Activitas Nervosa Superior Rediviva Volume 61 No. 3-4 2019

ORIGINAL RESEARCH

The effects of extremely low-frequency electromagnetic field on depression, anxiety, thyroid hormones, and gene expression in mice

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Submitted: 2019-11-21 Accepted: 2019-12-02 Published online: 2019-12-18

Key words: **Extremely low-frequency electromagnetic field; Depression; Anxiety; Thyroid hormones; Gene expression**

Act Nerv Super Rediviva 2019; **61**(3-4): 148–154 ANSR613419A08

Abstract

OBJECTIVES: Studies suggest that exposure to electromagnetic fields may increase the incidence of health problems such as mood disorders. In this study, we evaluated the effects of extremely low-frequency electromagnetic field on depression, anxiety, thyroid hormones, and gene expression in mice.

METHODS: In total, 240 male BALB/c mice were allocated into 4 groups of experimental and 4 groups of control animals, each including 30 mice. Experimental mice received 50 Hz electromagnetic field, 3 hours daily for 30 days. Control animals were placed inside the same room for the same time interval. We compared the depressive state by carrying out the Forced Swim Test, used a mouse Elevated Plus Maze to compare anxiety responses, assessed T3, T4, and TSH, and used real-time polymerase chain reaction to measure gene expression.

RESULTS: The duration of immobility posture in the swim test was less for the exposed mice. Also, in plus maze the animals treated with the field entered less frequently, and spent less time on the open arms, and showed less motor activity (all $P<0.05$). In addition, in the exposed mice T4 and TSH concentration increased significantly (both $P<0.05$); however, the increase in T3 was not significant ($p = 0.057$). We did not find any significant difference in gene expression between the groups (all $P>0.05$).

CONCLUSIONS: Extremely low-frequency electromagnetic field 50 Hz for 30 days decreases depression, and increase anxiety-like behavior in mice. In addition, exposure to the field activates thyroid hormone production; but it does not affect gene expression.

Introduction

Electromagnetic waves of different frequencies cause different biological effects (El-Hussein *et al.* 2018; Ciejka *et al.* 2017). Some studies suggest that exposure to extremely low-frequency (ELF) electromagnetic fields (EMF) would probably increase the incidence of health problems. Recent research indicated that ELF-EMF is associated with certain types of malignancies (Falone *et al.* 2018; Carlberg *et al.* 2017), mood disorders (Bagheri Hosseinabadi *et al.* 2018), Alzheimer's disease (Jalilian *et al.* 2018) and other neurological conditions (Huss *et al.* 2018; Zheng *et al.* 2017). However, different studies failed to show a strong relation between ELF-EMF and pathologies (Carlberg *et al.* 2018; Bua *et al.* 2018; Turner *et al.* 2017; Maes *et al.* 2016).

There is considerable uncertainty around the effects of ELF-EMF on mood and behavior. Some authors have considered ELF-EMF as a modulator of behavior (Mahdavi *et al.* 2016). They showed that ELF-EMF causes anxiety-like behavior (Djordjevic *et al.* 2017). Meanwhile, others reported that ELF-EMF therapy has clinical application for promoting recovery in post-stroke patients (Cichon *et al.* 2017). Researchers investigated the effect of ELF-EMF on power plant workers. They found that depression, stress, anxiety, and poor sleep quality are more prevalent among the exposed group (Bagheri Hosseinabadi *et al.* 2018). The ELF-EMF has been reported to enhance spatial learning and memory in animal models (Sakhaie *et al.* 2017; Akbarnejad *et al.* 2017b). Also, the results showed that ELF-EMF promotes neurogenesis in mice (Sakhaie *et al.* 2017). However, evidence from a large cohort study suggested that people exposed to ELF-EMF are at higher risk for dementia, motor neuron disease, multiple sclerosis and epilepsy, and that they are at lower risk for Parkinson disease(Pedersen *et al.* 2017).

