EFFECTS OF MODULATED VHF FIELDS ON THE CENTRAL NERVOUS SYSTEM*

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INTRODUCTION

The existence of brief epochs in which electroencephalographic and neuronal activities are strongly correlated has been repeatedly established in different areas of the brain. For example, periodic slow oscillations of neuronal membrane potentials, frequency related to the concomitant local electroencephalogram (EEG), have been recorded in various cortical areas[1-4] and in hippocampal and thalamic sites.[5,6]

Therefore, the EEG appears to reflect the attenuated undulations of the membrane potential of a surrounding population of neurons, and rhythmic electroencephalographic patterns could be generated by extracellular summation of simultaneous transient slow electrical events in a population of cells.

On the other hand, weak extracellular voltage gradients (1–5 mV/mm) have been shown to significantly affect the excitability, or firing thresholds, of stretch receptor neurons in the crayfish and spinal motor neurons in the cat.[7,8] As pointed out earlier by Nelson,[8] complex structural organization of brain tissues, as seen in the cerebrum, should be highly favorable for multiple electric field interactions, both in the intricate rate of overlapping dendritic trees and between the macromolecules of the extracellular space and the glycoproteins of the outer surface of the cell membrane.[9-14]

Indeed, extremely weak vhf fields [147 MHz, 1 mW/cm^2], amplitude modulated at brain wave frequencies, have been shown to strongly influence spontaneous and conditioned EEG patterns in the cat.[14,15] The hypothesis was offered that the weak electrical forces induced in the brain were modifying the excitability of the central neurons and that these changes were reflected in the recorded transient EEG episodes.

The extracellular electrical gradients could exert their forces on the multiequilibrium system that exists in the outer zone of the neuronal membrane, where mono- and divalent cations compete for binding sites on polyanionic macromolecules and polar ends of intrinsic membrane proteins.[16-20]

Local variations in the surrounding electric field could result in slight modifications of the negative binding sites, either by triggering configurational changes of the surface macromolecules or by inducing small displacements of the surface-bound cations. These alterations would, in turn, influence the activity of adjacent ions, which would disturb further the molecular arrangement of the surface macromolecules, thus propagating and multiplying the initial electrical disturbance.

Concurrent work in this laboratory[21] indicated that weak pulsed electric currents (2–5 mV/mm, 200 pulses/sec) applied across the cat cortex were able to trig-

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ger the release of previously bound radioactive calcium ($^{45}Ca^{2+}$). This, with earlier findings[22] that a local increment in extracellular calcium induces a local increase in the efflux of membrane-bound $^{45}Ca^{2+}$, appears to support the hypothesis of an intrinsic membrane amplification mechanism.

Intracranial injection of Ca^{2+} or Mg^{2+} [20 μl, 40 mM] in chronically implanted neonatal chicks resulted in an almost immediate synchronization of the hyperstriatal EEG, accompanied by behavioral depression.[23] During successive testing days, the animals appeared to recover behaviorally but never showed any sustained EEG arousal. By contrast, animals treated with sodium chloride recovered completely within the first hour after the injection.

The chick forebrain, being so highly sensitive to small perturbations of the extracellular concentrations of either divalent cations, was therefore chosen for investigating, *in vitro*, the possible interactions between extracellular weak voltage gradients, induced by vhf radiations, and ionic movements in cerebral tissue. In the present experiment, $^{45}Ca^{2+}$ fluxes from irradiated brains are compared at various frequencies of amplitude modulation of the carrier wave.

Materials and Methods

The experiments were conducted in an environmental chamber (ambient temperature, 37°C; relative humidity, 35%) specially adapted for the use of vhf fields. The screening procedures and implementation of the 147-MHz fields have been described elsewhere.[9,15] Briefly, the feedline from the transmitter was applied, via an antenna coupler, to the narrow apices of two large triangular aluminum plates (4100 cm^2). The applied power, monitored by two in-line wattmeters and a radio frequency microampmeter, was adjusted to provide field intensities of 1 to 2 mW/cm^2. Sinusoidal modulating frequencies at 0.5–35 Hz were fed into a power supply specifically designed to provide modulated high voltages to the transmitter. Modulation depths were kept between 80 and 90%.

Five hundred neonatal chicks, which ranged in age from 2 to 7 days, were used in these experiments. The animals were sacrificed by decapitation. The forebrains were rapidly dissected from the cranial cavities, and each cerebral hemisphere was incubated for 30 min in a polyallomer test tube that contained 1 ml of physiologic medium [37°C, 155 mM NaCl, 5.6 mM KCl, 2.16 mM $CaCl_2$, 24 mM $NaHCO_3$, and D-glucose (2 g/liter)] together with 0.2 ml of saline that contained 0.2 μCi of $^{45}Ca^{2+}$ (sp act, 1.39 Ci/mmole).

