Research Paper

Effect of Short-time Exposure of Local Extremely Low-Frequency Magnetic Fields on Sleepiness in Male Rats

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ABSTRACT

Introduction: Lack of high-quality sleep causes severe side effects like anxiety and changes in plasma concentration of oxalate. The current study investigated the impact of local extremely low-frequency magnetic fields (ELF-MFs) on inducing sleep (sleepiness) and anxiety in male rats.

Methods: In this experimental study, 40 male rats were divided into four groups (n=10 for each group). The ELF-MF exposure (0, 10, and 18 Hz) was applied with an intensity of 200μT for three days (10 min/d). The sham-treated animal did not receive ELF-MF. Serum levels of oxalic acid (OA) and sleepiness were measured before and after the last exposure to ELF-MF or sham. Anxiety, sleepiness, and OA were measured using the elevated plus maze, open-field test (OFT), and ELISA test.

Results: A comparison of oxalate levels before and after exposure to ELF-MF revealed that ELF-MF (10 Hz) decreased the serum level of oxalate ($P<0.05$). Comparing open/closed arm entry (in an elevated plus maze) between before and after exposure to ELF-MF revealed significant differences. Also, frequency, velocity, and distance moved were decreased in the open-field test.

Conclusion: Results of the present study demonstrated that ELF-MF with short-time exposure may modulate the metabolism of OA and may modulate anxiety-like behavior or kind of induction of sleepiness in male rats.

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Highlights

- Oxalate acid concentration may reduce after short time ELF-MF exposure.
- Locomotor activity in male rats may decrease after the ELF-MF exposure.
- Short time ELF-MF exposure may induce sleepiness in male rats that may be used to treat sleep disorders.

Plain Language Summary

It is necessary for a person to have good sleep to feel happy during the day. The usual way to treat the patient's sleep disorders is drug therapy, but there are some non-pharmacological treatments such as cognitive behavioral therapy and proper diet. In this study we decided to evaluate the effect of ELF-MFs on sleep induction (sleepiness) in male rats by assessing behavioral tests and measuring oxalate acid density. The results showed that the activity of rats and oxalate acid concentration reduced after ELF-MF exposure. This was consistent with results of the plus maze test and the reduction of velocity, frequency and in the open-field test can be attributed to sleepiness. The results of this research showed that ELF-MF with short time exposure may modulate the anxiety-like behavior or kind of induction of sleepiness in male rats. This effect may be used to treat sleep disorders and requires further human studies.

1. Introduction

A person must sleep well to feel happy during the day (Sadock, Flaherty, & Sadock, 2011). Insomnia is a general health disorder associated with occupational problems, interpersonal problems, driving accidents, educational problems, difficulty working night shifts, and anxiety (Bélanger, Morin, Langlois, & Ladouceur, 2004; Husby & Lingjaerde, 1990; Lader, 1999; Okada et al., 2000). According to studies, insomnia may develop at any age, although it is more common in aging (Friedman, 2006). Reduced sleep duration in humans has been associated with risk for metabolic disorders, diabetes, obesity, weight gain, and cardiovascular disease (Ayas et al., 2003; Di Milia, Vandelanotte, & Duncan, 2013; Schmid, Hallschmid, & Schultes, 2015; Singh, Drake, Roehrs, Hudgel, & Roth, 2005). The usual way to treat a patient's sleep disorders is drug therapy, but there are some non-pharmacological treatments such as cognitive behavioral therapy and proper diet (Morin et al., 2006).

