Alterations in Calcium Ion Activity Caused by ELF and RF Electromagnetic Fields*

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Introduction

Calcium ions play many important roles in biological systems. For example, calcium ion activity can be used as an indicator of second-messenger signal-transduction processes in cells. Intracellular stores of calcium are housed in the mitochondria, the endoplasmic reticulum and other membrane structures, and in calmodulin molecules, and are released upon triggering by signaling events that alter activities of various biochemical pathways and molecular events. Calcium ion activity also plays a role in membrane integrity and function, and is particularly critical for activity in both the central and peripheral nervous system, especially neurotransmitter release and action potential generation.

A number of studies of the action of electric and magnetic fields (EMF) on biological systems have used calcium ion activity as a monitor of effects. In one series of studies, calcium ion activity was initially demonstrated to be a surrogate for neurotransmitter release from the brain of cats (Kaczmarek and Adey, 1973, 1974). As a neurotransmitter surrogate, calcium ion activity was then used in a major series of studies by two independent groups (reviewed by Adey, 1992; Blackman, 1992) to characterize the effect of EMF on brain tissue removed from newly hatched chickens. Two other groups also used calcium ion activity to study the action of EMF on neuroblastoma cells in culture (Dutta et al., 1984, 1989, 1992) and on beating frog heart (Schwartz et al., 1990). The purpose of this presentation is to highlight the use of this chick-brain model system to identify critical exposure conditions that are required for EMF to influence nervous system preparations and to demonstrate similarities in biological response to EMF of widely differing frequencies.

Materials and Methods

Brain tissue from newly hatched chickens (one-to-seven days old) was used as a surrogate for mammalian brain tissue. Preparation of the tissue: both forebrain halves were removed from the chick, and labeled for 30 minutes in a salt solution containing tracer amounts of radioactive calcium ions. After thorough rinsing, paired brain halves from the same chick were place in separate tubes with similar salt solution, without radioactive calcium ions. One tube was place in the exposure chamber and the other tube was placed in a water bath. After treated for 20 minutes to EMF or sham (fields off) conditions the salt solutions containing the brain halves were assayed for the presence of radioactive calcium ions. Radioactive counts from samples in the exposure chamber were divided by the counts from the paired brain halves in the waterbath to normalize the data. The results were then evaluated by comparing the normalized release of radioactive calcium ion from exposed and sham-exposed brain tissues.

A variety of EMF exposure conditions were tested in order to identify critical characteristics that were required to produce changes in calcium ion activity. Two widely different frequency conditions were tested: (a) sinusoidal waves between 1 and 510 Hertz (Hz), defined here as ELF, and (b) sinusoidal

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waves at 50, 147 and 450 MHz radiofrequency radiation (RFR) was amplitude modulated (AM) at ELF, defined here as AM-RFR. Although these frequency conditions are very dissimilar from the view of field generation, it will be seen that the biological responses caused by these EMF appear to be very similar. For both frequency conditions, dose response data were obtained at selected frequency values to better characterize the possible mode(s) of action. Lastly, the unexpected non-linear responses to the frequency and intensity parameters motivated a search for other conditions that could influence these results. Two prominent studies examined the possible influence of the earth's magnetic field and the EMF exposure history of the incubating eggs on the responses of the brain tissue to EMF.

EMF was generated by two signal sources: function generators were used to generate ELF, and both function generators and RF signal sources were linked to generate AM-RFR. The exposure chambers for both ELF and AM-RFR consisted of TEM cells (Crawford cells) located in a temperature controlled environment. In one case, parallel plates were used to generate ELF electric fields.

Results

Changes in calcium ion activity in biological specimens have been shown to be a non-linear function of EMF intensity in both the ELF and the AM-RFR frequency ranges. ELF exposures of chick brains at 16 Hz have produced calcium ion activity changes in two very distinct intensity ranges bracketed by intensity ranges in which no changes occur (Figure 1). More detailed testing was conducted to investigate this non-linear response by our group and by Adey's group (see Adey, 1992 and Blackman, 1992 and references therein), using AM-RFR at 50, 147 and 450 MHz, AM at 16 Hz. The results of our effort demonstrated additional intensity regions in which calcium ion activity changes occur interspersed with no-effect intensity regions (Figure 2). These non-linear responses have been called response "windows." It was hypothesized by Grodsky (1976) and others, that the underlying basis for this window phenomenon involves cooperative processes of dynamical systems being influenced by EMF. Recently, Thompson et al. (1998) revised and simplified Grodsky's model and applied it to our data collected for AM-RFR at 50 and 147 MHz. With one adjustable parameter they were able to predict the outcome of 29 of our 30 experiments, carried out at different intensities (Thompson et al., Bioelectromagnetics, 2000, in press).