Relatively few studies have examined the effects of ELF-EMF on the endocrine system. A strong influence on morphometrical features of the pituitary ACTH cells has been recognized in the rats exposed to ELF-EMF. It was reported that ELF-EMF reduces the sizes of ACTH cells, the volume of their nuclei, and the mass of pituitary gland (Raus Balind *et al.* 2016). In an animal study, ELF EMF was shown to increase adrenal steroidogenesis in mice (Kitaoka *et al.* 2016). Results for studying the association of ELF-EMF with diabetes are contradictory (Li *et al.* 2016). To our knowledge, there is no recent report in the literature regarding the effect of ELF-EMF on thyroid gland activity.

The effects of ELF-EMF on gene expression are still debated. It has been reported that ELF-EMF decreases the expression of the telomerase reverse transcriptase gene (Fathi *et al.* 2017). Much research has been done concerning the genotoxic effects of electromagnetic waves. While the mechanism is unclear, ELF-EMF has been classified as "possibly carcinogenic"(Giorgi *et al.* 2017). However, some studies indicated that maybe ELF-EMF is effective for treating glioma cancer (Akbarnejad *et al.* 2017a).

The evidence from human studies regarding the health effects of ELF-EMF is weakened by methodological problems, such as selection bias. Observational data examining people in the workplace are of limited use because a large number of confounders reduce the validity of the conclusions. Moreover, underlying theories on pathological processes are not clear yet. The results described are sometimes contradictory and inconclusive (Jalilian *et al.* 2018; Rostami *et al.* 2016). Evaluation of the data is complicated by heterogeneity in the field intensity and duration of exposure (Samiee and Samiee 2017; Reale *et al.* 2016). Overall, the evidence concerning the health effects of ELF-EMF is not strong enough to be considered causal. Electromagnetic fields are increasingly used worldwide; however, until reliable information is collected, it is difficult to devise standard guidelines for protection against or to plan therapeutic protocols for using potential clinical advantages of ELF-EMF.

In the current study, we aimed to provide enough evidence regarding the effects of ELF-EMF on several biological processes in animal models. Our hypothesis was that ELF-EMF affects mood, thyroid function, and gene expression.

Materials and methods

Animals

In total, 240 male BALB/c mice (mean age of 60 days with the weight range of 25 – 30 g) were obtained from an animal breeding unit at a University of Medical Sciences. They were acclimatized for 2 weeks before the beginning of the experiment and were maintained at the temperature of 21 ± 2°C and the humidity of 45 ± 5%. Moreover, within this period of time, they were removed from their cages and were stroked for one minute in the palm of the experimenter's hand in order to decrease anxiety. The animals housed per group and had free access to a standard pellet diet and water ad libitum except for the time of exposure. The light source in the animal house was set to provide a 12 h light/12 h dark cycle (07:00 – 19:00 h, light on). The mice were exposed within the light phase of the cycle. We had 4 groups of experimental and 4 groups of control animals, each including 30 mice. The study was carried out in accordance with the Guidelines for the Care and Use of Experimental Animals. The ethics review board of Aja University of Medical Sciences approved the research.

Electromagnetic field exposure

We generated 50 Hz ELF-EMF with a device containing two exposure chambers. The device includes two solenoids (380 turns) connected to a power generator and a magnetic wire surrounding the chambers. The animal cages were made from Plexiglass. They were placed

inside the chambers and for 30 days a single stimulation was administered 3 hours from 09:00 to 12:00 to the mice. Also, control animals were placed inside the same room for the same period of time. The mice were protected from exposure to the environmental fields. We used aluminum foil (0.4 mm diameter) to make the protective shield, and checked existence of any effective radiation using a wave detector. During the stimulation, the temperature was kept at 25 ± 0.5°C. We monitored the strength of the EMF using a probe (Koshava, Wuntronic co., Germany) connected to a Telemeter. The generator was set at 50 Hz, and 1 millitesla for the experimental groups.

Experimental groups

The animals were randomly allocated to 8 groups each included 30 mice. The four groups of experimental mice were exposed to ELF-EMF, and at the end of the study, they were evaluated for depression, anxiety, thyroid function, and gene expression. The control groups were used for between-group comparisons. On the next day of the final exposure, the animals were evaluated for depression and anxiety. For the assessment of thyroid function and gene expression, the mice's blood was collected into heparinized tubes.

