

ORIGINAL ARTICLE

Common behaviors alterations after extremely low-frequency electromagnetic field exposure in rat animal model

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Naturally, the presence of electromagnetic waves in our living environment affects all components of organisms, particularly humans and animals, as the large part of their body consists of water. In the present study, we tried to investigate the relation between exposure to the extremely low-frequency electromagnetic field (ELF-EMF) and common behaviors such as body weight, food and water intake, anorexia (poor appetite), plasma glucose concentration, movement, rearing and sniffing in rats. For this purpose, rats were exposed to 40 Hz ELF-EMF once a day for 21 days, then at days 1, 3, 7, 14 and 21 after exposure, any changes in the above-mentioned items were assessed in the exposed rats and compared to the non-exposed group as control. Body weight of irradiated rats significantly increased only a week after exposure and decreased after that. No significant change was observed in food and water intake of irradiated rats compared to the control, and the anorexia parameter in the group exposed to ELF-EMF was significantly decreased at one and two weeks after irradiation. A week after exposure, the level of glucose was significantly increased but at other days these changes were not significant. Movements, rearing and sniffing of rats at day 1 after exposure were significantly decreased and other days these changes did not follow any particular pattern. However, the result of this study demonstrated that exposure to ELF-EMF can alter the normal condition of animals and may represent a harmful impact on behavior.

Keywords

Behavior, ELF, electromagnetic field, glucose, movement, sniffing

History

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Introduction

Few research were carried out to study the effects of extremely low-frequency electromagnetic fields (ELF-EMF) on the spontaneous brain activity that was recorded by EEG or the response to specific stimuli as measured by event-related potentials (Reiter, 1993; Reyes-Guerrero et al., 2006; Vázquez-García et al., 2004). Among them, studies examining the influence of ELF-EMF on common behaviors were not available. The results of various studies have questions and do not agree with each other, hence a result cannot be easily obtained.

Naturally mammalian brain emits waves with frequencies of 0.5–80 Hz, which are attributed to the neuronal discharges (Moore and Arikan, 2004). Based on the type of brain wave, activities in the brain are categorized into four groups: delta waves (below 3.5 Hz), which features a deep and peaceful sleep; theta waves (4–7 Hz) play a role in emotional stress, during anxiety (frustration), control voluntary movement and

memory; alpha waves (8 to 13 Hz) emitted during a lull in the waking state and the rest of the brain; and beta waves (14–80 Hz) which correspond to overactive nervous system, new conditions, or mental stress (Gotman et al., 1973). As well known, people were exposed to 40 Hz ELF-EMF at home and work environments routinely, which can affect their common behaviors (Del Giudice et al., 1988; Johnson and Guy, 1972). Changes in body weight, food and water intake, anorexia (poor appetite), plasma glucose concentration, movement, rearing and sniffing indicate profound changes in metabolic and central nervous systems (Chathu et al., 2008; Shearman et al., 2003; Woods et al., 1985). These changes can also be attributed to various diseases.

Pre- and postnatal exposure of animals and consequently behavioral or neurological effects after birth can be used as a sensitive system for the study. In Chung et al.'s study, pregnant rats were exposed to 60 Hz magnetic fields and several factors were investigated, such as growth, physical development, behavior and reproductive performance (Chung et al., 2004). They found no significant changes in behaviors parameters.

In the present study, we decided to investigate the influence of long-term whole body exposure with 40 Hz ELF-EMF on the common behaviors such as weight, food and

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water intake, anorexia, glucose, movement, rearing, and sniffing.

Material and methods

Animals

Animals (male Wistar rats) were purchased from Pasture Institute (Tehran, Iran) weighing 225–300 g ($n = 8/\text{group}$). The animals were housed in standard animal room for two weeks before starting the study for adaptation under a variable temperature between 18 and 22 °C and 12 h light: 12 h dark cycle with free food and water availability. All measurements were carried out at the same time between 12:00 and 14:00 h, and also each animal was tested only once. All procedures in this study were carried according to the Stem Cell Technology Research Center (Tehran, Iran) guidelines.

Experimental design and shielded room

To prevent any interference with prepared ELF-EMF, room spatial (including the ceiling, walls, and windows) has been protect by covering with aluminum foil (0.4 mm diameter). For further conformation this step was checked by a wave detector, and lack of effective radiation in shielded room was confirmed. Then, electromagnet-generating device antenna was fitted to the symmetrical (for waves uniformly irradiation) in the top boxes. All conditions were carried out for the control group except the irradiation.

