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- Epidemiology
- Standards
- Manufacturing
- Uses
- Reactions
- Sampling/Analytical Methods
- Reported Ambient Levels
- Measured Methods
- Work Practices
- Engineering Controls
- Biological Monitoring
- Methods of Analysis
- Treatment
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PART I. EFFECTS ON THE NERVOUS SYSTEM

INTRODUCTION: EFFECTS OF ELECTROMAGNETIC RADIATION ON THE NERVOUS SYSTEM

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In common with other biologic tissues, the nervous system responds sharply to raising or lowering its temperature, regardless of the means by which the change is achieved. Absorption of electromagnetic energy is inevitably associated with increased temperatures. Depending on the wavelength of the radiation and the geometry of the biologic preparation, significant thermal gradients may be established over quite small distances within the tissue. For these reasons, attention has been primarily focused on heating in evaluation of biologic effects of nonionizing radiation. Even a recent review body of the World Health Organization[1] decided after discussion to dismiss from its concerns possible biologic effects that might occur in the absence of significant heating.

It has become clear, however, that interactions with the mammalian central nervous system can be reliably produced by oscillating electric and electromagnetic fields without significant heating of tissues. Actual measurements in both intact brains and isolated cerebral tissue have shown that these fields are fully effective, with temperature changes of less than 0.1°C, and that brain temperature alterations that might be attributable to weak low-frequency electric fields that produce a variety of behavioral and physiologic responses[2–4] are far below the natural perturbations in brain temperature associated with eating, drinking, or environmental temperature shifts.[5]

If this separation of so-called thermal from nonthermal effects were merely a taxonomic propriety, necessary as a possible guide to hazardous exposures that cause actual tissue damage, no very exciting prospects would appear to lie ahead in future research on biologic, and particularly central nervous, interactions with nonionizing electromagnetic radiation. On the contrary, it now seems that impressed oscillating electric fields may be very powerful tools in arriving at an understanding of quite baffling problems in the structural and functional organization of the brain itself. Even from the viewpoint of potentially hazardous interactions, we must remember that the brain is an organ uniquely constructed of vast numbers of excitable elements and that it may be subtly influenced in ways that have no counterpart in liver, muscle, or kidney. We should also be aware that uniqueness in the structuring of brain tissue, as in the elaborate branching of dendritic fields of cortical neurons and the overlapping and functional contacts between adjacent dendritic fields, does not even occur in other parts of the mammalian central nervous system.[6] We may therefore anticipate that responsiveness to weak electromagnetic fields in cerebral tissue is a manifestation of collective properties of its numerous cellular elements, which may not be discernible in the separate behavior of isolated elements.

As pointed out elsewhere,[7] there is here a kinship with Heisenberg's uncertainty principle, not so much in the effects of measurement on the system being measured, but rather in the effects of experimental isolation of a tissue or its cellular elements,

in the hope that we may then better discern certain properties. These, in fact, may be miniscule properties of individual elements but substantive in systems as a whole. In other words, complexity of cerebral tissue may be an inherent and essential quality.

These studies of the sensitivity of the brain as a whole to weak electric and electromagnetic fields lead to the striking conclusion that mammalian central nervous functions can be modified by electric gradients in cerebral tissue substantially less than those known to occur in postsynaptic excitation and also substantially smaller than those presumed to occur with inward membrane currents at synaptic terminals during release of transmitter substances. For example, studies by Konig and Ankermuller[3] and Wever[4] in Germany, and in our own laboratory by Hamer[8] and Gavalas and colleagues,[2] have all reported behavioral and electroencephalographic effects with electric fields at 5–15 Hz with a peak amplitude of 2–10 V/m in air. Behavioral effects included shortening of human reaction times, of human circadian rhythms, and of subjective estimates of the passage of time in the monkey. Measurements in phantom monkeys in our studies showed that currents to ground of 0.8 nA were induced by the fields. Although no precise measurement of the intracerebral electric gradient produced by these fields has so far been technically feasible, if we assume a specific impedance for brain tissue at these frequencies of the order of 300 $\Omega \cdot$cm,[9] the expected electric gradient would be between 0.1 and 0.01 μV/cm in a monkey brain with a conducting cross section of approximately 10 cm^2 and a maximum linear dimension of 7 cm in the axis of a field of 10 V/m. Our experiments suggest that this intensity may be close to threshold for discernible behavioral and electrophysiologic effects.