Outcome measures

Depression

We compared the depressive state of the animals in exposed versus control group by carrying out the Forced Swim Test. The mouse was placed into a cylinder partially filled with water, and if it stopped swimming, this was considered as depression-like behavior. The exact duration of immobility posture was recorded in which the mouse make only small movements to keep its head above the water. The cylindrical tank was 25 cm in height, 10 cm in diameter, and was filled with 25°C water. The animal was not able to touch with its paws or tail to the floor of the tank. The test lasted 10 minutes, and the last 5 minutes used for the assessment. Then the test was stopped and the mouse was removed from the tank, dried, and returned to its cage. If a mouse failed to remain afloat, it was excluded from the study. An observer blinded to group assignment assessed all mice during the study.

Anxiety

We used a mouse Elevated Plus Maze to compare anxiety responses between experimental groups and control groups. Our plus maze is made of wood and consisted of two open, and two closed arms each 7 cm wide and 40 cm long that has been arranged to form a plus shape. The two closed arms are enclosed by 10 cm high walls. The apparatus is mounted on a base that elevates it 40 cm above the floor. The animal was placed in the intersection of the four arms of the elevated plus maze and its behavior was recorded for 5 min. We used a 100 W light source placed at 120 cm above the center of the maze. Activity in the open arms shows a conflict between the preferences for protected close areas and the motivation to explore a new environment. Each mouse was placed in the center of the maze facing an open arm. We recorded the number of entries into each type of arm and the time spent in each arm. A mouse was considered to have entered an arm when all four legs were on the arm. An observer blinded to group assignment assessed all mice during the study.

Open arms entries and the time spent on the open arms are positively correlated with the anxiolytic effect of interventions. We considered a significant change in anxiety if both the number of entries and time spent on the open arms showed unidirectional change and at least one of them was significantly increased or decreased.

Thyroid function

We assessed T3, T4, and TSH of the mice and compared them between the groups: exposed and control. Blood specimens were collected into ethylenediaminetetraacetic acid tubes and immediately sent to the university lab. At the lab, the sera were separated and kept at 2–8 °C at most for 5 days during which the concentrations of thyroid hormones were assessed. We used thyroid hormone ELISA kits (Pishtazteb co, Tehran, Iran) containing 6 standards and 2 high and low controls. There is no need to dilute the sera, and the test applies Sandwich enzyme immunoassay. The samples and the enzyme conjugates are added to wells coated with antibody. The sandwich is formed by the addition of a detector antibody; a substrate solution is added that reacts with the enzyme-antibody-target complex to produce a measurable signal. Thyroid hormones in the serum compete with enzyme conjugates for binding sites. Unbound conjugates are washed off. The intensity of this signal is directly proportional to the concentration of target present in the original specimen. The color intensity is inversely proportional to the concentration of the hormones in the sample. The color is compared with standard curves relating color intensity to hormone concentrations.

Gene expression

For collecting blood, the tail of the mouse was dipped in warm water and cleansed with the antiseptic solution. Then the tail was immobilized with the non-dominant hand and rotated 1/4 turn to access the lateral tail vein. The needle was aligned parallel to the tail with the beveled edge of the needle facing up. It was inserted into the vein from the tip of the tail and aspirated gently to collect 0.3 ml blood into an ethylenediaminetetraacetic acid tube. Then, the needle was removed, and the site of bleeding was pressed for 30 s with sterile gauze. The blood was sent to the biotechnology lab of the university.

We isolated RNA using RNX–Plus; a Guanidine/phenol solution. While Guanidine salt isolates RNA,

Tab. 1. Effects of ELF-EMF on mice behavior in the Forced Swim Test and the Elevated Plus Maze

Variable	Control	Exposed	*p*-value
Immobility (s)	202.4 (11.5)	189.1 (19.1)	0.002
Number of entries into open arms	4.2 (1.2)	3.3 (1.5)	0.013
Number of entries into close arms	2.4 (1.3)	3.4 (1.6)	0.010
Time spent on the open arms (s)	105.7 (24.8)	75.6 (23.0)	<0.001
Time spent on the close arms (s)	191.2 (12.2)	200.9 (18.7)	0.021
Number of locomotion per minute	8.0 (2.9)	6.3 (2.4)	0.016