The testing process was divided into two phases, and each phase lasted for 21 days. In this study, two frequencies of 1 and 5 Hz were used (once a day, 75 mW and 0.1 mT). In each phase, on days 1, 3, 7, 14 and 21, the animals were sacrificed for biological assessments. Blood samples, brain, adrenal gland were collected for further analysis (Figure 1). Eight animals were used in each experimental group.

Body weight

Body weight of the exposed and control animal groups was measured before starting EMF-ELF irradiation on each day by the scales with ± 0.1 g accuracy.

Water and food intake

Food and water were freely available to all the animals (400 grat chew and 150 ml tap water). During the period of study, 24 h before and after exposure with ELF-EMF, amount of food and water in each cage was calculated as water and food intake index.

Anorexia

To evaluate anorexia of exposed and control groups, rats were placed in the cages. Using a digital video camera the time of starting food intake was recorded, and the time interval between caging and eating had been applied as the anorexia.

Biochemistry analysis

To determine D-Glucose concentration in plasma samples by an ELISA method, blood samples were collected in the micro-tubes with 5% EDTA and were centrifuged in 4 °C for 5 min in 3000 RPM. The supernatant was collected for ELISA assay using the Glucose kites (PARS-AZMON, Tehran, Iran).

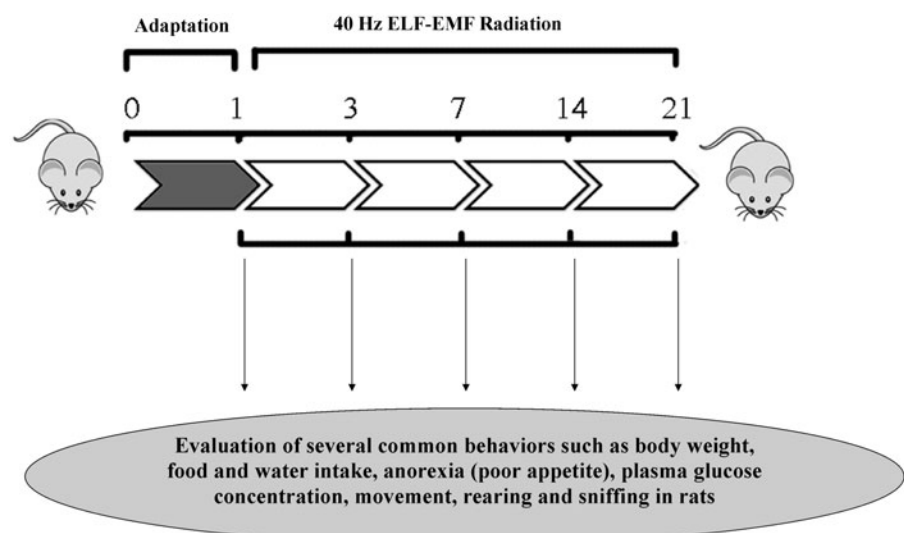
Movements, rearing and sniffing

To monitor the movements, rearing and sniffing of the rats exposed with ELF-EMF and control groups, they were placed in an open field container (30 × 30 × 40 cm high). Floor of container was divided into 16 equal-sized squares and highlighted with blue lines. Behaviors of rats in treated and control groups were recorded using a digital video camera which was placed on the top of the container at 120 cm heights. After 5 min (for adaptation), number of line crossing as activity of animals was recorded during 10 min.

Statistical analysis

For statistical analysis, two-way ANOVA was conducted using time and frequency as the factors. Further analyses for individual between-groups comparisons were carried out with *post hoc* Turkey's test. Data are showed as the mean \pm SD. In all comparisons, $p < 0.05$ was considered to be statistically significant. All statistical analyses were conducted using SPSS software, version 11.0 (SPSS, Chicago, IL).

Figure 1. Experimental design and overview of all this study steps.



Results

Weight change

As shown in Figure 2, at day 7 after exposure to 40 Hz ELF-EMF, rat's weight significantly increased in comparison to the control group. Although, weight of all irradiated rats increased in comparison to the control group, these changes were not significant.

Food and water intake

Food intake changes of animals were evaluated during 21 days after 40 Hz ELF-EMF exposure (Figure 3A), no significant changes were observed in irradiated rats compared with non-treated animals. Water intake of animals were measured, and results showed that food intake as well as water intake were not significantly changed during the period of study in irradiated rats compared to the control group (Figure 3B).

Anorexia and glucose level

As the weight of irradiated rats significantly increased after 7 day and food and water intakes were not increased significantly, to solve this problem we decided to evaluate anorexia and glucose concentration in rat's serum sample.

