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Effects of 3 Hz and 60 Hz Extremely Low Frequency Electromagnetic Fields on Anxiety-Like Behaviors, Memory Retention of Passive Avoidance and Electrophysiological Properties of Male Rats

Amin Rostami[1], Minoo Shahani[2], Mohammad Reza Zarrindast[3], Saeed Semnanian[4], Mohammad Rahmati Roudsari[5], Mostafa Rezaei Tavirani[2*], Hadi Hasanzadeh[6]

[1]Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran
[4]Department of Physiology, School of Medical Sciences, Tarbiat Modarres University, Tehran, Iran
[5]Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[6]Cancer Research Center and Department of Medical Physics, Semnan University of Medical Sciences, Semnan, Iran

***Correspondence to**
Mostafa Rezaei Tavirani, PhD;
Proteomics Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Tel: +98-2122439787;
Fax: +98-2122439787;
Email: tavirany@yahoo.com

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Abstract

Introduction: The effects of electromagnetic fields on biological organisms have been a controversial and also interesting debate over the past few decades, despite the wide range of investigations, many aspects of extremely low frequency electromagnetic fields (ELF/EMFs) effects including mechanism of their interaction with live organisms and also their possible biological applications still remain ambiguous. In the present study, we investigated whether the exposures of ELF/EMF with frequencies of 3 Hz and 60 Hz can affect the memory, anxiety like behaviors, electrophysiological properties and brain's proteome in rats.

Methods: Male rats were exposed to 3 Hz and 60 Hz ELF/EMFs in a protocol consisting of 2 cycles of 2 h/day exposure for 4 days separated with a 2-day interval. Short term memory and anxiety like behaviors were assessed immediately, 1 and 2 weeks after the exposures. Effects of short term exposure were also assessed using electrophysiological approach immediately after 2 hours exposure.

Results: Behavioral test revealed that immediately after the end of exposures, locomotor activity of both 3 Hz and 60 Hz exposed groups significantly decreased compared to sham group. This exposure protocol had no effect on anxiety like behavior during the 2 weeks after the treatment and also on short term memory. A significant reduction in firing rate of locus coeruleus (LC) was found after 2 hours of both 3 Hz and 60 Hz exposures. Proteome analysis also revealed global changes in whole brain proteome after treatment.

Conclusion: Here, some evidence regarding the fact that such exposures can alter locomotor activity and neurons firing rate in male rats were presented.

Keywords: ELF/EMFs; Locomotion; Memory; Locus Coeruleus.

Introduction

The effects of electromagnetic fields on biological organisms have been a controversial and also interesting debate over the past few decades, because modern civilization is overwhelmed by a broad range of electromagnetic fields, including extremely low frequency electromagnetic fields (ELF/EMF). Numerous investigations, from monitoring changes at molecular levels to the behavioral aspects, have been carried out in vitro and in vivo in order to illustrate different effects of ELF/EMF e.g., its impacts on cells,[1] hormones,[2-6] neurophysiological properties and sleep,[7,8] biochemical factors and metabolism,[9-11] pathology,[12,13] DNA damages and chromosome abnormalities,[14-19] reproduction and development,[20] and cancer.[21-23] On the other hand, the fact that central nervous system (CNS) as a very complicated electrochemical system may be influenced by electromagnetic fields attracts many researches interests. Vázquez-García et al reported that exposure to 60 Hz ELF/EMF can improves social recognition in male rats.[24] Cognitive performance in attention can be reduced in the presence of 50 Hz EMF according to a previous study.[25] In addition, high intensity electromagnetic can induce de-

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pression or metabolic disturbances.[26] It is also suggested that the exposure of 50 Hz ELF-MFs can cause oxidative stress-based nervous system pathologies associated with ageing[27] and increase blood brain barrier permeability.[28] Marchionni et al has reported that 50/60 Hz magnetic field can modify the firing rate of rat sensory neurons.[29] It has been shown that the cholinergic activity in the frontal cortex and hippocampus of the rat decreases immediately after exposure to 60 Hz magnetic field with different intensities[30] and 50 Hz ELF-EMF exposure can increase in vivo neurogenesis.[31] Zecca et al[12] reported that prolonged exposure to ELF/EMF increases the level of μ-opioid receptors in the rat brain. Some researchers reported that ELF-EMF altered the anxiety-like behavior in rats.[32,33] Despite the wide range of investigations, many aspects of ELF/EMFs effects including mechanism of their interaction with live organisms and also their possible biological applications still remain ambiguous.