Some studies showed that exposure to extremely low-frequency magnetic fields (ELF-MFs) might affect cognitive functions (Akbarnejad et al., 2018; Shafiei, Firoozabadi, Rasoulzadeh Tabatabaie, & Ghabaee, 2014; Sharma et al., 2017). Nevertheless, there are conflicting reports on the influence of MFs on sleep quality. For example, electromagnetic fields may affect sleep and cognition tasks in the rat (Dyche, Anch, Fogler, Barnett, & Thomas, 2012; Hassanshahi et al., 2017). Chronic exposure to pulsed ELF-MFs can alleviate the sleep disorders of patients with insomnia (Pelka Jaenicke, C., & Gruenwald, 2001) and affect sleep quality (Altpeter et al., 2006). On the other hand, Schmid et al. and Tworoger et al. did not report any significant effect on the sleep quality of young women (Schmid et al., 2012; Tworoger et al., 2004), even in diabetic patients with sleep disorders (Wrobel et al., 2008) and sleeping patterns of healthy people (Wagner et al., 2000). However, our latest studies showed that exposure to ELF-MFs can change EEGs recorded from some areas of the scalp and may even influence reaction time in young, healthy women (Ayoobi, Shamsizadeh, & Shafiei, 2017; Shafiei, Firoozabadi, Rasoulzadeh Tabatabaie, & Ghabaee, 2012). A good night's sleep after ELF-MF exposure had been reported by some participants who have sleep problems. However, the evaluation of this issue was not the focus of this research (Amiri Fallah, Firoozabadi, Shafiei, & Assadi, 2011; Amirifalah, Mohammad, Firoozabadi, & Shafiei Darabi, 2013). These studies have shown that local ELF-MFs may become a helpful therapeutic tool in treating some psychological symptoms (Shafiei & Firoozabadi, 2014).

Then again, the oxalic acid level is a good marker of sleep deprivation (Weljie et al., 2015). The primary sources of blood oxalate are a vegetarian diet, ascorbic acid decomposition, and endogenous synthetic pathways in the liver and erythrocytes (Hodgkinson & Zarembski, 1968). Endogenous substrates for oxalate synthesis include glycolate in peroxisome and hydroxyproline, and sleep deprivation for several days is related to hydroxyproline levels in mice (Koehl et al., 2006). Acute sleep deprivation eliminated oxalate from plasma via increased urinary excretion (Weljie et al., 2015).

Therefore, due to the importance of inducing sleep and the safety of extremely weak magnetic fields at very low frequencies, we decided to evaluate the effect of ELF-MFs on sleep induction (sleepiness) in male rats by assessing behavioral tests and measuring oxalate acid density.

2. Material And Methods

Study subjects

This study was obtained from the Central Animal House, Rafsanjan University of Medical Sciences, Iran. Forty male rats of the Wistar strain (160-210 g) were distributed into four experimental groups (n=40: three exposed groups [0, 10, and 18 Hz] n=10/group; sham exposed n=10) and were individually housed before and during experimental protocols. The animals were exposed to a stable temperature (22°C-24°C) and relative humidity of 45%-55%. They were kept under a cycle of 12h light and 12h darkness for at least 2 weeks in our laboratory before starting experiments. Rodents were kept awake at night and asleep during the day. In order to adjust the sleep-wake cycle of rats, we reversed 12h of light and 12h of darkness for these animals in the laboratory so that they have time to wake up during the day and we could do convenient experiments. Animals were fed daily, and cages cleaned.

ELF-MFs exposure

Helmholtz coils with 40 cm diameter separated by a distance of 20 cm were used to generate a nearly uniform magnetic field. A dodecagon made of acrylic with 25 cm diameter and 15 cm height was used and located between two Helmholtz coils. The animal was placed in this box, and a magnetic field with an intensity of 200µT was applied for 10 min/d for three days. ELF-MFs exposure was performed at 9 AM. The three experimental groups (0, 10, and 18 Hz) were differentiated in terms of the transmitted frequency, and in group four, all stages were performed like those of the previous groups, except that the set was turned off and there was no magnetic field (Figure 1). Food and water were unavailable during exposures.