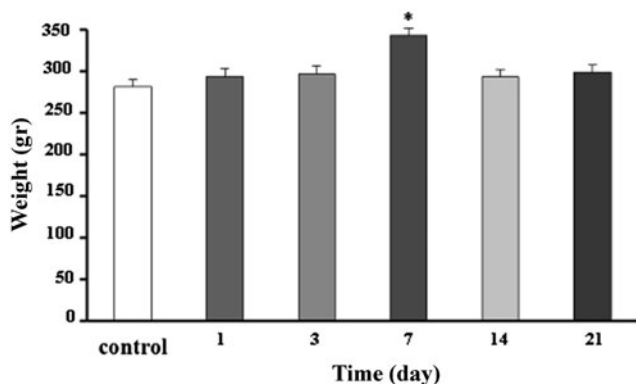


Figure 2. Weight changes of rats after exposed with 40 Hz ELF-EMF during 21 days. *A significant difference ($p < 0.05$) between group and control.

As shown in Figure 4, anorexia of irradiated rats was significantly decreased after 7 and 14 days of exposure in comparison to the control group. At other days anorexia changes were not significant in irradiated rats. To confirm further, serum glucose concentration was measured during the period of study and from day 3 their concentration increased (Figure 5). Only at day 7 this increase was significant in irradiated rats compared to the control group.

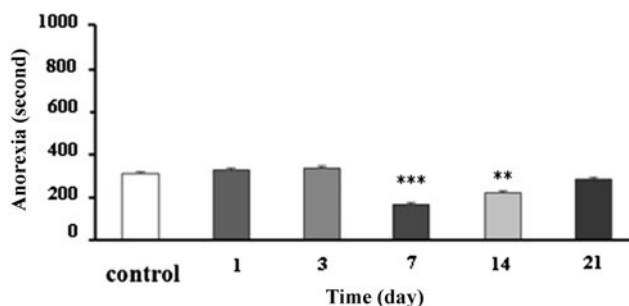


Figure 4. Anorexia levels of rats after exposed with 40 Hz extremely low frequency electromagnetic field during 21 days. ** $p < 0.01$ and *** $p < 0.001$ show a significant difference between the group and control.

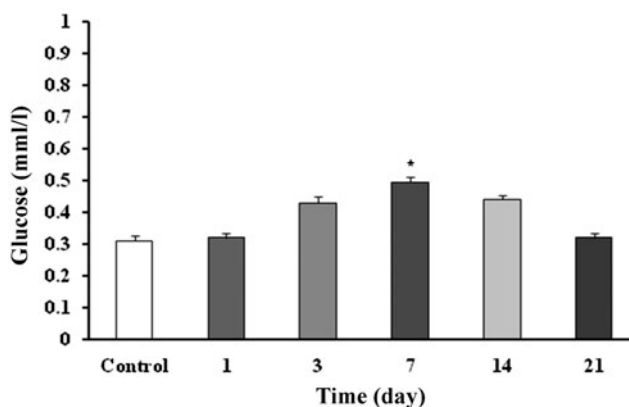


Figure 5. Glucose concentration of rats after exposed with 40 Hz extremely low frequency electromagnetic field during 21 days. *a significant difference ($p < 0.05$) between group and control.

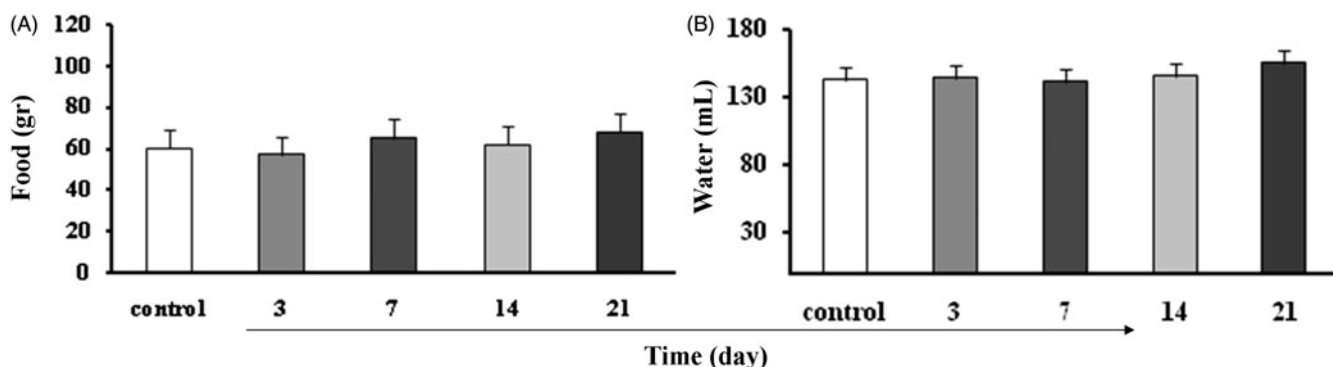


Figure 3. Food (gr, A) and water (mL, B) intake of rats after exposed with 40 Hz extremely low frequency electromagnetic field during 21 days.

