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Effect of Different Extremely Low Frequency Electromagnetic Fields on Mice Long Term Memory

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Abstract

Conclusive results of the effect of extremely low frequency magnetic fields (ELF MF) exposure on memory are controversial. The aim of the present study was to investigate and follow up the effect of exposure to 1, 2 and 3 mT ELF MF for 30 min daily for 7 days on mice long-term memory. Passive avoidance task was used, and assessments were carried out one day, one and two weeks after exposure. The results showed that all intensities impaired long–term memory compared to the control after one day and one week. 3mT group was more affected than 1 mT group after one day and more affected than 1 and 2 mT groups after one week. After 2 weeks of exposure, memory was significantly impaired only in 3 mT group in comparison to control and 1 mT groups. We concluded that ELF MF impair long-term memory, this impairment was more significant with the increase of exposure intensity. Memory impairment was temporary along the studied design in all studied exposure intensities.

Keywords: Electromagnetic exposure, different electromagnetic intensities, working memory, passive avoidance test

1. Introduction

Extremely low frequency magnetic field (ELF MF) is a magnetic field produced in the frequency range 1-300 Hz and originates primarily from distribution and usage of electricity [M. Mattsson et al., 2014]. The intensity and duration of exposure to ELF MF have been increasing nowadays. This is due to the widespread use of the applications of electric power and electrical facilities, that emit 50/60 Hz electromagnetic waves [Li-hua HE et al., 2011].

Although many studies focused on the changes in learning and memory due to the exposure of ELF MF with variety of conditions including; field intensity, duration, animal models and behavioral study methods, still a high debate about this issue exists [Kavaliers M et al., 1993, Sienkiewics ZJ et al., 2001]. There are no final, conclusive results from these studies.

Randa M et al. [2002] reported significant negative effects of chronic exposure (1 and 2 weeks) to ELF MF of 2 G intensity on memory in rats and related that to the increase in plasma corticosterone level. Similar results were obtained by Yuqing D et al. [2014] who reported that the ELF MF (50 Hz, 8 mT, 28 days) exposure induced alterations of the contents of amino acid neurotransmitters in the brain, which are associated with degenerative diseases of central nervous system, brain injury and cognitive impairment.

Contradictory, some results indicated that the chronic exposure of mice to 50 Hz MF at 2 mT for 60 min/day for 2 weeks, did not affect Y-maze performance but improved spatial learning acquisition and memory retention in the water maze task as shown in Wang X1 et al. [2013] study. Similarly cognitive function of mice was enhanced in Liu et al. [2008] study, which examined spatial learning and memory changes using the Morris water maze after 4 weeks of chronic exposure to an ELF MF (2 mT, 50 Hz ELF, 4 h daily).

Thus it is not yet confirmed whether ELF MF can either improve learning and memory or impair these cognitive functions. Furthermore, there was a study reported that the total exposure of 65 min to magnetic fields has no effect [Delhez M et al., 2004] on memory using the object recognition task.
Apart from the controversial about the exact effect of exposure to ELF MF on memory, the pattern of such effect whether temporary or permanent is another considerable point to be examined.

Our aim in this study is to examine the effect of chronic exposure to three different doses of ELF MF on mice long-term memory in order to help in reducing the previous results' confusion. It also aims to follow up on the memory of exposed mice at later times, in order to test whether these effects, if present, are permanent or temporary changes.

Processes of learning and memory in the mouse are analogous to those found in humans and nonhuman primates. Memory is classified as either explicit or implicit, with explicit memory involving the processes of encoding, storage, and recall. Both explicit and implicit memories can be assessed with regard to immediate, short-term (working), and long-term memory. [Kandel ER ET AL., 2000]

Long-term memory is recognized as a mechanism for storing information over a prolonged period of time that can last from several hours to a lifetime of the animal. [Haberlandt K et al., 2005]

2. Material and Methods

2.1. Magnetic field device

Magnetic field coil was designed and performed in Medical Biophysics department, Medical Research Institute, “Alexandria University”. The coil is of 850 loops, 2.5 cm radius, and resistance of 12 Ω. The coil was connected to electrical function generator (MCP Lab electronics, Model: LG110, Shanghai, China). An oscilloscope was attached to the system to verify delivery of the electric pulses.

The magnetic field intensity doses were calculated at the center of the coil mathematically, and confirmed practically using a homemade search coil (0.1 mm in diameter and 100 turns). The frequency was fixed at 50 Hz, and by varying the electric current we could achieve 1, 2 and 3 mT at the center of the coil.

The used doses of 1, 2, and 3 mT are thus 10000, 20000 and 30000 times the permissible magnetic dose for general public according to the guidelines of International Commission on Non-Ionizing Radiation Protection (ICNIRP), published in: health physics 1998.

2.2. Animals

Forty Albino mice weighing 20-25 g of 8-10 weeks of age were used in the experiment. The mice were kept in ordinary light conditions (12 h light / 12 h dark) in constant temperature at 23 ºC. Food and water were available ad libitum. All experimental procedures were carried out based on the ethical guidelines for care and use of laboratory animals of Alexandria University.

2.3. Exposure to ELF-EMF

The exposure was individually, at the time of exposure, each mouse was kept in plastic cage that fix his body while his head was free, the cage was placed in such matter that the head was in the center of the coil.

2.4. Animal groups

The animals were divided to four groups (n=10/group); Control group (CG): These mice were not exposed to any magnetic fields but placed in the same atmosphere as other experimental mice for the same duration, Group 1 (G1): mice exposed to ELF MF of 1 mT, Group2 (G2): mice exposed to ELF MF of 2 mT, Group 3 (G3): mice exposed to ELF MF of 3 mT. All exposed durations were 30 min daily for continuous 7 days.

