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A Role for the Magnetic Field in the Radiation-Induced Efflux of Calcium Ions From Brain Tissue In Vitro

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Two independent laboratories have demonstrated that electromagnetic radiation at specific frequencies can cause a change in the efflux of calcium ions from brain tissue in vitro. In a local geomagnetic field (LGF) at a density of 38 microTesla (μT), 15- and 45-Hz electromagnetic signals (40 $V_{p\text{-}p}$/m in air) have been shown to induce a change in the efflux of calcium ions from the exposed tissues, whereas 1- and 30-Hz signals do not. We now show that the effective 15-Hz signal can be rendered ineffective when the LGF is reduced to 19 μT with Helmholtz coils. In addition, the ineffective 30-Hz signal becomes effective when the LGF is changed to $\pm$25.3 μT or to $\pm$76 μT. These results demonstrate that the net intensity of the LGF is an important variable. The results appear to describe a resonance-like relationship in which the frequency of the electromagnetic field that can induce a change in efflux is proportional to a product of LGF density and an index, $2n + 1$, where $n = 0,1$. These phenomenological findings may provide a basis for evaluating the apparent lack of reproducibility of biological effects caused by low-intensity extremely-low-frequency (ELF) electromagnetic signals. In future investigations of this phenomenon, the LGF vector should be explicitly described. If the underlying mechanism involves a general property of tissue, then research conducted in the ambient electromagnetic environment (50/60 Hz) may be subjected to unnoticed and uncontrolled influences, depending on the density of the LGF.

Key words: ELF fields, calcium ions, magnetic fields, geomagnetic field, brain tissue

INTRODUCTION

Several reports demonstrate that the release of calcium ions from brain tissue can be altered by electromagnetic (EM) stimulation. Kaczmarek and Adey [1974] showed that calcium ions and GABA, a neurotransmitter, were released from the cat cortex in vivo during application of low-intensity, pulsed, electric currents to the cerebral cortex. This phenomenon was subsequently studied in a radiofrequency electric field, utilizing a tissue preparation from the forebrain of newly hatched chickens. Various frequencies of radiofrequency radiation caused a change in the amount of calcium ions released from this tissue preparation only when the radiation

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was within a certain range of intensities and was amplitude modulated at low frequencies characteristic of EEG recordings [Bawin et al, 1975, 1978; Blackman et al, 1979, 1980a,b, 1981; Sheppard et al, 1979]. Exposure of chicken-brain tissue to extremely-low-frequency (ELF) fields alone also caused a change in the release of calcium ions at specific ranges of signal intensity and frequency. For example, 15- and 16-Hz ELF fields were effective, but 1-, 30-, or 32-Hz fields were not [Bawin and Adey, 1976; Blackman et al, 1982, 1985].

Although the results of exposure of isolated chicken brains to ELF fields as published by Bawin and colleagues and by us agreed both in the frequency dependence and generally in the intensity dependence of the response, there was one major difference. Blackman et al [1982] reported an enhancement in calcium-ion efflux while Bawin and Adey [1976] reported a reduction in efflux. Because Bawin and Adey exposed the samples to an oscillating (AC) electric field whereas we used an AC electromagnetic field, it is possible that the difference in direction of efflux change was due to the AC magnetic component present in our system. We tested this hypothesis. The results identify the AC magnetic component as essential for the efflux enhancements observed in our laboratory. The demonstrated importance of the AC magnetic component led to an additional examination of a possible role for the local geomagnetic field (LGF) in this phenomenon.

MATERIALS AND METHODS

Exposure System

The basic exposure system has been described in detail [Blackman et al, 1982]. It consists of a transmission line exposure chamber (Instruments for Industry, Model 105s) terminated with a 50-ohm load, a function generator (Wavetek, Model 186) to provide the ELF signal, and associated instrumentation to monitor the frequency and intensity of the signal. This system was used to expose the samples to an AC field composed of both an electric and a magnetic component [Blackman et al, 1982].