After incubation, the samples were washed three times with nonradioactive solution. The brains were than bathed in 1 ml of physiologic medium for 20-min epochs each, during which time they were exposed to one of the experimental conditions. Assay of radioactivity was by liquid scintillation counting with 0.2 ml of the bathing solution diluted in 11 ml of Packard "Instagel."

The experiment included irradiation with sinusoidal modulation of the vhf field at 0.5, 3, 6, 9, 11, 16, 20, 25, and 35 Hz, also with an unmodulated carrier wave. Controls were run in the absence of fields. Ten half brains were tested simultaneously for each field condition and control. Every field condition was tested at least three times, to provide sufficiently large populations for further statistical analyses. In each experiment, three series of 10 samples were irradiated with the vhf fields modulated at three different frequencies, and one series of 10 half brains served as the control (no field condition). The order of presentation of the four conditions was randomized for each daily experiment. The radioactivities (cpm) of all samples were related to the mean value of the counts obtained with the 10 control samples, taken

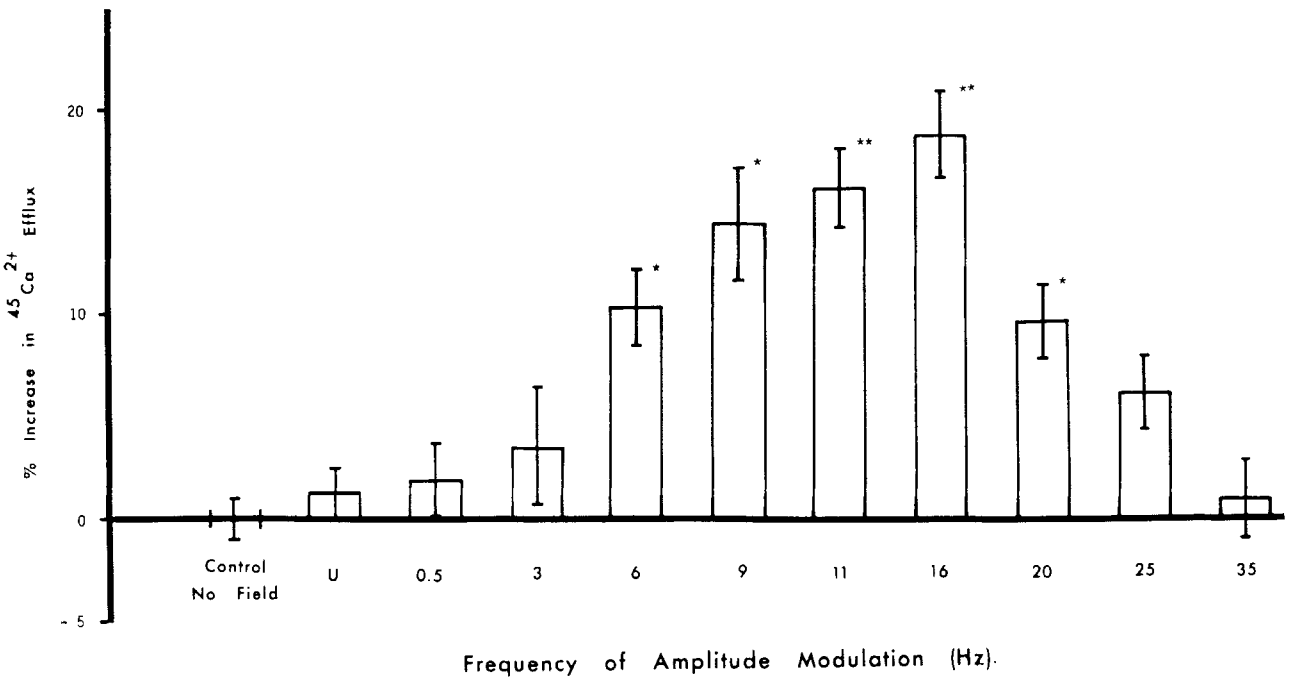

FIGURE 1. Effects of amplitude-modulated 147 MHz vhf fields on the $^{45}Ca^{2+}$ efflux from the isolated forebrain of the neonatal chick. The results, given ± SEM, are expressed as percentage of increase of the calcium efflux, by comparison with control condition, in the absence of fields. *, $p < 0.05$; **, $p < 0.01$.

as 100. All normalized data within each condition were then statistically compared with matched samples of control values.