In the present study, we followed the hypothesis that brain is a chemo-electromagnetic system that can be influenced by ELF/EMFs and some functional properties of the brain could be changed by these exposures. So, in order to investigate some of these possible consequences of ELF/EMFs exposures, we carried out a series of experiments including behavioral tests, electrophysiological properties and proteome analysis of the rat's brain exposed to 3 and 60 Hz ELF/EMF to show some possible interactions of these fields with brain functions. The selected frequencies (3 and 60 Hz) correspond to the delta and beta waves of brain, respectively.

Methods

Animals

A total of 113 adult Wistar rats (200-250 g) from the same colony were used. Animals were kept in groups of five in each cage with free access to food supplies and were maintained on an artificial light cycle (12 hours OFF: 12 hours ON, lights on at 7:00 AM) and room temperature (23 ± 2°C). Each animal was caged individually for 5 minutes before locomotion and stress test. All the experiments were conducted during the ON phase of the light cycle (9:00 AM to 4:00 PM). The study was performed according to institutional guidelines for animal care and use.

ELF/EMF Exposure System

Electromagnetic field was generated with ELF/EMF generator which was calibrated using a digital gauss meter (Magna MG-701, Magna Co. Ltd, Tokyo, Japan). The antenna of the generator was placed at the center of an aluminum mesh shielded room with dimensions of 1.5×2×2 m. This room was used as animal exposure room. Four groups of rats were placed in their usual plastic cages without any metallic cover and exposed to ELF/EMF with frequencies of 3 Hz and 60 Hz and intensity of 4 mT for 2 h/day (9:00 to 11:00 AM) consisting of 2 exposure periods of 4 days separated with a 2 days interval. The center of each cage was placed at a distance of 40 cm from effective radiative surface of the antenna on a wooden stage with a distance of 50 cm from the ground (Figure 1). Sham exposed animals were maintained for an equal period of time inside the exposure room with the generator off. Experiments were carried out at relatively constant room temperature (23 ± 2.0°C).

For electrophysiological recording, each animal group was exposed to either 3 Hz or 60 Hz for 2 hours in the electrophysiological set up.

Anxiety-Like Behaviors

The method used here has been well explained previously.[34] In this study, 60 animals were randomly divided in 3 experimental groups as sham, 3 Hz exposure and 60 Hz exposure. The plus-maze test was a wooden, cross-shaped maze consisting of four arms arranged in the 'plus' sign shape. Two across arms have no side or end walls (open arms; 50×10 cm) and the two other arms had side walls and end walls, but were open on top (closed arms; 50×10×40 cm). At the center of the apparatus where four arms intersect, there was a square platform of 10×10 cm. The maze was elevated to a height of 50 cm. In order to increase total arm entries, 5 minutes prior to maze testing rats were placed in a wooden test arena (50×50×35 cm) facing the close arm. One and two weeks after the exposure period, the effects of exposure to the 3 Hz and 60 Hz ELF/EMF were determined in the plus-maze test. The percentage of open arm entries and open arm time that are introduced as the standard anxiety indices was calculated as follows: (*a*) %OAT (the ratio of times spent in the open arms to total times spent in any arms × 100); (*b*) %OAE (the ratio of entries into open arms to total entries × 100). Total crossing of each entries by forepaws were measured as a relative pure index of locomotor activity.

Retention Test of Passive Avoidance Learning

The dark box and lighted box with the same measures (20×20×20 cm) are the main compartments of the passive avoidance apparatus. The boxes are separated by a guillotine door (8×8 cm). The lighted box was illuminated with a lamp (60 W, positioned above the apparatus). The floor of the dark compartment was made of stainless steel (0.5 cm diameter) separated by a distance of 1 cm. Intermittent electric shocks (50 Hz, 5 seconds), 1.5 mA intensity were delivered to the grid floor of the dark compartment

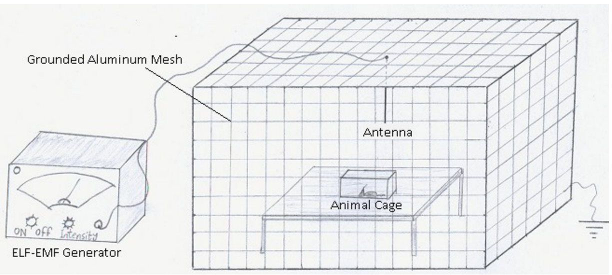

Figure 1. Irradiation setup in which ELF-EMF generator is shown on the left and its antenna is entered into aluminum cage from its ceiling to expose animals in their home-cage.