To expose the samples to an AC electric field alone, the 50-ohm load was removed from the transmission line and the input signal was adjusted to produce the desired electric-field intensity in the exposure chamber. For a 40 $V_{p\text{-}p}$/m, 59.5 nTesla rms (nT[rms])field, removal of the load reduced the intensity of the AC magnetic component to approximately 15 pT (rms), a factor of 2.5×10^{-4}, as measured by a gaussmeter (Bell, Model 640), probe (Bell, Model SAB4-1808), and spectrum analyzer (Hewlett-Packard, Model 3582A). To simplify the notation, this value of the residual magnetic component will not be cited further in discussions of exposure to electric fields alone.

To expose the samples to an AC electromagnetic field under altered LGF conditions, a DC magnetic field was generated by a pair of Helmholtz coils, 18 inches (0.457 m) in radius and separated by 18 inches. The wire coils with 100 turns each were powered by a DC source that was monitored with a multimeter (Fluke, Model 8060A). The Helmholtz coils were placed around the transmission line and produced a uniform magnetic field within the exposure chamber that was parallel to the local vector of the geomagnetic field, which was inclined at 85°. The gaussmeter was used to set the desired density of the LGF in the chamber in the absence of samples. Introduction of samples did not change the density reading. It was assumed that the sample magnetization, M, was essentially zero, and that the relationship $B = \mu_0 M +$

$\mu_0 H$, where B is the magnetic induction density, μ_0 is the permeability, and H is the magnetic field strength [Halliday and Resnick, 1967], reduces to $B = \mu_0 H$. The measured LGF density, B, can then be assumed also to represent the density in the tissue.

Preparation of the Tissue Sample

Cerebral hemispheres from newly hatched chickens (Gallus domesticus) were prepared as described by Blackman et al [1982]. After decapitation, the entire forebrain was removed from the skull, sliced along the midline, and placed in physiological medium (pH 7.8) composed of 155 mM NaCl, 5.6 mM KCl, 2.4 mM $CaCl_2$, 2.5 mM $NaHCO_3$, and 11.1 mM glucose. For labeling with radioactive calcium ions, the medium was supplemented with 1.0 μCi/ml of Ca-45 (New England Nuclear NEZ-013). The paired brain halves from each chicken were labeled together at 37 °C for 30 min, then rinsed in nonradioactive medium to remove excess radioactivity, placed in separate tubes each containing 1 ml of nonradioactive medium, and sealed with a silicon stopper. The tube containing one brain-half was placed in the exposure chamber maintained at 37 °C and the tube containing the other half was placed in a water bath at 37 °C for the 20-min treatment. Four tubes containing individual brain halves along with six tubes containing medium to an equivalent column height were treated at a time as described previously. The six additional tubes were shown to cause the brain samples in the four remaining tubes to respond to a broader range of intensities when they were exposed to modulated 147-MHz radiation [Blackman et al, 1980a]. The additional tubes were included in studies with ELF fields to keep our procedures invariant. We have no evidence that they serve a similar function during exposure to either ELF electric, magnetic, or electromagnetic fields. The metabolic status of this tissue preparation and the influence of several chemicals on the radiation-induced response have been described [Bawin et al, 1975; Bawin et al, 1978; Blackman et al, 1981].

Exposure, Sampling, and Assay

The exposure, sampling, and assay for radioactivity were performed as described by Blackman et al [1982]. Four brain-halves were exposed at a given time either to a 16-Hz electric field alone at 6, 10, or 40 $V_{p\text{-}p}$/m (in air) or received a sham treatment (0 $V_{p\text{-}p}$/m). This combination of exposure and of sham treatment for four brain-halves constituted one set of the basic procedure. Each set was repeated seven to eight times at the three electric field intensities. The test at 40 $V_{p\text{-}p}$/m was repeated a second time 8 months later. This experimental design directly tested the influence of exposure to a particular set of applied field conditions against exposure to zero applied field.

