LONG-TERM LOW-LEVEL ELECTROMAGNETIC RADIATION CAUSES CHANGES IN THE EEG OF FREELY-MOVING RATS

H.S. MOHAMMED, N.M. RADWAN**, NAWAL A. AHMED***

*Biophysics Department, Faculty of Science, Cairo University, Giza, Egypt, e-mail: haitham_sharaf@yahoo.com
**Zoology Department, Faculty of Science, Cairo University, Giza, Egypt

Abstract. In the present study the effect of long-term exposure (1 hour/day for 4 months) to electromagnetic radiation field (EMF) (900 MHz, 0.02 mW/cm^2, SAR: 1.165 W/kg) on the EEG of freely moving rat is investigated. The spectral analysis of the EEG bands was done after 1, 2 and 4 months of daily EMF irradiation and after 1 month of stopping irradiation that extended daily for 4 months. The quantitative analysis of EEG revealed a clear shift from high frequency (beta) to lower frequency (delta) in irradiated animals as compared to unexposed animals, which indicated the change in the animal arousal. The cumulative effect of non-thermal EMF has been suggested for chronic exposure to radiation. It could be suggested that EMF cause changes in central cholinergic system that change the cortical excitability that reflected in slowing down of the EEG frequencies.

Key words: Electromagnetic, radiation, EEG, rat, freely moving.

INTRODUCTION

During recent years, mobile communication systems have experienced wide and rapidly growing use all over the world. Ever since, considerable concerns have been expressed about possible health risks to the human organism and, in particular, to the brain.

The nervous system has been thought to be most sensitive to external EMF since there is a tremendous electrical activity in neural processes. Information processing and transmission in nervous system is based on bioelectromagnetic phenomena. Therefore, it can be expected that EMF effects appear most likely in the nervous system.

Electroencephalography (EEG) is one of the major tools used to investigate the average electrical activity of the brain in both animal and human. EEG served as a sensitive tool in quantifying the effects of various factors on the brain.

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Several investigators have reported that low-level exposure produces alterations in the EEG signal and brain behavior [1, 4, 7, 8, 12, 14, 20, 21] while others conclude that exposure to EMF field does not alter resting EEG [11, 18, 27, 37].

The aim of the present study was to investigate the effect of chronic exposure to low level (0.02 mW/cm^2) 900 MHz electromagnetic radiation field on the EEG of freely moving rats.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

A total of 48 experimental animals (male adult albino rat, *Rattus norvegicus*) were used in this study. The average weight of the animals was 250– 350 g. The animals obtained from local suppliers were housed in groups of 6 to 8 animals in separate cages for two weeks before starting experiments. The food and water were available for the animals ad libitum. All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center, Egypt.

The experimental animals were divided into 4 groups. Each group consisted of 12 animals was divided equally into irradiated and control animals.

To record EEG signals from the animals, an electrode implantation surgery was done as described in our previous work [26]. Briefly, the animal was anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg). Then the animal was positioned in the stereotaxic machine (David Kopf instruments, Tujunga, California, USA) and three holes were drilled by using a dental drill in the following places: 2 mm interior and 2 mm lateral to the Bregma, 2 mm posterior and 2 mm lateral to the Bregma and the third electrode was placed 1mm posterior to the Lambda. The electrodes were self threading stainless steel screws with 1mm diameter and 3 mm height. The electrodes fixed in their place and isolated by a layer of dental cement (zinc polycarboxylate, Spofa-Dental-Praha, Czech Republic). After the surgery the animals were individually housed in separate cages for 7 to 10 days to recover from the surgery. In the EEG recording session the animal was placed in a sound attenuated and electrically isolated cage for 10 min before the session's start. The recoding session lasted for one hour for each animal.

EXPOSURE SETUP

The electromagnetic radiation (900 MHz) was generated by a radiofrequency (RF) oscillator (Aeroflex company, model: 2025, UK) with an RF output level up to 13 dBm and a frequency range of 0–2.5 GHz. A power amplifier (Stealth

Microwave model, SSB Technologies Inc.), was used to supply a power intensity of 0.02 mW/cm^2 at the rat location. The EMF was delivered to the animals *via* a monopole antenna placed at the center of the equally radiated container. A field survey meter (Narda, EMR200, frequency from 0 to 4 GHz, Germany) was used to measure the power density of the EMF at the place of the irradiated rats. Readjustment of the RF generator intensity was made at the beginning of the exposure session to insure the constant value of the radiation power density during the course of the experiment.