Specific frequencies of EMF have also been shown to have a differential effect on calcium ion activity. AM-RFR exposures cause changes in calcium ion activity only when the RFR is amplitude modulated, and then only within a certain AM frequency region around 16 Hz. Effects occurred for modulations of 6, 9, 11, 16 and 20 Hz, but no effects occurred for modulations of 0.5, 3, 25 and 35 Hz. More detailed testing of ELF frequencies between 1 and 510 Hz have shown a series of frequency windows of effects, separated by no-effect frequencies (Figure 3). It was subsequently shown that both the intensity (Figure 4) and the orientation of the earth's magnetic field during exposure can alter effects at specific frequencies. These results led to the development of ion resonance models and tests of their predictions (Blackman, 1985; Liboff, 1985).

A separate experiment was conducted to investigate whether the EMF frequency environment for a developing chick embryo could influence the frequency and intensity window responses of the brain tissue from the hatched chicks. Eggs were exposed to 10 V/m electric fields of either 50 or 60 Hz for their entire 21 day incubation period, and the brain tissues from hatched chickens were tested for changed calcium ion activity. It was found that the exposure of the eggs changed the responses of the brain tissues obtained from the hatched chickens (Blackman et al., 1988).

Other biological systems have shown non-linear calcium ion activity as a function of intensity and frequency. Schwartz et al. (1990) found enhanced release of calcium ions from beating frog-heart preparations only within two intensity regions separated by no-response regions when exposed to 240 MHz amplitude modulated at 16 Hz. Further, Dutta et al. (1984, 1987, 1992) found non-linear changes in calcium ion activity from human neuroblastoma cells in culture when exposed to ELF fields. Thus, the non-linear nature of the calcium-ion activity response to EMF exposure has been observed in several biological systems and is well established.

Discussion

The work with EMF-induced changes in calcium fluxes was initiated by a publication in 1968 describing ELF-induced changes in response time in a behavioral test of human subjects (Hamer, 1968). Subsequent publications from this research group with monkeys showed similar changes in response time as observed for human subjects, and also changes in EEG patterns. This work led to studies on cat EEG patterns and studies monitoring changes in neurotransmitter release and calcium ion release (these experiments have been reviewed in Blackman, 1999). In turn, those results led to the work discussed above with chick brain preparation and, additionally, to brain cell culture and innervated heart studies. Lastly, a theoretical model proposed in 1976 has recently been revised, reformulated, and applied to the intensity window phenomenon (Thompson et al., Bioelectromagnetics, 2000, in press). This model predicts 29 out of 30 data points in our AM-RFR experiments with a single fitting parameter; a highly significant result. Thus, there is a demonstrated linkage for EMF-induced effects from human behavioral changes, through changed brain electrical activity and biochemical changes associated with brain electrical activity to theoretical models for EMF-induced changes in calcium ion activity. This body of work represents a major accomplishment connecting human behavioral effects from EMF exposure to mode of action explanations.

Several independent laboratories have observed this non-linear calcium-ion activity phenomenon in nervous system specimens and other tissues. The phenomenon is not due to thermal loading from exposure to EMF. However, these results have not been properly evaluated nor utilized by many organizations currently attempting to set exposure standards to protect public health.

The dismissal of this extensive body of coordinated experimental and theoretical work by most current standards setting committees needs to be re-evaluated. This re-evaluation should be strongly motivated by recent laboratory reports that exposure to cell phone transmissions causes changes in response times in humans. Are these results connected in any way to those changes already observed in the late 1960s and early 1970s in humans and in monkeys?

The data presented here on EMF-induced changes in calcium ion activity and coordinated biological studies underscores the value of shifting from the historical engineering-based approach to a biologically-based public health reference frame. In the past, dominance of the engineering paradigm has caused coordinated studies in the ELF and the RFR frequency bands to be evaluated by different groups, thereby diminishing information that would otherwise have provided powerful justification for additional inquiry

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Figure 1 Calcium ion activity in the chick brain preparation as a function of intensity of the 16-Hz magnetic fields. Between 28 and 32 samples were used to obtain the exposed and sham values for each intensity. There are two well-defined intensity regions that produce statistically significantly differences in calcium ion activity. Note: 1 mGrms = 0.1 µTrms.