To compare directly the consequences of exposure to two different applied field conditions, the intervening sham exposures were eliminated from the experimental design. Thus, to test the influence of the AC magnetic component, exposure to 16-Hz electric fields, 40 $V_{p\text{-}p}$/m was alternated with exposure to 16-Hz electromagnetic fields of 40 $V_{p\text{-}p}$/m and 59.5 nT(rms) for a total of nine replicates. The influence of the LGF was also tested directly by a similar strategy. Exposure of four brain-halves at a time to 15-Hz, 40 $V_{p\text{-}p}$/m, 59.5 nT(rms) fields under an LGF density of 38 μT was followed by four more samples treated similarly but at an LGF density of 19 μT. These two conditions were repeated eight times. This test was then repeated a second

time during the next week. For exposure to 30-Hz fields 40 V_{p-p}/m and 59.5 nT (rms), each set of four brain-halves treated at the ambient LGF density was paired with a comparable set treated at the adjusted LGF density. Selection of the sequence of treatment conditions for each pair was determined by a random number generator with a different seed for each field density. These two conditions were repeated eight to ten times for each LGF density. Two of the conditions were tested a second time either 2 weeks or 5 months later. Thus, this experimental design, without the use of sham exposure, directly tested the influence of the one condition that was different in the two exposures. Six net altered LGF densities were selected to be compared with the ambient LGF: ± 25.3, ± 78, $+50.7$, and -81.7 μT. These altered DC magnetic field conditions were chosen because they were consistent with a generalized resonance relationship that will be discussed below. The exposure period was 20 min.

Following a treatment, a 0.2-ml aliquot of medium was removed from each tube containing a brain-half, mixed with 5.0 ml of scintillation cocktail (National Diagnostics, LCS), and counted in a scintillation counter (Packard Instruments, Model 2660) to estimate the amount of radioactive calcium released by the tissue. Ten thousand counts were collected for each vial to ensure a standard deviation of ± 100 counts or 1%. The scintillation counter automatically converted the counts, with a correction for dead time, to counts per minute (cpm). The mean activity calculated from the means of all the treatment conditions is 1,787 $\pm$ 268 SD (n = 60) cpm. A sample-channels ratio was always measured to ensure uniform counting efficiency.

Data Analysis

The data were analyzed as described by Blackman et al [1982]. The radioactivity in the control sample was used to normalize the radioactivity in the paired experimental sample to adjust for possible influences caused by differences in brain mass among animals, in specific activity of the labeling solutions, and in age and sex of the animal. Previous results based on normalization by brain mass [Blackman et al, 1979] confirmed the validity of this approach [Blackman et al, 1980a]. Data from exposed and sham-exposed samples were compared for each exposure condition. Exceptions to these procedures occurred in those circumstances where no sham treatments were performed. In those cases, the data from two different exposure conditions were compared.

The data collected for each test of the various experimental conditions were analyzed by a two-way analysis of variance for a replication main effect and for a replication-by-exposure group interaction. Neither the replication main effect or replication-by-exposure group interaction was significant in any experiment, so a one-way analysis of variance was used to test for effects of exposure.

Replicate experimental tests separated by a week to as much as 8 months were conducted for the following conditions: 1) 16-Hz, 40 V_{p-p}/m electric versus 40 V_{p-p}/m, and 59.5 nT (rms) electromagnetic fields; 2) 15-Hz, 40 V_{p-p}/m, and 59.5 nT (rms) electromagnetic fields at two LGF densities; and 3) 30-Hz, 40 V_{p-p}/m, and 59.5 nT (rms) electromagnetic fields at two combinations of LGF densities. For each of these conditions the data were combined and an analysis of variance for a partially nested design was used to test for time and replication-within-time main effects and time-by-exposure group and replication-by-exposure group within time interactions. None of the main effects or interactions was significant. This implies that the data from the

experiments done at different times can be combined into one analysis. Therefore, a one-way analysis of variance of the combined data was used to test for effects of exposure.

To ensure that a slight shift from a normal distribution for the ratio values did not affect the conclusions, a log transform of the ratios, which produced a normally distributed sample set, was also analyzed.