An additional series of 40 chicks was used to compare field effects on the $^{45}Ca^{2+}$ efflux from brains bathed in physiologic medium and brains poisoned, after incubation, with 10^{-4} M NaCN. Five poisoned samples were tested simultaneously with five normal brains for three field exposures (unmodulated radiation and fields modulated at 0.5 and 16 Hz) and control condition. Each condition was tested twice, and the data were normalized to the control mean values.

Possible field effects on the $^{45}Ca^{2+}$ efflux from a variety of biologic tissues are now being investigated. Preliminary experiments have been conducted with skeletal muscles (lateral head of the gastrocnemius, 25 animals). Muscle and cerebrum dissected from the same animal have been tested simultaneously for two field exposures (6-Hz modulation, 10 samples; 16-Hz modulation, 15 samples) and no field, 20-min epochs, for control.

Results

$^{45}Ca^{2+}$ Efflux from Brain Tissues Bathed in Physiologic Medium

Unmodulated radiations and fields modulated at 0.5 and 3 Hz failed to induce any significant changes in the $^{45}Ca^{2+}$ efflux, by comparison with unirradiated control brains (three repetitions, 30 samples for each condition). By contrast, there was a progressive increase in the $^{45}Ca^{2+}$ efflux from the brains exposed to the fields modulated at 6 Hz (40 samples, 10.1%, $p < 0.05$), 9 Hz (30 samples, 14.3%, $p < 0.05$), 11 Hz (50 samples, 16.0%, $p < 0.01$), and 16 Hz (80 samples, 18.5%, $p < 0.01$). These effects gradually decline at higher frequencies. Exposures to 20-Hz sinusoidal modulation lead to a small increase of the $^{45}Ca^{2+}$ efflux (30 samples, 9.5%, $p < 0.05$). The results obtained with 25-Hz modulation (30 samples, 6%) were not statistically significant, and the fluxes observed with 35-Hz modulation did not differ from the controls.

These findings are illustrated in Figure 1. The data are expressed as percentage of increase of the $^{45}Ca^{2+}$ efflux for the various experimental conditions, by comparison with the no field condition.

$^{45}Ca^{2+}$ Efflux from Brain Tissues Poisoned with 10^{-4} M NaCN

The results with poisoned brains were identical to those observed simultaneously for the samples bathed in physiologic solution. The field effects observed previously were not altered by the cyanide treatment, which strongly suggests that the $^{45}Ca^{2+}$ effluxes from the cerebral tissues are independent of any ongoing metabolism. The $^{45}Ca^{2+}$ fluxes from poisoned brains and normal samples are compared in Table 1. The data have been normalized to the mean value of the radioactivity counts obtained in the absence of the vhf field with unpoisoned brains.

$^{45}Ca^{2+}$ Efflux from Skeletal Muscle

There exists a much greater variability in the radioactivity counts obtained with muscular tissue than with cerebral tissue, within each experimental condition. This

TABLE 1

EFFECTS OF AMPLITUDE-MODULATED VHF FIELDS ON $^{45}Ca^{2+}$ EFFLUX FROM THE ISOLATED CHICK BRAIN*

	Relative $^{45}Ca^{2+}$ Efflux†	
	Normal Brain	Poisoned Brain
Control, no field	100.0 ± 4.0 (15)	96.7 ± 3.6 (15)
Unmodulated field	103.7 ± 6.0 (10)	102.3 ± 5.3 (10)
0.5-Hz modulation	100.5 ± 4.6 (10)	98.7 ± 5.0 (10)
16-Hz modulation	114.2 ± 6.4 (10)‡	118.9 ± 7.7 (10)‡

*Effects of 10^{-4} M NaCN at spot frequencies of the modulation.

†The number of determinations is given in parentheses. The data referred to the mean control value of 100 are given ± SEM.

‡$p < 0.05$.

TABLE 2

EFFECTS OF AMPLITUDE-MODULATED VHF FIELDS ON $^{45}Ca^{2+}$ EFFLUX FROM BIOLOGIC TISSUES*

	Relative $^{45}Ca^{2+}$ Efflux†	
	Brain	Muscle
Control, no field	100.0 ± 5.4 (10)	100.0 ± 5.8 (10)
6-Hz modulation	115.0 ± 4.2 (10)‡	105.1 ± 9.8 (10)
16-Hz modulation	119.1 ± 6.2 (10)‡	100.1 ± 3.9 (15)

*Comparison of brain and skeletal muscle at two spot frequencies of the modulation.

†The number of determinations is given in parentheses. The results referred to the mean control value of 100 are given ± SEM.