Open-field test

Anxiety-like behaviors and locomotor activity were measured with an open-field and behavioral tracking analysis system; we used a box 50cm in height, and 70cm in diameter, with a video camera, installed 2.5m above the apparatus. Each rat activity was digitally monitored and then analyzed using EthoVision XT software, version 7.1, Noldus Information Technology, Wageningen, The Netherlands. The interior area of the box was divided into inner and outer zones. First, the rat was placed in the center of the box, and its activity was recorded for 5 min. Then the following behavioral parameters were scored: velocity (cm/s), frequency, and total distance moved (cm) in the inner zone. After removing the rat from the open field, the experimental chamber was cleaned with diluted ethanol (10%) and dried (Crawley, 1999).

Elevated plus maze test

we used the elevated plus maze test to measure the rat's movement and anxiety. It comprised 2 open arms (50×10 cm) and 2 enclosed arms (50×10×40 cm) that extend from a common central platform (10×10 cm). A wooden apparatus was elevated 50 cm above floor level. Testing was conducted between 9.00 am and 1.00 pm in a quiet room. The rats were placed in the center of the maze facing an open arm individually, and 5 min were allowed for free exploration. All sessions were filmed. The floor was cleaned after each test with distillate water. The animals were tested before the first ELF-MF exposure and after the last ELF-MF exposure. Measurements were made from the time spent in the maze's central, open and closed parts. In the absence of anxiety, the animal moves more and has a great deal of activity in the open arm. However, if the rat experiences anxiety and sleepiness, it will remain in the closed arm (Rodgers & Dalvi, 1997).

Oxalic acid (OA) measurement

The total serum metabolic oxalic acid (OA) was measured by oxalate assay kit (Colorimetric-KA4532, Abnova, Taiwan) to evaluate how systemic metabolism is impacted by sleep restriction. Blood was taken in the morning one day before the first exposure to ELF-MF (before behavioral tests) and one day after the last exposure to ELF-MF (after behavioral tests). The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of OA in samples. OA is added to a monoclonal antibody enzyme well which is pre-coated with OA monoclonal antibody; this is subject to incubation, and then OA antibodies labeled with biotin are added and combined with streptavidin-HRP to form the immune complex. Next, incubation is carried out, and the material is re-

Figure 1. Schematic view of Helmholtz coils

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washed to remove the uncombined enzyme. To close, chromogen solution A and B is added, and the color of the liquid changes into blue, and with the effect of the acid, the color finally becomes yellow. The chroma of color and the concentration of the substance OA in the sample were positively correlated.

Procedure

Figure 2 represents all of the protocols on different days. This protocol was implemented at different frequencies (0, 10, and 18Hz) for these groups and the sham group, in which the signal generator did not produce any electrical signals, and thus there were no ELF-MFs in the sham group.

Statistical analysis

The obtained data were analyzed by SPSS software version 20.0. The quantitative data were reported as Mean±SD and qualitative data in percentages. A paired t-test was used to compare open-field scores, elevated plus maze, ELF-MF, and mean oxalic acid before and after the last exposure. Differences among groups were compared using 1-way ANOVA across all groups. The significance level in all tests was $P<0.05$.

3. Results

ELF-MF exposure and anxiety

The average presence of rats in the open arm decreased after the last ELF-MF exposure, and there were significant differences in the ELF-MF exposure 10Hz (P=0.03) and ELF-MF exposure 18Hz groups (P=0.007). On the other hand, the average presence of rats in the closed arm increased significantly after the last ELF-MF exposure in the ELF-MF exposure 18Hz group (P=0.01) (Figure 3).