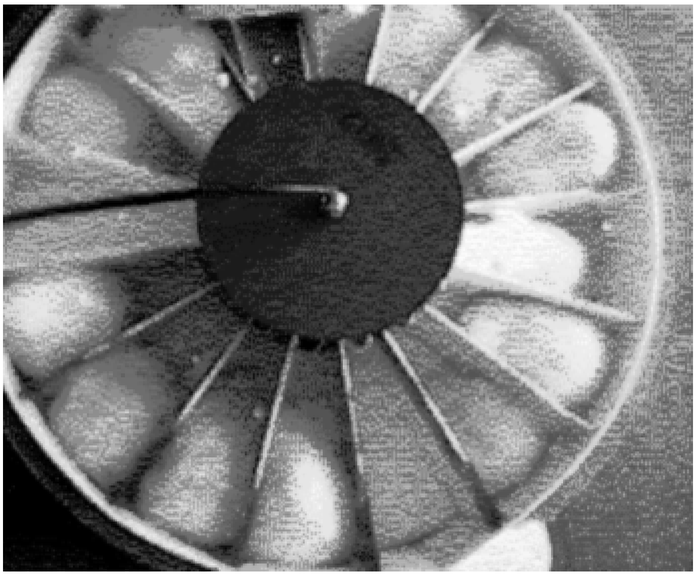

Fig. 1. Photo of the irradiation setup.

As shown in Fig. 1 circularly radiated containers were manufactured from a special kind of opaque plastic for the purpose of animal irradiation. The container, which has a diameter of 40 cm, is divided into 16 sectors each sector of 14 cm in length and with a cone shape and covered with transparent plastic sheet, which contains small openings (7 mm) necessary for breathing for each animal. The length of the monopole antenna placed at the center of the container was set so that the antenna is resonant at the operating frequency. The antenna had an omni-directional pattern in the azimuth plane through which the rats were to be uniformly distributed. The average SAR was 1.165 W/kg as calculated by the finite-difference-time-domain (FDTD). A geometric/electric model was constructed for the animal's head from the stereotaxic atlas of Paxinos and Watson [24]. An ellipsoid model with the internal anatomic layers was used. The electric properties were assigned to each layer. The animal head model was then subjected to irradiation with the electromagnetic field with a power density as that measured by the field survey meter through the experimental exposure process. The FDTD algorithm was then applied to calculate the electric field distribution everywhere inside the head model. The SAR was then calculated at the desired points as $E^2/2\sigma$, where E is the electric field value at the point and σ is the conductivity of the tissue at this point.

The control group of animals was placed in the same exposure conditions of the exposed animals without the radiation antenna. The EEG recording was started for the groups of animals which are irradiated continuously for 1 hour/day for one month, two and four months. The EEG recording of the fourth group was started after four months of exposure and another month without exposure (recovery group).

DATA ANALYSIS AND STATISTICS

The average power spectrum for each EEG recording session (one hour) was obtained by Biobench software (USA) and this spectrum was divided into four frequency bands: Delta (0.5–3Hz), Theta (4–7Hz), alpha (8–12 Hz), beta (13–20). The band power was calculated for each EEG frequency band, and then the percentage of each band was estimated as follows:

$$\frac{\text{Band power}}{\text{Total power}} \times 100 \tag{1}$$

The total power is the summation of the power in all EEG frequency bands.

The mean and the standard deviation for each group of animals were calculated and compared using the Student's t-test (statgraph, V2, 1985).

RESULTS

The data presented in Table 1 showing the changes in spectral band power of different EEG frequencies after 1, 2 and 4 months of EMF exposure and after another month of recovery for which the radiation exposure was stopped.

The 1 hour/day continuous irradiation of animals for one month induced a marked increase in the spectral power of Delta band (+29.65 %), a slight decrease in Theta and alpha bands (–7.08 % and –4.81%, respectively) and a marked decrease in Beta-1 band (–38.82 %) with respect to the sham exposed animals.

After two months of radiation exposure the one month obtained results emphasized by a significant increase in the spectral power of Delta band (+43.43 %). Accompanied to this increase in EEG slow wave (Delta) a decrease in Theta and alpha bands (–7.4% and –16%, respectively) and a further marked decrease in Beta band (–51.07%) had been observed with respect to the sham exposed animals.

Table 1

Effect of EMF irradiation on the normalized band power of different EEG frequencies of freely moving rats

	Exposure period	Irradiated	Control	% Difference
Delta wave	1 month	38.30 ± 0.093	29.54 ± 0.043	29.65
	2 months	42.37 ± 0.055	30.50 ± 0.033	38.92*
	4 months	44.98 ± 0.032	28.32 ± 0.046	58.82*
	4 + 1 month recovery	35.84 ± 0.13	29.44 ± 0.044	21.74
Theta wave	1 month	31.49 ± 0.049	33.89 ± 0.093	–7.08
	2 months	31.38 ± 0.036	32.90 ± 0.087	–4.62
	4 months	32.16 ± 0.033	34.88 ± 0.066	–7.79
	4 + 1 month recovery	26.03 ± 0.094	34.32 ± 0.098	–24.16
Alpha wave	1 month	16.02 ± 0.035	16.83 ± 0.038	–4.81
	2 months	14.12 ± 0.026	17.33 ± 0.033	–18.52
	4 months	17.76 ± 0.018	16.33 ± 0.50	8.76
	4 + 1 month recovery	10.20 ± 0.012	16.76 ± 0.76	–39.14*
Beta wave	1 month	9.44 ± 0.047	15.43 ± 0.14	–38.82
	2 months	10.49 ± 0.016	18.98 ± 0.05	–44.73*
	4 months	7.55 ± 0.018	16.76 ± 0.098	–54.95*
	4 + 1 month recovery	7.05 ± 0.038	14.89 ± 0.033	–52.59

Mean ± SD of the irradiated and control animal groups with the percentage difference between them is represented; the number of animals for each group equals 6; * Significant changes at $P < 0.05$ compared to sham exposed animals.