by an isolated stimulator.
A total of 30 rats were randomly divided to the groups of sham, 3 Hz and 60 Hz exposure and allowed to habituate to the laboratory environmental conditions 1 hour prior to each training or testing sessions. All training tests were performed between 08:00 AM and 14:00 PM. During training, each animal was kept in the lighted box; 10 seconds later the door between the compartments was opened and the latency to enter the dark (shock) compartment with all four paws was recorded. The criterion for excluding animals from the experiment was waiting more than 100 seconds to cross to the other side. Once the animal crossed all four paws to the next compartment, the door was closed and a 1.5 mA foot shock was administered for 5 seconds. Then animal was removed from the apparatus and returned to its home cage.
In order to determine long term memory, a retention test was performed 24 hours after training. The experiment was carried out similarly to the acquisition trial, except that the guillotine door did not close when the rat entered the dark compartment and the shock was not applied to the grid floor. If animal remained in a light compartment and did not cross within 300 seconds to the dark compartment, (where the foot shock had been given) the session was ended and score of 300 was assigned.

Electrophysiological Recording

The method used in the present study is similar to the method described previously.[35] Briefly, a total of 23 animals were anesthetized with urethane (1.2-1.5 g/kg body weight, i.p. injection) and were placed in a stereotaxic instrument. Body temperature was maintained at 35.5–36.88°C by a thermistor-controlled heating pad. A 2 mm diameter hole in the skull above LC (according to the atlas of Paxinos) was drilled, and the dura was reflected. Glass micropipette (2–4 mm tip diameter, 2–10 MΩ impedance) filled with 2% pontamine sky blue dye in 0.5M sodium acetate was used to obtain extracellular recording from individual neurons which was stereotaxically advanced into locus coeruleus (LC). Unit activity was amplified by a microelectrode amplifier (Nihon Kohden Co Ltd, Tokyo, Japan) and displayed continuously on a storage oscilloscope (Tektronix Co Ltd, TDS1000-EDU, Beaverton, OR, United States) as unfiltered and filtered (300 Hz–3 kHz band pass) signals, and also monitored with an audio monitor. Action potentials were isolated from background activity with a window discriminator (WPI) which generated output pulses for signals that crossed a lower voltage gate, peaked below an upper voltage gate. The discriminator output signals were linked to a computer for online data collection. The output signals were saved as number of output signals as spikes in unit of time. The unit activity was calculated by computer as an average frequency.

Statistical Analysis

Anxiety like behavior data were expressed as mean ± SE and data were analyzed using SPSS 16 (SPSS/PC Inc., Chicago, IL, USA). After verifying the normality and homogeneity of variables, analysis of variance (ANOVA) was performed with a 95% CI ($P<0.05$) and differences between experimental groups at this level were considered statistically significant.
The obtained single unite recording results are expressed as mean ± SE. Firing rates before and after exposures were compared using student's paired *t* test and for multiple comparisons, one-way ANOVA was used with Tukey post hoc ($P<0.05$).

Results

Anxiety Like Behavior

Figure 2 shows the effects of ELF/EMF exposure at the above mentioned exposure protocol on anxiety like parameters in the elevated plus maze tested 1 and 2 weeks after the exposures. One-way ANOVA analysis revealed that ELF/EMF with frequencies of 3 Hz and 60 Hz exposure did not alter percentage open arm time and percentage open arm entries in neither 3 Hz nor 60 Hz exposed groups ($P>0.05$) (Figure 2A and 2B), but indicated a significant decrease in locomotor activity immediately after

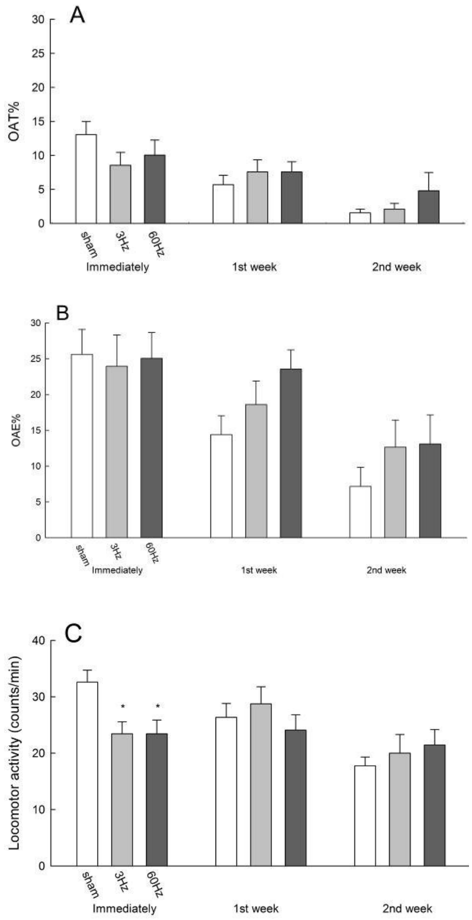

Figure 2. The effects of 3 and 60 Hz ELF/EMF whole body exposure of two groups of rats for the selected exposure protocol on anxiety-like behavior. The test was performed 1 and 2 weeks after exposures. Each bar is mean ±SE of 20 animals. Percentage open arm time (A), percentage open arm entries (B) or locomotor activity (C). *$P<0.05$.