ELF-MF exposure and locomotor activity

The open-field test was used to determine anxiety-like behaviors and locomotor activity in different groups before and after ELF-MF exposure. For locomotor activity, the traveled distance of movements decreased after the last ELF-MF exposure, and the traveled distances were

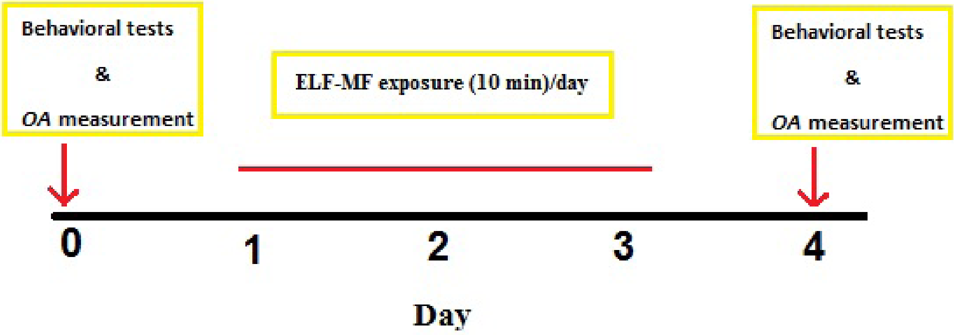

Figure 2. Schematic view of the protocol of study on different days

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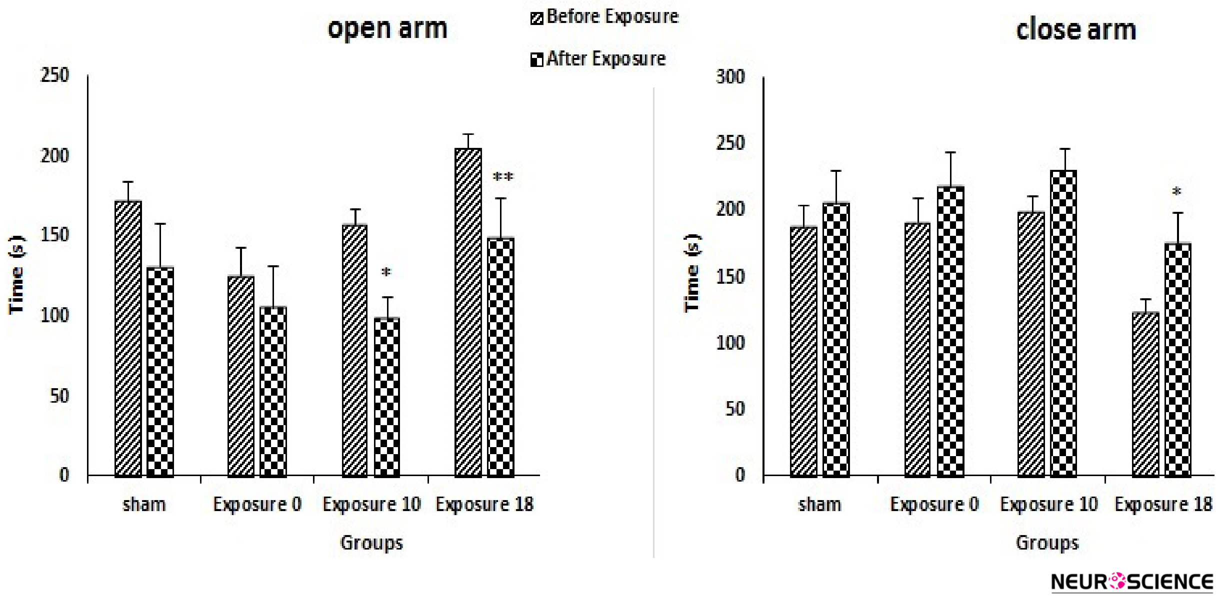

Figure 3. Elevated plus maze test in all groups

There was a significant effect between the groups before the first and after the last ELF-MF exposures.

* P<0.05; ** P<0.01.

significant between before the first and after the last ELF-MF exposure in the direct current (DC) magnetic field exposure, in which frequency was 0Hz (P=0.02), ELF-MF exposure 10Hz (P=0.002) and ELF-MF exposure 18Hz groups (P=0.000). Also, the velocity decreased after the last ELF-MF exposure and was significant in ELF-MF exposure 0Hz (P=0.02), ELF-MF exposure 10Hz (P=0.002), and ELF-MF exposure 18Hz groups (P=0.000). Frequency decreased after the last ELF-MF exposure, and it was significant in all groups (P=0.01) except the sham group (P=0.48) (Figure 4).

