, in summary, data from *in vivo* research do indicate "something is there" in terms of the physiology of whole animals, and data from *in vitro* research do indicate "something is there" in terms of the molecular biology of cells. The inability to replicate bioelectromagnetic phenomena in detail presumably indicates that important details simply are not understood. It is imperative to understand the mechanism of action for bioeffects in bioelectromagnetics.

Researchers interested in microwaves have established one fundamental mechanism of action, heating. If exposure is sufficient to heat tissue to about 45°C, damage will result, including lethality. Debate continues as to whether or not there is any such thing as a "nonthermal" or "athermal" microwave bioeffect. The bulk of the evidence indicates that important bioeffects do not occur in the absence of heating.

Because much of this research has been directed at questions of health and safety, exposure intensities usually are selected based on environmental relevance. However, researchers conducting *in vitro* studies have used very strong magnetic fields, e.g., 1 T and greater, on living organisms in order to obtain reliable, robust bioeffects, allowing study of possible mechanisms of action.

There is no widely accepted known biological mechanism by which ELF electric or magnetic fields can affect physiological functions. The only known interaction between electric or magnetic fields and the living body is the eddy current induced in the body. Indeed, induced current is supposed to be a candidate for responsible factor to elucidate the mechanisms of action of the magnetic fields.

Levels of electric fields, magnetic fields, and electromagnetic fields in our living environment from human-made sources have increased steadily over the last 100 years. The introduction of electric power in the late 19th century and the rapid adoption of mobile telephony in the late 20th century are but two examples. In addition to these existing technologies, utilization of electromagnetic energy will be accelerated further in many areas of technology. There are several new technologies, such as maglev transportation and improved medical imaging systems, that will benefit society in the coming years.

To mention a few examples in the medical field, X-ray apparatus brought revolution for diagnosis and treatment of diseases in the early 20th century, followed by computer tomography in the 1970th. Magnetic resonance imaging (MRI) apparatus was developed in the 1980s, based on the principle of magnetic nuclear resonance that was discovered independently in 1946 by F. Bloch and E. M. Powell. MRI has been used extensively in some developed countries, and its use is expected to spread to many other countries.

In the transportation area, magnetic levitated (Maglev) trains are in the final development stage in Japan and Germany. Commercial service using the German technology started late 2002 in Shanghai, China.

In the area of communication technology, mobile phones use has expanded explosively in recent years, becoming an integrated part of modern society. This telecommunications technology is being adopted rapidly worldwide. The dramatic increase in the number of mobile telephones necessitates the installation of many more base stations. Furthermore, rapid development of the technology means many forms of wireless communication are being developed, so there is a great diversity of frequencies and modulation schemes in use. The engineers are developing new technologies far faster than the biologists can assess the advantages and disadvantage, if any, of the various schemes.

These new situations are likely to further increase the levels of electric fields, magnetic fields, and electromagnetic fields, although not uniformly over space and time. Electro-, magnetic- or electromagnetic energies at various frequencies will be used increasingly in our living environments. To cope with these situations – and to promote human life more comfortably and efficiently — achieving a scientific understanding the interactions of these fields with living organisms is highly desirable, from a variety of viewpoints, including economic, ethical, legal and practical.

Index