RESULTS

Exposure to 16-Hz electric fields alone at 6, 10, or 40 $V_{p\text{-}p}$/m produced no significant differences in efflux compared with the corresponding sham treatments (Table 1). The analysis of the combined 40 $V_{p\text{-}p}$/m data similarly demonstrated no significant difference (P = .471). These results are displayed in Figure 1 along with results from Blackman et al [1982], showing enhancement caused by electromagnetic fields at 6 and 40 $V_{p\text{-}p}$/m (8.9 and 59.5 nT [rms], respectively), and Bawin and Adey [1976], showing reduction caused by electric fields at 10 $V_{p\text{-}p}$/m. Although the differences are apparent, the data are not conclusive because a direct comparison was not performed in a single experiment.

The result of the direct comparison between 16-Hz electric and electromagnetic fields is given in Table 2 and indicates that the results of the two treatments are significantly different. The elevated mean value for efflux caused by the electromagnetic field is consistent with the data obtained at 40 $V_{p\text{-}p}$/m in the earlier study [Blackman et al, 1982]. The data demonstrate that the AC magnetic component must be present in the 6- and 40-$V_{p\text{-}p}$/m fields to induce an enhanced efflux.

The results of exposure to various multiples of the ambient LGF density compared to the ambient density of 38 μT are shown in Table 3, along with the P value calculated for the normalized values and for the log transform of the normalized values. The results demonstrate that a normally effective 15-Hz signal is ineffective when the net density of the LGF is reduced to half the ambient, and an ineffective 30-Hz signal is effective when the net density is changed to .67× or 2.0× ambient but not when it is increased to 1.33× or to 2.185× ambient.

The comparable statistical results with the normalized values or their log transforms in Tables 1, 2, and 3 demonstrate that the ratio values do not deviate

TABLE 1. Mean Relative Quantity of Calcium Ions Released by Brain Tissue Pairs When Exposed to a 16-Hz Electric Field

Intensity ($V_{p\text{-}p}$/m)	Treatment	N	Mean[a]	SE	P	P(LN)[b]
6	Sham	32	1.021	0.047		
	Exposed	32	1.075	0.042	0.394	0.303
10	Sham	28	1.004	0.047		
	Exposed	28	.995	0.057	0.902	0.721
40	Sham	32	1.031	0.036		
	Exposed	32	1.116	0.056	0.209	0.285
40	Sham	32	1.048	0.039		
	Exposed	32	1.025	0.035	0.666	0.707

[a]Ratio of the counts per minute in the treated sample compared to the control sample.
[b]Probability value for the natural-log transformed data.

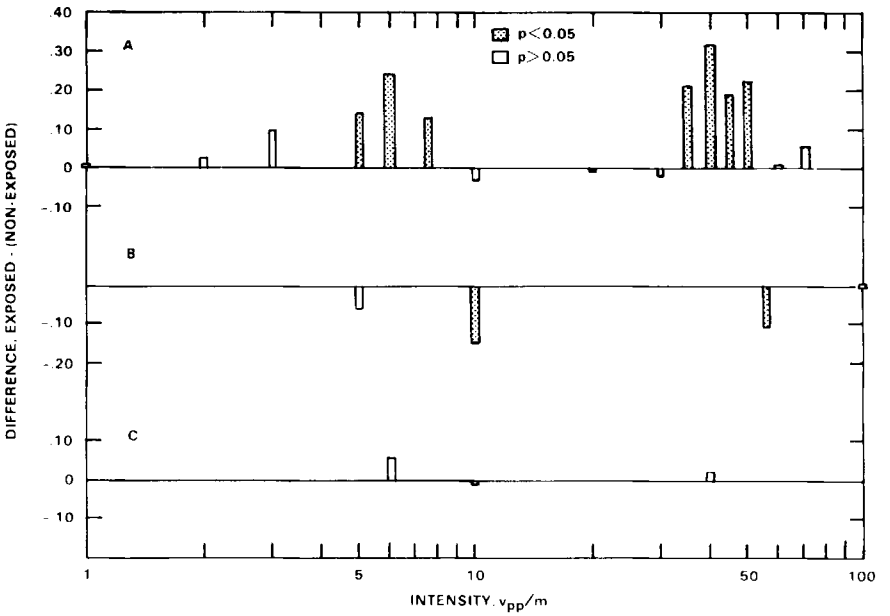

Fig. 1. Effect of 16-Hz fields on the efflux of calcium ions from chicken brain tissue as a function of intensity. Efflux is calculated as the difference in amount of calcium ions released from the exposed tissues as compared with the nonexposed tissues. A) Data from Blackman et al [1982] for an electromagnetic field. B) Data from Bawin and Adey [1976] for an electric field only. C) Data from this study, Table 1, for an electric field only. The 40 $V_{p\text{-}p}$/m data have been combined.