ELF-MF exposure and OA density

The serum levels of OA were measured by ELISA. The mean serum level of OA was 2.10±0.56 nmol/µL before ELF-MF exposure, and it was significantly higher as compared after ELF-MF exposure (1.28±0.47) in the wave 10Hz group (P<0.05). No significant difference was observed among the other groups before and after ELF-MF exposure (Figure 5). All results are summarized in Table 1.

4. Discussion

Our previous human experiments suggested that exposure to 200µT MFs with extremely low-frequency may improve sleep disturbance (insomnia) caused by anxiety (Amirifallah et al., 2011; Amirifalah et al., 2013). This study used magnetic fields at very low frequencies to evaluate the impact of DC (0Hz), 10Hz, 18Hz, and 200µT MF on sleepiness induction rates.

We suggest that the reduction in the frequency, velocity, and distance moved in open-field tests after exposure (Figure 4) and the decrease in the average presence of rats in the open arm (Figure 3) were due to a kind of induction of sleepiness. Therefore, the findings of this study are consistent with those of our previous studies, confirming our hypothesis that the ELF-MFs can induce sleepiness (Table 1).

Ayoobi et al. examined the effect of ELF-MF on sleepiness induction in healthy students, especially females. They used 200µT MF (3 min duration) at three frequencies (18, 14, and 10Hz), applied to the skull in areas C4, CZ, and C3, respectively, on 32 males and 31 females. Their study showed that exposure to local ELF-MFs might influence minimum reaction time in young, healthy females (Ayoobi et al., 2017). Their findings are consistent with the results of this study.

However, some studies, like Kurokawa et al., had inconsistent findings. They evaluated the effects of continuous exposure to 50Hz, 20µT MF on 20 subjects for 55 min. The reaction time in time perception tasks did not change with ELF-MF exposure (Kurokawa, Nitta, Imai, & Kabuto, 2003). In this study, the magnetic field used was 10 times less than the magnetic field in this study.