protein and DNA are precipitated in the phenol phase. At the end of the procedure, the aqueous phase contains all types of genomic RNA. In brief, one ml ice-cold RNX–Plus solution was added to a 2 ml tube containing the homogenized sample and incubated at room temperature for 5 min. Next, chloroform was added, the solution was mixed by shaking, incubated on ice, and centrifuged at 12000 rpm for 15 min. The aqueous phase was transformed to new RNase-free 1.5 ml tube and isopropanol added; the solution was mixed, incubated on ice and centrifuged again. The supernatant was discarded, 1 ml of 75% Ethanol was added, and the vortex was carried out. Then, it was centrifuged at 7500 rpm; the supernatant was discarded; the pellet was dried at room temperature for several minutes and dissolved in diethylpyrocarbonate-treated water by placing the tube in the 55-60°C water bath. One µL RNase inhibitor was added to the solution and spectrophotometry was carried out. The quality of the isolated RNA was assessed by agar jelly.

Real-time polymerase chain reaction (PCR) was done to measure gene expression. The procedure was performed by adding: 2X SYBR Buffer III 7.5 µL, Takara ExTaq HS 0.3 µL, Prime Script RT 0.3 µL, reverse and forward primers 40 µM each, ROX Dye II 0.3 µL, RNA 1µL (100 nanogram), and infusible water up to the total volume of 15 µL. Denaturation took place at 95°C for 3 min, then 40 cycles at 95°C for 5 s; annealing at 60°C for 34 s, and elongation at 72°C for 30 s.

Statistical analyses

Data are given as mean (standard deviation) for continuous variables. The data were tested on normality with the help of histograms and the comparison of means and medians and the Shapiro-Wilk test. Variables comparisons were performed using t-tests. Point estimates, 95% confidence intervals, and p-values were calculated. P-values less than 0.05 were considered significant. Statistical analyses were done with R version 3.5.0. Gene expression data from the quantitative real-time PCR were analyzed with REST 2009 Software (Technical University Munich).

Results

Table 1 shows the effects of 30 days exposure to ELF-EMF on the behavior of the mice. The results indicate that ELF-EMF significantly affected mice behavior. The exposed animal had significantly less immobility duration than the control. In addition, the number of entries into, and the time spent on the closed arms of the elevated plus maze was significantly more in the exposed mice. Meanwhile, group control entered into the open arms more frequently, spent more time on the open arms, and had more number of locomotion per minutes.

Figures 1 to 3 demonstrate the effects of ELF-EMF on the concentration of thyroid hormones. Between-group analyses showed that T4 and TSH increased significantly in the group exposed (both *p*-values < 0.001). The mean concentration of T3 was higher in the exposed group; however, the difference was not statistically significant ($p = 0.057$). The results showed that 30 days of exposure to ELF-EMF significantly affected thyroid hormone concentration.

Table 2 shows the effects of 30 days of exposure to ELF-EMF on gene expression. Gene expression increased for PROK and Cyp 17 in the exposed group however the difference was not significant. The results

Tab. 2. Effects of ELF-EMF on gene expression based on the output of REST 2009 Software (Technical University Munich) for the analysis of gene expression data from the quantitative real-time PCR.

Gene	Type	RE	Expression	SE	95% CI	*p* value
mACTB	Reference	1.0	1.000			1.00
PROK	Target	1.0	1.119	0.340–4.427	0.026–9.853	0.740
Cyp 17	Target	1.0	1.896	0.265–9.308	0.010–399.863	0.213