the exposures in both 3 Hz and 60 Hz groups ($P<0.05$) in comparison with sham group. According to these findings, relatively long exposure of both 3 Hz and 60 Hz ELF/EMF can alter locomotor activity but have no effect on anxiety.

Memory Retention

In order to investigate the impacts of 3 Hz and 60 Hz ELF/EMF exposures on long term memory, animals were subjected to retention test of passive avoidance learning test. As illustrated in Figure 3, exposure at applied exposure protocol had no significant effect on retention memory in male rats tested by passive avoidance apparatus ($P>0.05$).

Electrophysiology

In these experiments, we examined whether ELF/EMF exposure can affect neural activity of LC.

Effects of 3 Hz Exposure on Unit Activity of LC

After isolating each LC unit and determining the stability of its firing rate, anesthetized animals were subjected to 3 Hz ELF/EMF exposure for a period of 2 hours which was adjusted in the extracellular recording cage. As it is shown at Figure 4, the overall unit activity of a total of 14 isolated neurons in LC decreased significantly immediately after 2 hours whole body exposure of 3 Hz ELF/EMF ($P<0.05$) compared with its pre-exposure unit activity. In fact, statistical analyses revealed that 8 out of 14 nuclei showed a significant decrease in firing rate after 2 hours exposure of 3 Hz ELF/EMF ($P<0.01$).

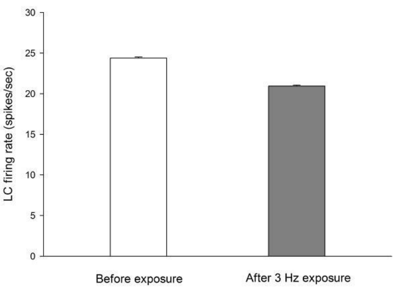

Figure 3. The effects of 3 and 60 Hz ELF/EMF whole body exposure of two groups of rats for selected protocol (9:00 to 11:00 AM) on memory retention. The test was performed immediately after exposures. Each column represents the mean ±SE of data acquired from 10 rats ($P>0.05$).

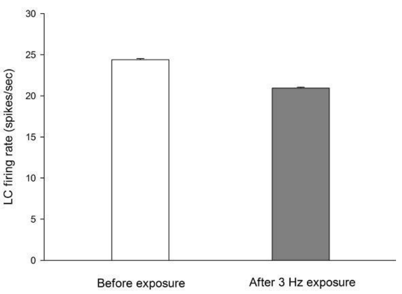

Figure 4. The average of LC neurons activity before and after 2 hours exposure to 3 Hz ELF/EMF.

Effects of 60 Hz Exposure on Unit Activity of LC

In order to investigate effect of 60 Hz on LC activity, a procedure similar to 3 Hz exposure was carried out. At the end of 2 hours exposure, a significant decrease was found in LC's neurons total activity compared to pre-exposure neurons activity (Figure 5). In fact, 5 out of 9 nuclei showed a significant decrease in the firing rate ($P<0.01$). These results indicate that short time exposure of both 3Hz and 60Hz ELF/EMFs can suppress LC neurons unit activity significantly immediately after 2 hours exposure (Figure 6).

Proteome Analysis

Immediately after exposures, rat brains were extracted and protein extraction was performed. At the end of 3 Hz ELF/EMF exposure at selected protocol and using 2-DE technique, proteome analysis of rat's whole brain revealed changes in the expression of some proteins. In fact, primary proteomics analysis showed that the expression of 43 proteins changed in 3 Hz exposed group compared with sham group. Among these proteins, expression of 27 proteins were suppressed in the exposed group while 10 new proteins were expressed (data are not presented here).

Body Weight and Mortality

No significant change in body weight was observed 6 months after the 3/60 Hz exposures. Furthermore, no clinical disorders or mortality have been recorded during the 6 months monitoring after 3/60 Hz exposures.