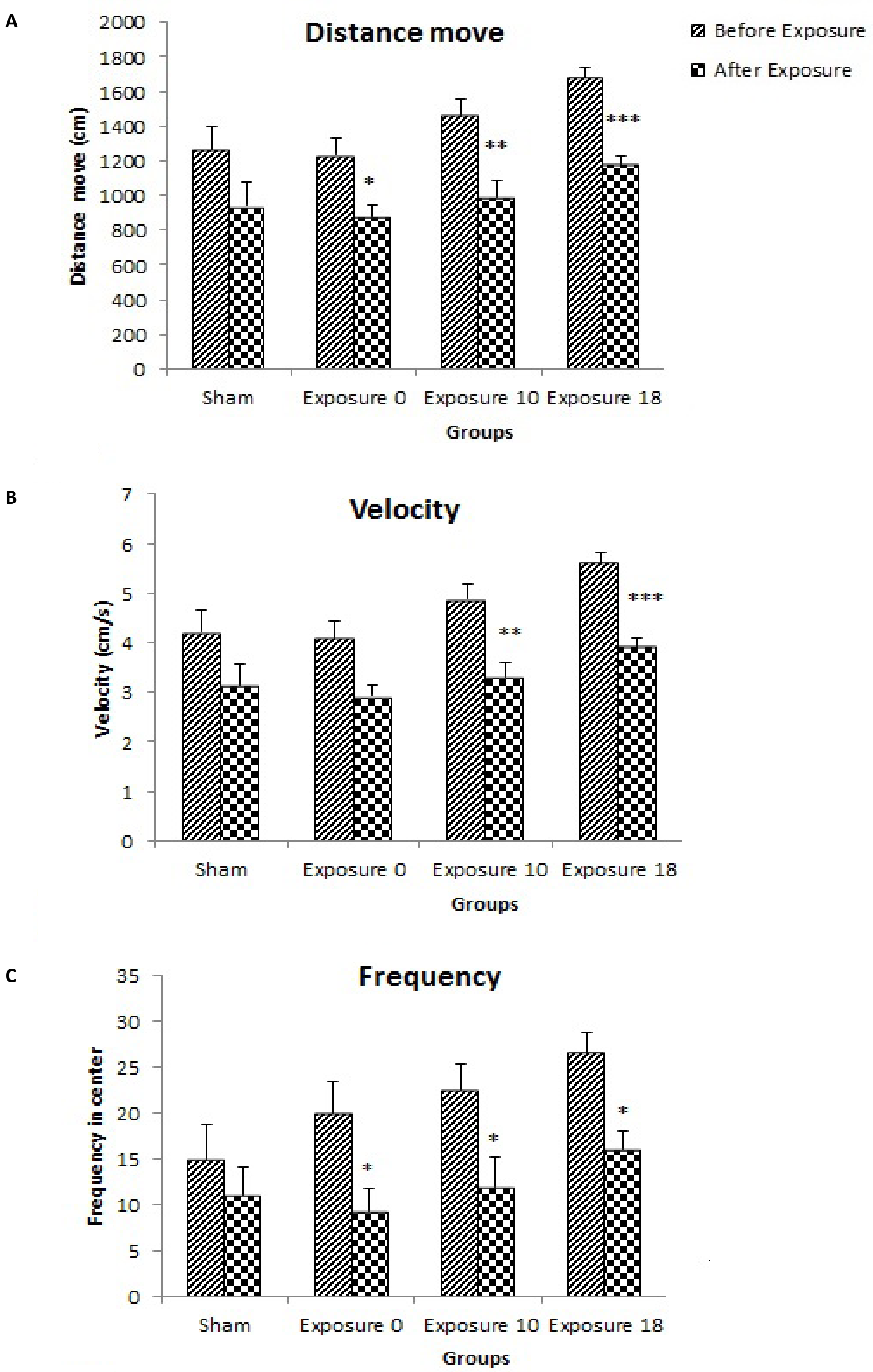

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Figure 4. Open-field tests in all groups

There was a significant effect among the groups before the first and after the last ELF-MF exposures.

* P<0.05; ** P<0.01; *** P<0.001.

Table 1. Comparing all tests before the first and after the last ELF-MF exposures

Tests			Mean±SD			
			Sham	Exposure 0	Exposure 10	Exposure 18
Elevated plus maze test (s)	Open arm	Before	171.22±35.68	124.65±55.49	156.67±28.66	204.44±27.28
		After	130.71±69.58	105.14±80.88	97.81±42.18 *	148.10±79.78 **
	Close arm	Before	187.33±51.35	190.92±58.24	198.00±34.84	123.00±31.56
		After	205.22±78.35	217.51±81.17	229.71±53.21	175.71±71.53 *
Open-field test	Distance moved (cm)	Before	1264.41±375.82	1233.51±257.88	1463.85±258.55	1683.34±174.31
		After	940.35±377.01	877.33±169.85 *	991.16±240.35 **	1179.85±154.59 ***
	Velocity (cm/s)	Before	4.24±1.25	4.11±0.86	4.88±0.86	5.61±0.58
		After	3.13±1.26	2.92±0.57	3.30±0.80 **	3.93±0.52 ***
	Frequency	Before	15.00±10.54	20.00±8.88	22.57±7.37	26.66±6.12
		After	11.00±8.49	9.29±6.75 *	12.00±8.14 *	16.11±5.58 *
Oxalic acid (OA)	Density (n/mol)	Before	1.51±0.24	1.96±0.20	2.10±0.56	1.89±0.53
		After	1.52±0.36	1.30±0.41**	1.28±0.48***	1.42±0.22

* Significant difference between before the first and after the last ELF-MF exposures.
* P<0.05; ** P<0.01; *** P<0.001.