Neither these observations nor models of cerebral organization that arise from them are nihilistic to the impressive body of synaptic physiology. Rather, they invite consideration of hierarchies of excitatory organization in which synaptic mechanisms represent but one level. How may information be processed in brain tissue? Fiber conduction and synaptic activation are clearly essential elements in brain function. On the other hand, at least three other modes of information handling in cerebral neurons deserve equivalent attention. They include dendrodendritic conduction, neuronal-neuroglial interactions across the intercellular space, and the sensing of weak stimuli that modify the immediate environment of the neuron. The last class would include sensitivity to weak electric (and perhaps magnetic) fields and to minute amounts of chemical substances, which include drugs, hormones, and neurohumors. Susceptibility of brain tissue to drugs, such as D-lysergic acid diethylamide (LSD), in body fluid concentrations of 10^{-9} [10] are well known and generally accepted. Hormone concentrations that predictably modify brain function are even lower.

It is therefore surprising that such scant consideration has been given to the possibility that brain tissue may be sensitive to field potentials in the environment of the neuron, including the intrinsic fields of the electroencephalograph. Our reluctance to proceed with studies that might reveal these sensitivities is understandable, however, as long as our viewpoint remains focused on classic synaptic pathways as the sole and sufficient mechanisms of neuronal interaction.

The neuronal membrane surface is characterized by outer coats of fragile, highly hydrated macromolecular material that appears to be glycoprotein in nature and polyanionic, with numerous negative fixed charge sites.[11,12] These outer coats or "glycocalyces" of cell membranes[13] blend with other macromolecular material in the intercellular space. Although polyanionic in character, it binds strongly to acidic solutions of phosphotungstic acid.[14,15] Weak electric currents pass through the extracellular space as a preferred pathway, so that only a small portion of any ex-

tracellular current penetrates either neuronal or neuroglial membranes.[16] Impedance measurements in cerebral tissue therefore primarily reflect conductance in the extracellular space, and it is noteworthy that conductance changes in cortical and subcortical structures accompany a variety of learned responses, which suggests that the cell surfaces and intercellular macromolecular material may be one site of structural change in information storage and its retrieval.[17–19]

This membrane surface glycocalyx greatly extends the effective membrane thickness, perhaps to as much as 2000 Å, in what has been described as the "greater membrane."[20] In this greater membrane model, a sensing role has been proposed for the material of the glycocalyx, with specialized receptor sites for hormones and neurohumoral substances effective in minute amounts and in the binding of transmitter substances. The effectiveness of substances in minute amounts at the membrane surface may involve conformational changes in macromolecules at the binding site. Thereafter as a transmembrane effect, molecular "switches," such as prostaglandins, may trigger a transmembrane response in the presence of calcium ions,[21] with activation of powerful metabolic enzymes in energy-releasing mechanisms.[22] Clearly, this sequence of events in chemical sensing involves major "membrane amplification" between the initial surface binding and the release of metabolic energy.

Does a comparable mechanism of membrane amplification underlie central nervous sensing of weak electric fields? It is possible that the broad surface sheet of macromolecular material with its numerous fixed negative charges may function as a sensor of these fields. These negative charges bind cations as a "counterion" layer at their surface. Katchalsky[23] noted that divalent cations are more powerfully bound than monovalent ones, with the exception of hydrogen ions, and that calcium is much more powerfully bound to macromolecular polyelectrolytes than other divalent cations, including magnesium. Our data support the possibility that the binding of calcium to membrane surface polyanions may be classed as a "cooperative" process, with a weak trigger at one point initiating macromolecular conformational changes over considerable distances and perhaps triggering metabolic energy release through transmembrane signals. Schwartz and associates[24–27] have proposed a cooperative mode of this type for linear biopolymers, such as poly-L-glutamic acid, with development of cooperativity by assuming that immediately neighboring segments of the polymer are more likely to be found in like charge states than unlike ones. If this occurs on the membrane surface, decremental conduction of slow waves in neuronal dendrites could be based on a "virtual" wave of altered calcium binding, traveling longitudinally on dendritic structures, which would leave modified states of binding sites on the macromolecular sheet behind the advancing wave, but would involve only small displacements of calcium ions to adjacent sites.[6] Einolf and Carstensen[28] have pointed out that lateral cationic movement along a porous surface having radially oriented fixed charges is associated with dielectric constants between 10^4 and 10^6 at frequencies less than 1.0 kHz.