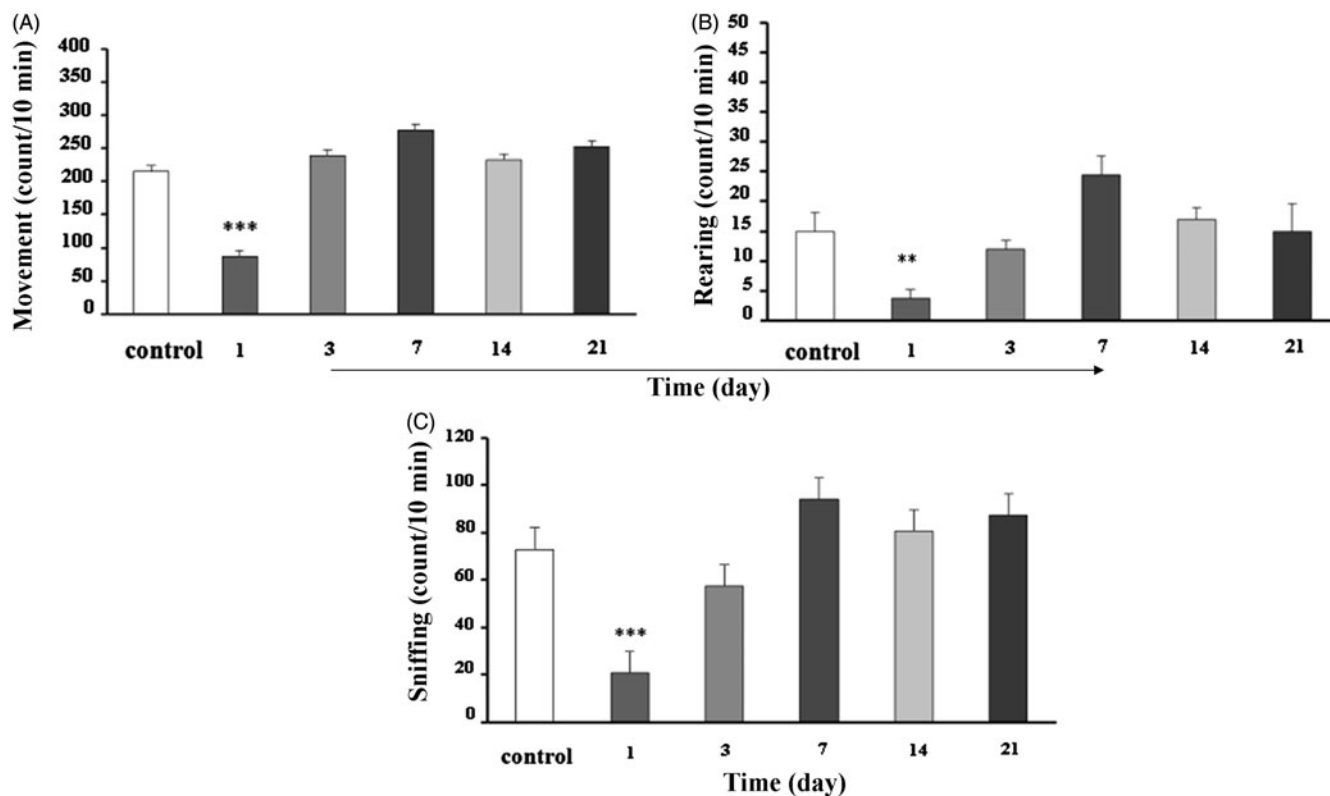


Figure 6. Movement (A), rearing (B) and sniffing (C) of rats after exposed with 40 Hz extremely low frequency electromagnetic field during 21 days. ** $p < 0.01$ and *** $p < 0.001$ show a significant difference between the group and control.

Movement, rearing and sniffing

Changes in irradiated rat movement were significantly decreased at one day after exposure to 40 Hz ELF-EMF compared to the control group (Figure 6A). During other days, these changes were not significant in comparison to the non-treated animals. One day after exposure to 40 Hz, significant decrease was observed in rearing of irradiated rats compared to the control group (Figure 6B). At day 7, rearing of irradiated rats were increased in comparison to the control group, but these changes were not significant. Sniffing is a parameter related to the attention, only one day after exposure to 40 Hz ELF-EMF significantly decrease was observed in irradiated animals compared to the control group (Figure 6C). At other days, sniffing of irradiated animals was changed but not significant. Results of two-way ANOVA analyses with p values for all different experiments are shown in Table 1.