TABLE 2. Mean Relative Quantity of Calcium Ions Released by Brain Tissue Pairs When Exposed to 16-Hz, 40 $V_{p\text{-}p}$/m Electric or Electromagnetic† Fields

Signal	N	Mean[a]	SE	P	P(LN)[b]
Electric	36	1.041	0.039		
Electromagnetic	36	1.191	0.042	0.011*	0.010*

†Intensity of magnetic component is 59.5 nT (rms).
[a]Ratio of counts per minute in the treated sample compared to the control sample.
[b]Probability value for the natural-log transformed data.
*$P < .05$.

sufficiently from normality to affect the conclusions of the statistical analyses. Analyses of the log transformed data in a recent study [Blackman et al, 1985] produced the same conclusion.

DISCUSSION

Exposure to isolated electric fields at certain intensities in contrast to electromagnetic fields did not produce a significant change in efflux of calcium ions. This finding holds for our previous results (Fig. 1), and when tested directly (Table 2). Our results contrast with those of Bawin and Adey [1976] in two ways: 1) we observed enhanced efflux with 16-Hz electromagnetic fields while they observed reduced efflux and 2) they did and we did not detect a significant difference in calcium-ion efflux when just the AC electric component is present at 10 $V_{p\text{-}p}$/m. With

TABLE 3. Mean Relative Quantity of Calcium Ions Released From Brain Tissue as a Function of the Local Geomagnetic Field Density

Net Field[a]	Applied Field[a]	N	Mean[b]	SE	P	P(LN)[c]
15 Hz						
0.5	−0.5	32	1.045	0.049		
1.0	0	32	1.199	0.054	0.040*	0.043*
0.5	−0.5	32	0.997	0.042		
1.0	0	32	1.236	0.046	<0.001***	<0.001***
30 Hz						
2.0	1	32	1.309	0.078		
1.0	0	32	1.040	0.038	0.003**	0.005**
1.33	0.33	32	1.058	0.049		
1.00	0	32	1.048	0.037	0.870	0.986
0.67	−0.33	40	1.248	0.054		
1.00	0	40	1.020	0.038	<0.001***	0.001**
0.67	−0.33	28	1.208	0.033		
1.00	0	32	0.981	0.042	<0.001***	<0.001***
−0.67	−1.67	32	1.143	0.048		
1.00	0	32	1.041	0.032	0.085[d]	0.110[d]
−0.67	−1.67	32	1.323	0.068		
1.00	0	32	1.065	0.040	0.002**,[d]	0.003**,[d]
−2.0	−3.0	32	1.300	0.089		
1.0	0	32	1.028	0.035	0.006**	0.007**
−2.185	−3.185	32	1.076	0.036		
1.0	0	32	1.002	0.049	0.226	0.148

[a]Multiple of the normal local geomagnetic field density (38 μT).
[b]Ratio of counts per minute in the treated sample compared to the control sample.
[c]Probability value for the natural-log transformed data.
[d]Combined data from both tests of −0.67× gave $P < 0.001$ and $P(LN) < 0.001$; see text.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

respect to the direction of field-induced efflux, we still agree with our earlier statement that the "discrepancy in the direction of efflux change will probably be resolved once the mechanism responsible for the ELF-induced efflux changes is understood" [Blackman et al, 1982]. The lack of agreement on the effectiveness of the 10 $V_{p\text{-}p}$/m field may reflect slightly different absolute measurements of voltage reported by the two groups (see Fig. 1). A more detailed intensity series should be performed to address this issue. Thus, further research is necessary to investigate these discrepancies.