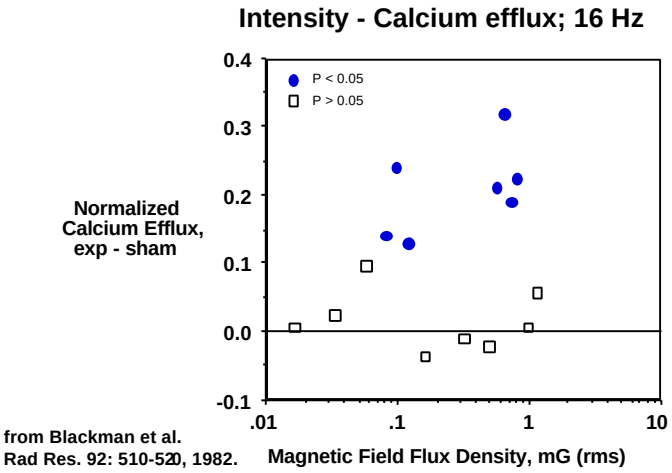

Figure 2 Response of chick brain tissue to 50 MHz RFR, amplitude modulated at 16 Hz. Log P represents the log of the probability that exposed and sham exposed samples are from the same population as a function of AM-RFR intensity; if the samples are not from the same population, the probability function is small (log probability more negative) and indicates an effect region. Between 28 and 32 samples were used to obtain the exposed and sham values for each power density. Black and striped bars indicate statistically significant differences at $p < 0.05$; white and gray bars indicate no-effect regions. The ratio of the difference between adjacent power densities producing maximal effects, i.e., a/b, b/c, c/d, are all equal to 1.38. This observation was not thoroughly understood at the time, but it appears to reflect results of the model developed by Thompson et al. (1998). Note: 1 mW/cm^2 = 10 W/m^2.

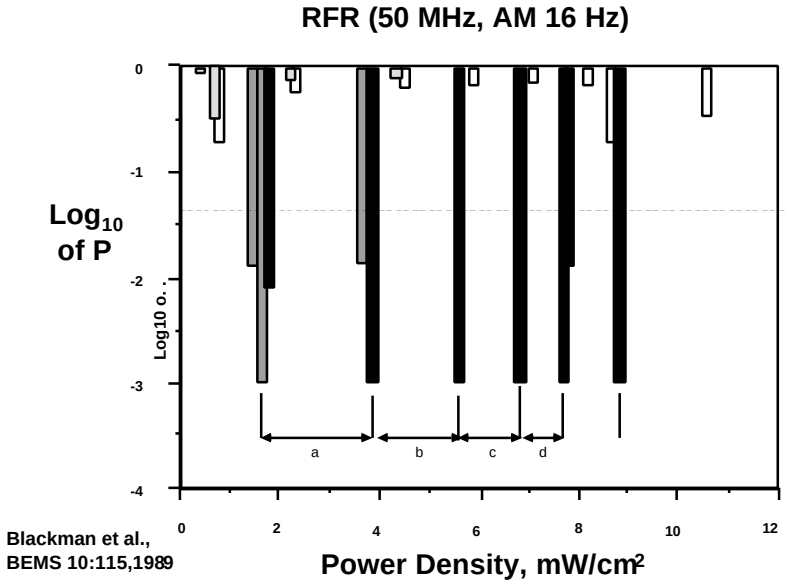

Figure 3 Response of chick brain tissue to different frequencies of ELF from 1 to 510 Hz, at a constant field strength of 0.69 mG (0.069 µTrms). Between 28 and 32 samples were used to obtain the exposed and sham values for each frequency. Solid circles indicates statistically significant changes in calcium ion activity in exposed compared to sham-exposed samples ($p < 0.05$).

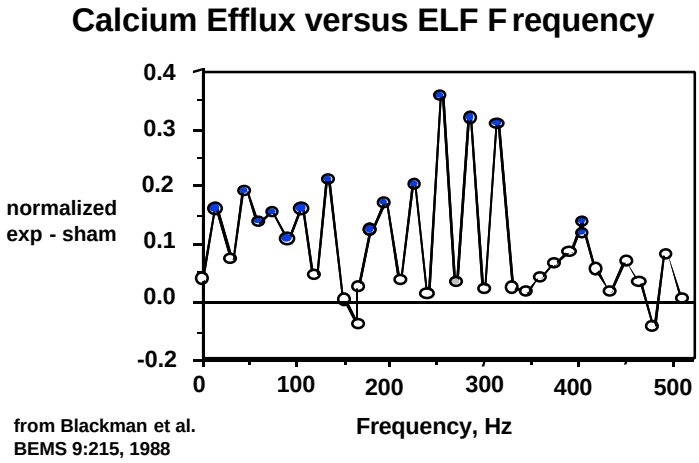

Figure 4 Influence of DC magnetic field on ELF frequencies that produce statistically significant differences in calcium ion activity in the chick brain preparation. Between 28 and 32 samples were used to obtain the exposed and sham values for each data point. The dashed vertical line at 380 mG (38.0 µT) indicates the ambient, DC magnetic-field flux density; frequency effects occur at 15 and 45 Hz (darkened symbol) but not at 30 Hz (open symbol). Changing the DC magnetic-field flux density to specific values causes 30 Hz to become effective and 15 Hz ineffective in altering calcium ion activity.