Discussion

Here, we tried to investigate 40 Hz influence 75 mW and 0.1 mT on the common behaviors of male rats during 21 days. Body weight, water and food intakes, locomotors activity, rearing and sniffing and also serum glucose concentration were evaluated at days 1, 3, 7, 14 and 21 during the period of study. Results of this study demonstrated that although the weight of irradiated rats increased in comparison to the control group and as these changes are significant at day 7, some changes of food and water intakes in rats after exposure were not significant. Anorexia is a medical term related to the lack of appetite and is also a serious eating disorder

(Hoek, 2006), anorexia of irradiated rats significantly decreased at days 7 and 14, which may be justified by increased weight in rats after exposure to ELF-EMF.

Glucose assessing can be useful in detecting various disorders (Lillioja et al., 1988; Videbech, 2000), interestingly glucose concentration also significantly increased in serum samples of irradiated rats. These changes could be due to the food intake or catabolic action changes. In a previous study, we were evaluated 1 and 5 Hz ELF-EMF radiation affects on behavioral, hormonal, and metabolic of rats (Mahdavi et al., 2014). Results indicated that weight of irradiated rats did not change, and also no alter was observed in food and water intakes of rats after exposure to EMF-ELF in comparison to the control group. Evaluation of plasma glucose concentration showed an increase in exposed rats with 5 Hz agreed to this present study, and a decrease was detected in the animals irradiated with the 1 Hz despite of this study. It can be interpreted that, with increasing ELF-EMF frequency, the plasma glucose concentrations will significantly increase. Several research were carried out to study the influence of whole body exposure to ELF-EMF on human and animal health. Roman et al. (2005) reported the reduction in cell proliferation under exposure to ELF. Researchers also identify an increase in the concentration of Na^+ , K^+ and osmolarity after irradiated with ELF, and suggest that there is a relationship between these factors and inhibition of cell growth. Many volunteer studies showed that EMF intensity is sufficient to induce a feeling of understandable vision. The threshold for this effect is dependent on the frequency and intensity of the EMF, although the radiation of EMFs and the 50 or 60 Hz, 1 mT incomprehensible reserved. Generally, any

Table 1. Results of two-way ANOVA analyses with *p* values for all different experiments.

	Intra-group		Inter-group		Inter-Intra groups interactions	
	<i>F</i> _(4,70)	<i>p</i>	<i>F</i> _(1,70)	<i>p</i>	<i>F</i> _(4,70)	<i>p</i>
Weight						
40 Hz	2.10	0.09	3.99	0.05	1.99	0.1
Food Intake						
40 Hz	3.28	0.027	1.54	0.22	0.11	0.95
Water						
40 Hz	0.39	0.76	0.73	0.39	0.39	0.76
Anorexia						
40 Hz	78.45	0.000	154.53	0.000	60.07	0.000
Glucose						
40 Hz	0.815	0.52	1.175	0.282	0.551	0.69
Movement						
40 Hz	5.59	0.001	10.68	0.002	3.51	0.01
Rearing						
40 Hz	12.24	0.000	87.46	0.000	12.85	0.000
Sniffing						
40 Hz	12.237	0.000	87.46	0.000	12.85	0.000

change in learned behaviors is related to learning and memory, except it does not show a strong field. Several studies reported that the ELF-EMF has no effect on cognitive behaviors such as attention, performance and memory span (Cook et al., 2006; Kurokawa et al., 2003; Trimmel and Schweiger, 1998). Another study also reported that long-term radiation exposure to 50 Hz and 500 μ T has significant effect on the mobility of irradiated animals (Harris et al., 1998). Taken together, these results confirm that the ELF-EMF is able to induce behavioral changes under certain conditions (Ossenkopp et al., 1986).

Furthermore, movement, rearing and sniffing of irradiated animals are evaluated as the main variables in the ongoing search. It was interesting that, all three parameters were significantly decreased at day 1 during the period of study in irradiated rats compared to the control group. Since 40 Hz represents a new condition, psychological pressure is considered as overactive CNS. It can be interpreted that, on the first day after radiation, the animals were subjected to shock treatment, but after that the animals were adapted to the EMF.

In conclusion, result of the present study demonstrated exposure to ELF-EMF can alter normal condition of animals and may indicate the cause of many behavioral abnormalities in societies, although further studies are needed.

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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