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Generally, reduced time spent in the central area in the open-field test (Prut & Belzung, 2003) and reduced time spent in the open arms plus maze (Lister, 1987) have been established as an index of increased anxiety behavior. Some animal studies indicated that high intensity and chronic exposure to ELF-MF might induce anxiety-like behavior in rodents (Kitaoka, Kitamura, Aoi, Shimizu, & Yoshizaki, 2013; Liu, Wang, He, & Ye, 2008), and in this study, we expect them to be sleepy because of short exposure.

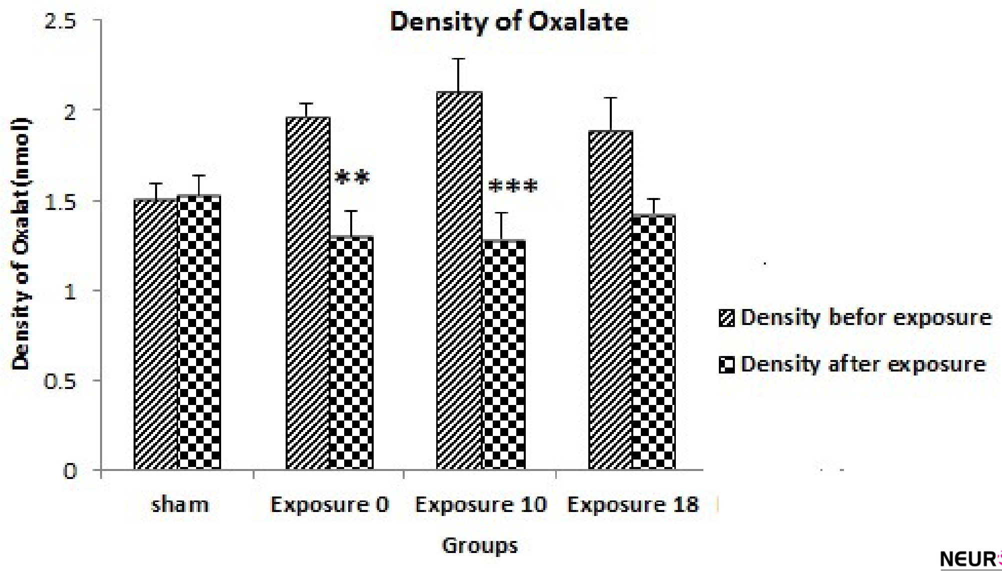

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Figure 5. Comparing serum levels of oxalic acid before the first and after the last of the ELF-MF exposure groups

Serum OA levels in wave DC and wave 10Hz group after ELF-MF exposure were significantly less than before the ELF-MF exposure. No significant difference was observed among the other groups. ** P<0.01, *** P<0.001.

Hi, et al. conducted an experimental study and showed that 4 h/d of chronic exposure to ELF-MF significantly affects adult rats' anxiety level and spatial memory. However, 1 h/d exposure to ELF-MFs had no effect in any test (He et al., 2011). ELF-MF exposure of rats 4 h/day increased the anxiety-like behaviors in the open-field test and the elevated plus maze test without altering locomotor activity but had no observed effect in the light/dark box test. Moreover, 1 h/d of ELF-MF exposure did not affect any test. Increased exposure to ELF-EMF may directly and significantly affect anxiety (Hosseinabadi, Khanjani, Ebrahimi, Haji, & Abdolahfard, 2019).

Generally, in a short time exposure, evidence shows that ELF-MF exposure had no significant effects on anxiety induction. Furthermore, the results of the meta-analyses have shown that exposure to ELF-MFs has no considerable effect on cognitive functions (Barth, Ponocny, Ponocny-Seliger, Vana, & Winker, 2010).