The results of this study also demonstrate that the density of the LGF directly affects the frequency-specific response of brain tissue to AC electromagnetic (EM) fields. Exposure of chicken brain tissue to a 15- or 16-Hz, 40 $V_{p\text{-}p}$/m (59.5 nT [rms]) electromagnetic field can cause significantly enhanced calcium-ion efflux under the ambient, LGF conditions in our laboratory, whereas 1- and 30- or 32-Hz fields do not [Blackman et al, 1982, 1985]. The 15-Hz data in Table 3 show that a reduction in the LGF to 19 μT (half the ambient density) was sufficient to eliminate the effect. Alternatively, the 30-Hz data in Table 3 show that a normally ineffective 30-Hz,

40 $V_{p\text{-}p}$/m (59.5 nT [rms]) field caused enhanced calcium-ion efflux when the density of the LGF was appropriately altered; however, the direction of the applied field generated by the Helmholtz coils was critical. For an applied magnetic field antiparallel to and 0.33× the density of the ambient LGF, a 30-Hz electromagnetic field induced enhanced efflux; the same magnetic field applied parallel to the LGF prevented the induced efflux. Thus, it is the density of the net magnetic field that was critical; increased efflux resulted at LGFs + 0.67× ambient and of ± 2.0× ambient when compared to the lack of enhancement for ambient conditions. Although one of the tests conducted at −.67× ambient was not quite statistically significant ($P = .085$), the combined data, which the analysis allowed, did demonstrate a difference at $P < .001$ for both the ratio and the log transformed data. A slight increase in the density of the LGF from −2.0× to −2.185× the ambient was sufficient to remove the enhancement conditions. Although both the density and direction of the magnetic field generated by the Helmholtz coils were independent variables in these experiments, the net DC magnetic field vector is the independent variable directly involved in the mechanism underlying this phenomenon.

These data can be rationalized by considering a generalized relationship that characterizes the interaction of magnetic fields with some molecular system(s) in which an excitatory frequency, F, is coupled to the density of the magnetic inductance, B, by the equation, $F \propto B\ (2n + 1)$, $n = 0,1$. The data from Table 3 and this generalized relationship, plotted in Figure 2, are in agreement for $n = 0$ and $n = 1$. This relationship also predicts that a 45-Hz electromagnetic field should produce enhanced efflux under ambient conditions; this result has been demonstrated [Blackman et al, 1985]. While larger values of n predict additional effective frequency and intensity combinations, we have not yet tested those conditions with altered LGFs.

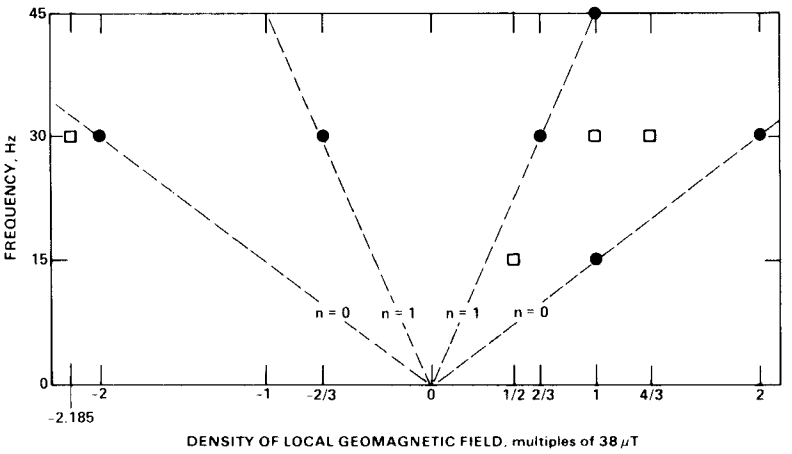

Fig. 2. Effect of the local geomagnetic field (LGF) on the induction of calcium-ion efflux from brain tissue by 15-, 30- and 45-Hz electromagnetic fields. Results at altered LGF densities, given as multiples of the normal ambient value, were always compared to coincident results obtained at the ambient value (38 μT). The closed circles indicate statistically significant differences, while the open squares indicate a nonsignificant difference between the two LGF conditions. The data for 15-, 30-, and 45-Hz exposures compared to sham exposures at an LGF density of 38 μT (1.0×) are taken from Blackman et al [1985]. The lines display a generalized, heuristic relationship between the frequency of the oscillating field and the LGF density times an index, 2n + 1, where n = 0, 1. Initial conditions were established as n = 0 for the excitatory 15-Hz field and the ambient LGF density (1.0×).