To be useful, such a model must be necessary and sufficient to explain phenomena not adequately accounted for by other schemes. Central nervous system interactions with weak electric and electromagnetic fields noted in our laboratory invite serious consideration of this type of model, because they occur at energy levels far below those seen in classic synaptic activation. It is a model with hierarchic organization. Molecular events at the membrane surface would influence the excitability of a particular neuron. In turn, this neuron would influence others in its domain through conduction processes. These processes appear to involve dendrodendritic conduction of the large neuronal "slow waves," unique to cerebral neurons, from

one cell to another and propagation of axonal spikes. Joint activity with other neurons produces a volume-conducted slow-wave field through the domain. In turn, this field would again modify the environment at each neuronal surface.

In a search for more definite evidence that the membrane surface might transduce weak extracellular fields as a step in excitation, my colleagues and I have studied the effects of vhf electromagnetic fields, amplitude modulated at electroencephalographic frequencies.[29] The results are presented elsewhere in this monograph by Dr. Bawin and coworkers. Our general hypothesis is related to the strong asymmetry in fixed charge distribution on surface macromolecular sheets with respect to extracellular fluid and to deeper layers of the membrane. Such a phase partition would be expected to demodulate the envelope of a carrier wave, much in the fashion of a semiconductor, although remaining unresponsive to the carrier frequency itself. Differential effects at specific brain sites would then be dependent on particular modulating frequencies. Our findings with 147-MHz 1.0-mW/cm^2 fields, amplitude modulated at 0.5–30 Hz, strongly support this hypothesis. Field modulation at frequencies identical with electroencephalographic rhythm "signatures" in single brain structures sharply reinforced the occurrence of these rhythms, in both conditional and unconditional behavioral situations.

Moreover, our studies in cerebral neurochemistry strongly implicate the binding and release of calcium ions to membrane surface macromolecules as an important step in these field interactions. These studies with Dr. Kaczmarek have followed three lines of related research. First, calcium in cerebral cortex triggers its own release and the release of the transmitter agent γ-aminobutyric acid (GABA) in a highly nonlinear fashion, which is consistent with displacement of bound calcium from polyanionic sites on the membrane surface.[30] This sharp nonlinearity in the release of bound calcium by a small increase in extracellular calcium suggested the possibility of triggering calcium release with a weak electric gradient. In this second series of experiments, pulsed electric stimulation of cat cortex with gradients in the range 20–60 mV/cm increased the efflux of both calcium and GABA.[31] If these weak fields acted through classic processes of transmitter release, important questions may be raised. If a typical synaptic terminal is 0.5 μm in diameter, the extracellular gradient imposed by these fields is, at most, 2.5 μV across the terminal. It is unclear how such a weak stimulus may affect the transmembrane potential of 50 mV sufficiently to influence transmitter release. The applied fields in these experiments are more than four orders of magnitude less and are in the range of naturally occurring gradients. This supports the hypothesis that cortical neurons are sensitive to the natural electric field gradients that surround them.

These positive findings of calcium release with weak electric stimulation have led Drs. Bawin and Kaczmarek to test weak modulated vhf fields on freshly isolated chicken brains, including those killed with potassium cyanide, as described by Dr. Bawin and coworkers at this meeting. There is a remarkable "tuning curve" for different modulation frequencies, with increased calcium efflux from the cortex at modulation frequencies between 9 and 20 Hz, but very little increase outside this frequency band. Previous studies in our laboratory have demonstrated persistence of membrane fixed charges after cyanide poisoning of cultured neurons, so it seems reasonable to assume that the binding of calcium and its subsequent efflux relate to persistent properties of membrane surface polyanions.

It is against this background of rapidly evolving knowledge of membrane structure and function, particularly a new awareness of the sequence of steps that initiate excitation, that we begin this Conference on the Biologic Effects of Nonionizing Radiation. Without this knowledge as a required background in attempts to under-

stand subtle interactions with the nervous system, it would be difficult to plan further research, and we might remain indefinitely at the level of phenomenology. Aided by these recent developments in molecular biology, we may reasonably aspire to the use of nonionizing radiation as a significant new tool in our search for the keys to information transmission, transaction, storage, and retrieval in brain tissue. We do not yet know many of the ways by which the brain transacts information, although these first studies with impressed fields hint strongly that the method may already have opened a tiny crack in the door of a "second signaling system" at least as potent locally in cerebral tissue as the classic axonal substrate for the passage of pulse-coded signals over longer paths.

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