Ouyang et al. measured plasma concentrations of oxalic acid in free-ranging wild birds' blood as a sleep restriction biomarker based on earlier studies (Ouyang et al., 2017; Weljie et al., 2015). The findings showed that oxalic acid levels reduced strongly in birds with their highest activity levels at night. They assumed it is likely that night activity may cause sleeplessness and, as a result, a decrease in the concentration of oxalic acid (Ouyang et al., 2017).

Our results suggest that the activity of rats and oxalate acid concentration reduced after ELF-MF exposure (Figure 3). ELF-MF is assumed to cause severe anxiety in rats, resulting in oxalate decrease (Table 1). These results are consistent with the results of the plus maze test (Figure 1) and the reduction of velocity frequency in the open-field test (Figure 2). However, such a hypothesis is incompatible with the evidence obtained in our previous human research, which showed that healthy female participants experienced good sleep after the ELF-MF exposure. The result of Figure 2 (plus maze) can be attributed to sleepiness.

In 2011, Akhtary et al. analyzed the effects of ELF-MFs on learning, memory, and pseudo-anxiety behaviors in giant white laboratory mice. Field groups lived for 28 days in special fiberglass cages with a magnetic field of 10 or 100 µT. The anxiety was measured by the elevated plus maze test. The groups showed no significant difference (Akhtary, Rashidy-Pour, Vafaei, & Jadidi, 2011). It can be concluded that a 10µT field with chronic conditions could not induce anxiety. These findings are similar to the results of the present study.

In another study, 60 diabetic patients were divided into two groups, and 32 were subjected to a magnetic field with a frequency of 180Hz to 195Hz with an intensity of about 100µT for 20 min, 5 times per week. The results showed that ELF-MFs did not affect sleep disorders and serum level oxalate (Wrobel et al., 2008).

Monazzam et al. reviewed the quality of sleep and general health of employees exposed to ELF-MFs in a petrochemical complex. They examined the relationship between the levels of ELF, sleep quality, and the general health of 40 EMF-exposed workers and 22 controls. The results showed that 28% of the subjects were in poor health, and 61% had a sleep disorder. No significant difference ($P<0.01$) was found among the case and control groups regarding sleep quality and general health (Monazzam et al., 2014).

In 2011, the effects of ELF-MFs on mouse anxiety levels were investigated. A magnetic field of 50Hz was used with two groups of animals for 1 and 4 hr. Pseudo-anxiety behaviors were evaluated using an open-field experiment and evaluated plus maze. Mice from the 4-h exposure had more pseudo-anxiety behaviors and static motor activities followed by increased anxiety (He et al., 2011). This difference can be due to the difference in the exposure time and frequency of the magnetic field in these two studies.

The limitations of our study were not measuring melatonin levels. Some reports suggest that melatonin production is slightly increased in exposed animals (Altpeter et al., 2006; Dyche et al., 2012). There are many studies in this field, but when we examined them, there was a significant discrepancy and even a negative one.

5. Conclusion

This study showed that ELF-MF with short-time exposure might modulate the anxiety-like behavior or kind of induction of sleepiness in male rats. This Effect may be used to treat sleep disorders and requires further study.

Ethical Considerations

Compliance with ethical guidelines

The research was approved by the Ethical Committee of Rafsanjan University of Medical Science (RUMS).

Funding

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Authors' contributions

Conceptualization and Supervision: Fatemeh Ayoobi, Ali Shamsizadeh, Amir Moghadam-Ahmadi, Seyed Ali Shafiei and Alireza Khoshdel; Methodology: Fatemeh Ayoobi, Ali Shamsizadeh, Amir Moghadam-Ahmadi, Seyed Ali Shafiei; Investigation, Writing–original draft, and Writing-review & editing: All authors; Data collection: Elnaz Azizi, Fatemeh Ayoobi; Data analysis: Elnaz Azizi, Fatemeh Ayoobi, Ali Shamsizadeh, Amir Moghadam-Ahmadi, Seyed Ali Shafiei, and Alireza Khoshdel; Funding acquisition and Resources: All authors.

Conflict of interest

The authors declared no conflict of interest.

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