It should be remembered that the results presented here are the first ones obtained that link the frequency of an ELF electromagnetic field with the density of the LGF in the production of a biological response. These results must be independently corroborated and extended to establish the underlying principles governing this phenomenon and the ramifications for other biological specimens. The model presented above is purely a heuristic relationship. We hope it will serve as a guide in future experiments to identify exposure conditions that should be studied in a detailed manner.

Our results indicate a probable cause for highly variable results and for inability to corroborate effects reported by different investigators. If magnetic resonance is the underlying basis by which low frequency EM fields cause biophysical changes, as has been suggested [Jafary-Asl et al, 1983], then effective frequencies may be directly proportional to the intensity and direction of the ambient LGF. Any replication of phenomena involving oscillating EM fields may be difficult unless the ambient LGF vector is explicitly utilized in the selection of both the appropriate stimulatory frequency and the orientation of the exposure apparatus. Also, it is not known whether the oscillating electric or magnetic component is the effective agent. Nevertheless, the intensity and orientation of the geomagnetic field can be different at various geographical sites as well as at different locations within the same room due to the building construction. Thus, the LGF vector may be a fundamental experimental variable that has not been recognized and controlled in previous studies.

There are a number of reports describing various biological changes caused by low frequency electric, magnetic, and electromagnetic fields. It is possible that the LGF is directly involved in those biological responses that are frequency dependent. Exposure to radiofrequency radiation, amplitude modulated at specific frequencies below 100 Hz, can alter the release of calcium ions in a synaptosome preparation from the rat brain [Lin-Liu and Adey, 1982], in cultures of neuroblastoma cells derived from mouse and human brain tumors [Dutta et al, 1984], and in the cerebral cortex of an alive, awake cat [Adey et al, 1982]. Furthermore, non-CNS tissue is also affected by radiofrequency radiation, modulated at specific frequencies. Such fields have been shown to alter the rate of calcium-ion release in rat pancreatic tissue slices [Albert et al, 1980] and in frog heart muscle [Schwartz et al, 1983] and to cause a reduction in the cytotoxic activity of mouse T-lymphocytes [Lyle et al, 1983]. Sinusoidally oscillating low frequency magnetic fields of low intensity reduce the respiration rate and lengthen the mitotic cycle of *Physarum polycephalum*, a myxomycete [Goodman et al, 1979], alter the oviposition of *Drosophila* eggs and the subsequent viability of the eggs and pupae [Ramirez et al, 1983], enhance the synthesis of DNA in cultures of human fibroblasts [Liboff et al, 1984], and lower the core temperature in rats [Smith, 1983]. Pulsed magnetic fields also cause biological changes in a variety of systems [Dixey and Rein, 1982; Delgado et al, 1982; Ramirez et al, 1983; Ubeda et al, 1983; Chiabrera et al, 1979; Goodman et al, 1983; Luben et al, 1982]. Fields emitted by electric power lines have caused inconsistent results in various studies designed to detect and evaluate biological changes; these variable results have been a source of concern [Graves et al, 1979]. It is possible that different values of the LGF contribute to these variable results and to difficulties encountered in attempts at replication. Future studies of biological effects induced by low-intensity ELF fields should be designed to evaluate the potential influence of the LGF conditions at the testing sites.

Finally, detailed evaluation of exposure parameters may provide information on the fundamental nature and site of interaction. Information essential for identification and characterization of the initial reaction site(s) could come from defining both the effective oscillating frequencies and the influence of the intensities and relative orientation of the steady-state and oscillating fields. The ramifications of these results for biological processes in general have yet to be determined. Moreover, theoretical studies describing potential mechanisms of action may need to be revised to include the possible involvement of the LGF.

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