RE: Reaction Efficiency; SE: Standard Error; CI: Confidence Interval

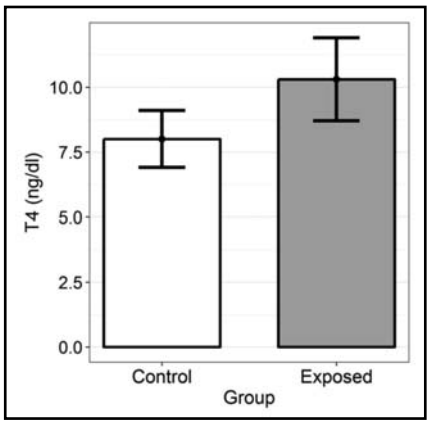

Fig. 1. Effects of 30 days exposure to ELF-EMF on the concentration of T4

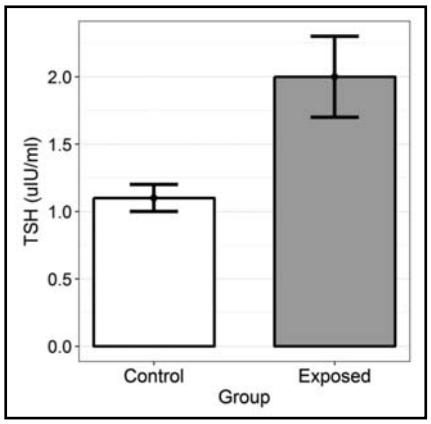

Fig. 2. Effects of 30 days exposure to ELF-EMF on the concentration of TSH

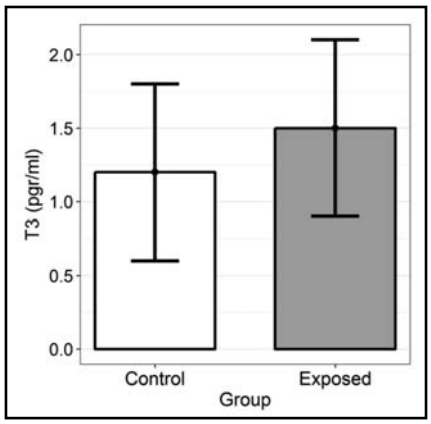

Fig. 3. Effects of 30 days exposure to ELF-EMF on the concentration of T3

showed that 30 days of exposure to ELF-EMF did not affect gene expression significantly.

Discussion

In the present study, we aimed to investigate whether ELF-EMF affects biological processes in male BALB/c mice. We hypothesized that ELF-EMF affects mood, thyroid function, and gene expression. We found that the duration of immobility posture in the Forced Swim Test was less for the exposed mice. Also, in Elevated Plus Maze the animals treated with ELF EMF entered less frequently, and spent less time on the open arms, and showed less motor activity, compared with control mice. It seems that the electromagnetic field influences the mood significantly. Our experience revealed that ELF-EMF decreases depression and increases anxiety-like behavior in the mice. Moreover, thyroid function test indicated that exposure to ELF-EMF activates thyroid hormone production. Both serum T4 and TSH showed an increase in concentration. This also might explain why anxiety increases in the experimental group. It should be noticed that a relative increase in T3 was seen, however, the increase was not significant. In addition, we did not find any significant difference in gene expression between experimental and control groups.

It has been suggested that magnetic fields influence the behavior mediated by dopaminergic and serotonergic systems (Janac *et al.* 2009). In a study on Wistar rats, they were continuously exposed to 50 Hz ELF-EMF for 1, 3, and 7 days; and the activity of serotonin 5-HT (2A) receptors in the prefrontal cortex, and dopamine D1 and D2 receptors were assessed (Janac *et al.* 2009). The affinity of serotonin 5-HT (2A) receptors decreased while their density increased in the prefrontal cortex of the exposed animals. Particularly, the decrease in affinity was time-dependent. However, the affinity and density of dopamine receptors did not show significant change. It was concluded that ELF-EMF affects serotonergic neurotransmission in the brain. In another study, Wistar rats exposed to 10 Hz ELF-EMF for 15 days, 3 h daily showed a significant reduction in the concentration of 5-hydroxyindolacetic acid in raphe nucleus (Shahbazi-Gahrouei *et al.* 2016). Serotonin depletion is thought to induce depression. Selective serotonin reuptake inhibitors are commonly prescribed antidepressants, as well. We exposed our animals for 30 days and the duration of the exposure was sufficient to induce a change in the serotonergic activity in the mice. Our experiment implies that ELF-EMF might have the potential to be used as an antidepressant in the clinic.

In an animal study, the effect of 7 days ELF-EMF on anxiety-like behavior was investigated (Djordjevic *et al.* 2017). Ten adult male rats were divided into two groups of control and exposed. After exposure to 50 Hz ELF-EMF, the rats were elevated with a plus maze for anxiety-like behavior. The results showed that anxiety-like behavior and oxidative stress increases in exposed animals. The exposed rats spent more time in closed arms of the plus maze [95.80 (1.26) s] compared to the control group [87.05 (3.24) s], and the difference was significant ($p < 0.05$). Movement time was more in controls and inaction time was more in the exposed rats, too. Our study also showed that anxiety-like behavior increased in the exposed group. Of course, we exposed our mice for 30 days to a 50 Hz field and included enough animals in each group.