Continuing the exposure for 4 months resulted in an increase in the spectral power of Delta band (+15.03%), a little decrease in Theta band (–5.1%), a little increase in the Alpha band (+5.52%) and a marked decrease in Beta band (–32.01%) with respect to the sham exposed animals.

After stopping radiation exposure for one month there was still a marked increase in the spectral power of Delta band (+21.32%), a marked decrease in Theta band (–23.2%), a significant decrease in Alpha band (–39.4%) and a marked decrease in Beta band (–54.3%) with respect to the sham exposed animals.

DISCUSSION

The results, in the present study, showed that the long term exposure of the adult male rat to low level EMF has an effect on the EEG of the exposed animals with respect to the sham exposed animals.

There was an increase in the low frequency EEG bands (0.5–3 Hz, delta band) accompanied by a simultaneous decrease in the high frequency EEG bands (13 – 25 Hz, Beta band) after one, two and four months of exposure and also after stopping of exposure for one recovery month. These findings are in agreement with Takashima *et al*. [33] who reported a decrease in high frequency EEG bands and an increase in low frequency bands after chronic exposure of the male rabbit to Radiofrequency field. Similarly, acute exposure of rats and rabbits to continuous microwaves increased EEG delta activity [29]. In addition, Chizhenkova [3] reported that the heads of unanesthetized rabbits exposed to 2.4 GHz for 1 min at 40 mW/cm^2 demonstrated EEG spindle-shaped firings and an increase in the number of slow waves. In a recent study, Barcal and Vozeh [2] found that the EMF exposure showed a distinct shift to lower frequency in ECoG components in mice. In human, some studies on the effects of high frequency EMF on the EEG have reported the appearance of slow wave activity during and after the field exposure [14, 17].On the other hand, no uniform changes in EEG power spectra were also reported following exposure to continuous microwaves [22]. Röschke and Mann [27] were unable to detect any differences in EEG spectra related to exposure to mobile phone signal.

The increase in delta power was frequently reported following exposure to EMFs in animals (see [10]); this was usually attributed to the thermal effects of EMFs in the brain. In the present study the increase in delta power cannot be attributed to thermal effect since, in this study, low intensity EMF radiation is applied. However, this result emphasizes that the EMF at non-thermal range can have a similar effect as thermal intensity on the electrical activity of the brain. Non-thermal effects of EMF on the nervous system have been reported in several studies [6, 25, 28, 29, 32]. Lass *et al*. [20] and Hinrikus *et al*. [12] demonstrated the influence of the low-level microwaves on human EEG and mental behavior.

In the present study, it can be suggested that the chronic exposure of low-level radiation fields has a cumulative effect. This is shown in the continuous increase of delta band power and the decrease of beta band power during the time period of radiation exposure in this study. Research has shown that the effects of EMF on the nervous system can cumulate with repeated exposure. Several lines of evidence suggest that responses of the central nervous system to EMF could be a stress response [19]. Stress effects are well known to cumulate over time and involve first adaptation and then a possible breakdown of homeostatic processes. This suggestion may explain why some of the low-level EMF studies that applied for short term have failed to find positive results on brain's electrical activity. In a recent study, Eberhardt *et al*. [6] have reported nerve cell damage in rat brain 14 and 28 days after low-level exposure to microwave. In a study from our laboratory, Khadrawy *et al*. [16] reported that EMF exposure of the same parameter as the present study induced a delayed effect on the cortical amino acid neurotransmitters which have a direct effect on the cortical excitability.

In the present study, the simultaneous enhancement in slow frequency (delta) and suppression in fast frequency (beta) could be evidence for the decrease in the level of arousal and drive-related behaviors resulted from the interaction of EMF with the nervous system of the irradiated animals. Dumansky and Shandala [5] reported a cortical synchronization and slow waves (arousal deficit) after prolonged exposure of rats and rabbits to EMF of different power densities. Testylier *et al*. [34] reported a disturbance in cholinergic neurotransmitter acetylcholine (ACh) after exposure to EMF in rats and this disturbance could change brain excitability since the release of ACh in the cortex, produced by innervations from basal forebrain nuclei, is fundamental to promote and maintain cortical arousal, memory and neuronal plasticity [23, 31]. It could be suggested the decrease in level of arousal, in the present study, due to the disruption of central cholinergic system as a result of the interaction of the electromagnetic radiation with the nervous system.

CONCLUSION

The present study suggested that the EMF chronic exposure at such a low level, similar to that emitted by mobile phone handset, can affect the electrical activity of the brain and causes shift to lower frequencies. This shift to lower frequency indicated a change in the arousal state of the animal. This study emphasized also the non-thermal effects of the EMF especially for chronic exposure. The study gave also evidence for the delayed effects of EMF which can be continued for a long period of time after cessation of EMF exposure. However, further studies are needed to verify these effects and investigate their significance to brain functions.

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