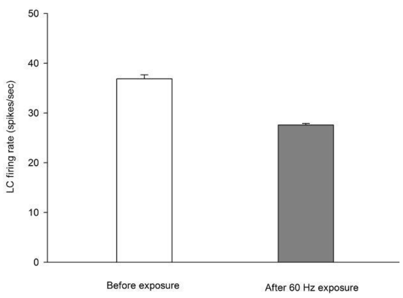

Figure 5. The average of LC neurons activity before and after 2 hours exposure to 60 Hz ELF/EMF.

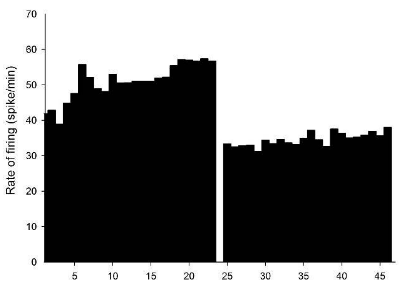

Figure 6. A typical unit activity of LC neuron in response to 2 hours exposure to 3 Hz ELF/EMF. A significant decrease in the unit activity of LC occurred after 3 Hz ELF/EMF exposure (The gap between two series of spikes in each graph represents 2 hours exposure time).

Discussion

Effects of ELF/EMF on biological systems have been the subject of debate over past few decades. Many studies revealed that ELF/EMF exposures can alter some animal behaviors.[24,25,32,33] Since most of behaviors are controlled by different brain's nuclei and neurotransmitter systems, it seems that ELF/EMFs do not interact with all part of CNS or at least do not affect all parts of it in the same way.
In the present study, effects of ELF/EMF exposure with frequencies of 3 Hz and 60 Hz on anxiety like behaviors, long term memory and unit activity of LC were studied. The results mainly indicated: (1) A decrease in locomotor activity following exposure to both 3 Hz and 60 Hz ELF/EMF while other parameters of anxiety like behaviors did not alter; (2) A decrease in unit activity of LC after 2 hours exposure to these frequencies.
The first result shows that the locomotor activity of rats can be affected by exposure to 3 Hz and 60 Hz ELF/EMF while other parameters of anxiety like behaviors stayed unchanged (Figure 2). Alteration in locomotor activity is in contradiction with findings of some researchers that reported no change in locomotor activity of rats after exposure to ELF/EMF.[24,26] On the other hand, a significant decrease in swim speed of exposed animal to 60 Hz MF tested by water-maze was reported[36] which can be in accordance with our findings.
In order to shed light on some aspects of this finding, the unit activities of LC was measured following both 3 Hz and 60 Hz exposures. Second finding indicates that the unit activity of LC decreased significantly after exposure of these two frequencies (Figures 4 and 5) which can confirm the effects of ELF/EMF exposures on the brain and the probable following change of its activity.
LC contains a large aggregation of noradrenergic (NE) neurons and has wide projections throughout the brain. So, it can influence the activity of many brain areas and modulate different basic behavioral and physiological processes, such as sleep, waking, and arousal.
According to its function, it can be expected that decrease in LC nucleus activity after exposure to ELF/EMF resulted in some lassitude in exposed animals and causes the following decrease in locomotor activity.
The mechanism by which ELF/EMF exposures can alter locomotion activity could also be explained partly by some reports showing that the number of opioids receptors[37] and overall activity of this system could be increased following ELF/EMF exposures,[38] which can lead to increase or decrease in performance depending on the site of its activation. Activation of this system can decrease the activity of cholinergic system in hippocampus and frontal cortex which is related to learning and arousal. Furthermore, Opioids peptides can decrease the level of noradrenalin in the brain and thus have some negative effects on amygdale which can lead to some depressant effects. A change in the mood of the animal may explain the observed decreasing locomotor activity in exposed animals.
According to some reports, ELF/EMF can improve social recognition (short term memory) in rats[24,39] while Trimmel et al reported a reduction in memory performance following 50Hz EMF exposure.[25] According to our findings, exposure of the animals to ELF/EMF with either frequencies of 3 Hz or 60 Hz had no effect on long term memory (Figure 3). Electromagnetic fields might alter short term memory and have mild or no effects on long term memory. However, testing long term memory using some techniques milder than passive avoidance is inevitable.
On the other hand, our results showed that exposure to ELF/EMF can alter the patterns of protein expression in rat's whole brain. In fact, now almost all researchers accepted that EMFs can alter gene expression and protein synthesis in living organism. From this point of view, one should consider that proteins widely define the functions of an organism, so any change in proteome caused by EMFs can alter the cell or organism's behavior. It is highly suggested to investigate the effects of ELF/EMFs on different animal tissues using omics technologies (e.g., proteomics) in order to elucidate the pathways involved in living organism's responses to these exposures.

Conflict of Interest

The authors declare no conflict of interest, financial or other exist.

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