‡$p < 0.05$.

variability may be due to small differences in the weight of the samples, and the results would probably be more homogeneous if expressed in radioactivity counts per minute per gram of tissue. However, the preliminary results obtained after exposures to the vhf radiations modulated at 6 and 16 Hz do not indicate any changes in the $^{45}Ca^{2+}$ efflux, by comparison with unirradiated muscles. The brain samples tested concurrently with the muscular tissues gave results identical to these reported above.

Comparisons between the $^{45}Ca^{2+}$ effluxes from both tissues are given in TABLE 2, for the two field exposures tested so far. The experimental data have been related to the mean value of the radioactivity counts obtained with unirradiated muscles and brains.

DISCUSSION

The experimental data indicate that weak vhf fields, amplitude modulated at brain wave frequencies, are able to increase the calcium efflux from the isolated brain of the neonatal chick.

The need for additional investigation of calcium fluxes in a variety of tissues is obvious. Nevertheless, it is worth noting that the two frequencies of modulation tested in the preliminary experiment reported here did not trigger significant changes in calcium efflux from striated muscle, even though both frequencies were effective for the brain samples tested at the same time.

The $^{45}Ca^{2+}$ fluxes from the brain were not influenced by cyanide treatment. The results obtained with the poisoned control samples, in the absence of field, as well as with the poisoned irradiated samples, were not different from those obtained with brains bathed in physiologic medium. Therefore, it may be assumed that the ionic exchanges observed in this experiment were independent of ongoing metabolic processes. This finding seems to favor the divalent cation, present in the highly complex border zone where the cell membrane is in contact with extracellular macromolecular material, as a possible mediator of the observed phenomena.

As mentioned in the introduction, the electrochemical equilibrium that exists in cerebral tissues between ions, polyanionic macromolecules, and glycoproteins of the cell surface can be disrupted by small variations of either surrounding ionic concentrations or local electrical gradients. Thus, rhythmic modulations of the radio frequency energy, reflected as slow undulations of the extracellular electric field, could easily affect the binding of calcium to the neuronal membrane.

A small displacement of these calcium ions, which would result in cooperative interaction between modified adjacent binding sites, could play an important role in the propagation and amplification of local electrical events.

The fact that unmodulated radio frequency energy failed to induce any change in the fluxes, in addition to the findings that the changes observed at 11 and 16 Hz progressively decreased in magnitude for ascending and descending frequencies of modulation, seems to indicate that the calcium ion movements under observation were critically related to very specific slow components of the irradiation.

This finding is in accordance with many previous studies,[24-28] in which the pulse repetition rate of radio frequency energies has been shown to be of critical importance in eliciting specific biologic field effects, and it supports further the hypothesis that the specific frequencies of modulation were responsible for the specific changes seen in the cat EEG. In this context, it might be of significance that the frequencies that lead to the largest calcium efflux (11 and 16 Hz) are constituents of the EEG of the aroused neonatal chick.[29,30]

Finally, because the binding and release of calcium has been linked to inhibition and excitation in the cerebral cortex of the cat,[22] the mode of interaction between external electric fields and central neurons proposed here could explain, at least partially, our previous electroencephalographic results.

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Discussion

Dr. J. C. Sharp (*Walter Reed Army Institute of Research, Washington, D.C.*): Can an interaction from the radio-frequency field be picked up by the electrode on the brain tissue?

Dr. Bawin: We thought that we might be able to induce some current in the brain through the electrode. Nevertheless, as the cable is shielded up to the head, most of the radio-frequency energy picked up on the cable shielding is returned to ground. Only a short length (about 3 cm) of unshielded electrode was present beyond the connection to the shielded cable. This was only a small fraction of a wavelength at 147 MHz. Our best argument exists in the EEG records. We found no propagation phenomena between electrodes; that is, the field effect that we describe as "sharpening" of a response toward certain frequencies was only associated with a very short transient rhythm similar to that in the on-going EEG. The evoked rhythm in the conditioned cat subsided after a long time in extinction trials. It vanished completely from the EEG while the animal was in extinction trials in the presence of radio-frequency fields. It would not be reasonable to assume that we are inducing a current in the brain that will only materialize when the animal is responding to the conditioning stimulus. There was no cross talk between one electrode and another. I generally monitored four or five electrode sites simultaneously, and I conditioned rhythms in only one or two specific locations. I detected no rhythm propagation into any other leads.

Dr. J. A. Elder: Upon turning the modulated vhf fields off, does the calcium again become bound to the brain tissue? Is the effect reversible or irreversible?

Dr. Bawin: I don't know. The calcium is constantly exchanged back and forth, and I only measured at a particular point in time how much calcium had been exchanged from the brain to the saline milieu.