Some of our results regarding the effects of ELF-EMF on human mood state are consistent with previous findings reported in the literature. In a recent cross-sectional study, the effects of chronic exposure to ELF-EMF were investigated in power plant workers (Bagheri Hosseinabadi *et al.* 2018). Two groups of people; exposed (n=132), and unexposed (n=143) were included. The outcome measures were slept quality, stress, depression, anxiety, and the intensity of the electromagnetic field. There were significant differences between exposed and unexposed groups in the

mean total score of the Pittsburgh Sleep Quality Index (p=0.049), and depression (p=0.039) using Depression, Anxiety and Stress Scale (p=0.039). The differences were not significant in anxiety (p=0.686) and stress (p=0.176). However, the data showed that there were significant trends for the outcome measures in different job categories of the group exposed, as the office workers were less affected than the technicians. The trend was apparent in depression (p=0.019), anxiety (p=0.008), and stress (p=0.002); but not in the quality of sleep (p=0.110). The results indicated that long-term exposure to ELF-EMF increases depression, stress, and anxiety.

There is no recent study on the effects of ELF-EMF on thyroid function. In the literature, the evidence is insufficient and comes from a few outdated reports with some studies suggesting significant effect and others no effect for electromagnetic fields other than ELF. Of the studies suggesting significant effect, some indicated an increase and others showed a decrease in the hormone concentration. Increase in circulating thyroid hormones can be caused by the effect of ELF-EMF on the hypophysis or directly on the thyroid gland. The underlying physiologic mechanism is not understood. However, the association between thyroid hormones and anxiety is well established. The development of anxiety-like behavior in our mice may be partly owing to the increase in thyroid hormone concentration.

Studies showed that 2.5 GHz band radio-frequency EMF waves increase the expression of acetylcholinesterase mRNA in rats (Obajuluwa *et al.* 2017). Also, it has been reported that ELF-EMF modulates gene expression of estrogen receptor beta in the olfactory bulb of female adult rats (Reyes-Guerrero *et al.* 2010). A study indicated that 60 Hz ELF-EMF up-regulates the expression of genes in a subset of Th17 cells (Lee *et al.* 2016). Spermatocyte-derived GC-2 cells of mice, intermittently exposed to 50 Hz ELF-EMF for 72 h (5 min on/10 min off), showed different miR-26b-5p expression with different field intensities (Liu *et al.* 2016). Beside of changes in the cell cycle, miR-26b-5p and the ELF-EMF affected the mRNA expression of CCND2. Researchers found that 50 Hz ELF EMFs increase the number of cells with micronuclei and nuclear buds and that the fields may induce chromosome instabilities (Maes *et al.* 2016). In our study, we did not find a significant change in gene expression. Yet, it seems that the outcome of the exposure depends on cell type, the quality of the exposure (intermittent versus continuous), the duration, and the intensity of EMF.

The size of our study was large enough and we tried to follow standard procedures. To our knowledge, there was no other recent study comparable to ours regarding the effects of ELF-EMF on thyroid function in mice. However, we did not evaluate the outcomes at follow-ups for longer than 30 days. Therefore, we could not verify the long-term effects of ELF-EMF on physiological features. Besides, we did not carry out a dose-response study. Further research on different doses and different durations of exposure may, therefore, be warranted.

Conclusion

Our experience showed that ELF-EMF 50 Hz for 30 days decreases depression and increase anxiety-like behavior in mice. In addition, exposure to ELF-EMF activates thyroid hormone production. We did not find any significant change in gene expression after exposure to ELF EMF.

Author contributions

ME conceptualized and designed the study, helped in behavioral assessments, and supervised the work. MS helped in literature review and performed thyroid tests. KMA contributed to the concept and carried out genetic assessments. AK helped in designing the study and performed statistical analyses. HSB reviewed the literature and carried out the behavioral tests.

All the authors participated in drafting and its final approval.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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