2.5. Learning and long term memory assessment; Passive Avoidance Task (PAT): [Sakurai M et al., 2008]:

PAT is a fear-aggravated test used to evaluate learning and long-term memory. In this test, mice learn to avoid an environment in which an aversive stimulus (a foot-shock) was previously delivered. The testing apparatus was formed of two compartments, lightened white compartment and black dark compartment that were separated by a sliding guillotine door. The dark compartment floor is connected electrically to a shock generator. The shock intensity used in training is the minimal amount needed to motivate the animal, which was about (0.2-0.3mA). Illumination was provided by a fluorescent lamp mounted above the light chamber. After each session of the PAT, the white compartment was cleaned using 10% ethanol solution to ensure that behavior of animals was not guided by odor cues. The task was formed in 2 phases; training phase or acquisition trial and testing phase that was done one day, one and two weeks after acquisition training to follow up their memory status.

2.5.1. Acquisition trial

Each mouse was placed 30 s in the white compartment facing away the dark compartment for exploration. After 30 s door was opened. The time taken (initial latency) to enter the darkened compartment was recorded (from the time the door was opened). When the animal steps into the dark compartment with all four paws, the door was closed, and a 2 s foot-shock was given (0.2-0.3 mA). 30 s after been shocked, the mouse was removed and placed in its home cage.

2.5.2. Testing Trial

Twenty-four h, one week and two weeks after acquisition, each mouse was returned to the white compartment facing away the dark compartment. 5 s after, the door was opened. The time taken; retention latency (RL) to enter the dark compartment was recorded, but the animal was not shocked if entered. The mouse was then removed and placed in its home cage. The task was stopped when the mouse had not entered the dark compartment within 300 s; this duration is known as the test ceiling.

Mice with normal memory function will not reenter the dark compartment in the testing trial that extends to 300 s (test ceiling). This is because it learnt to avoid an environment in which an aversive stimulus (a foot-shock) was previously delivered. Reentering the dark compartment thus, denotes impaired memory function in a way that the less time the mouse takes to reenter the dark compartment (RL), the more the memory is impaired.

2.6. Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using mean, and standard deviation. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test. It revealed normal data distribution thus parametric tests were applied. Comparison between the studied groups was analyzed using F-test (ANOVA) and Post Hoc test (LSD) without adjustment for multiple comparisons. Significance of the obtained results was judged at the 5% level. [Kotz S et al. 2006]

3. Results

3.1. Initial latency (IL).

Comparison of the initial latency in all animal groups revealed no significant difference in the time taken by the mice to enter the dark compartment before any stimulus was delivered (foot shock). The mean duration of these latencies shown in (Table 1) reflects the normal behavior of mice in exploration and preference to the dark environment.

Table 1. Comparison between the different studied groups according to retention latency.

Retention latency	Control	Group I	Group II	Group III	p
Initial	11.30 ± 3.89	11.40 ± 3.20	11.80 ± 3.68	11.40 ± 3.41	0.990
1 day	298.0 ± 6.32	$63.10^{a} \pm 30.45$	$50.40^{a} \pm 46.84$	$30.80^{ab} \pm 19.28$	$<0.001^{*}$
1 week	300.0 ± 0.0	$126.70^{a} \pm 72.50$	$148.90^{a} \pm 96.03$	$57.50^{abc} \pm 52.56$	$<0.001^{*}$

Table 1. (Continued): Comparison between the different studied groups according to retention latency.

2 weeks	300.0 ± 0.0	300.0 ± 0.0	288.60 ± 20.70	268.70^{ab}±45.71	0.026^{*}
p_1	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	

Note: p is p value for F test (ANOVA), Sig. between groups was done using Post Hoc Test (LSD)
p_1: Stands for adjusted LSD p-value for ANOVA with repeated measures for comparison between initial with each other periods
a: significant with control
b: significant with group I
c: significant with group II
*: Statistically significant at $p \leq 0.05$

3.2. Retention latency (RL)

Retention tests were performed 24 h, one week and two weeks after the acquisition trial. RL after 24 h showed a significant decrease in all experimental groups in comparison to the control group. Also, there was a significant decrease in its duration in G3 in comparison to G1. One week after acquisition, the RL was still significantly lower in all experimental groups in comparison to the control group. In addition, RL was significantly decreased in G3 in comparison to its duration in G1 and G2. After two weeks, there was no significant difference in RL between CG, G1 and G2. But it was significantly decreased in G3 compared to the previously mentioned groups.

3.3. Follow up examination

Follow up to each experimental group was done to examine the deterioration or improvement of the mice memory function (Figure 1). In CG, the retention latency was significantly increased 24 h, 1 week and 2 weeks after the shock in comparison to the initial latency. This increase almost reached the maximum test duration (300 s) after 24 h and reached the maximum test duration after one and two weeks. That’s to say that almost all rats didn’t reenter the dark compartment after 24 h and literally all of them did not reenter the dark compartment after one and two weeks. There was no significant difference in RL among the examined time intervals in this group.

G1 showed somehow similar results, where the retention latency was significantly increased 24, one week and two weeks after shock in comparison to the initial latency, but it only reached the maximum duration time (300 s) after two weeks. Results also showed a significant increase in RL duration along time, as it was significantly longer after1 week in comparison to 24 h and after two weeks in comparison to after one-week duration.

On the other hand, there was some difference in results regarding G2 and G3. Where in G2 there was no significant difference in RL after 24 h in comparison the initial latency, but it was then significantly increased after 1 week and after 2 weeks in comparison to initial and 24 h after shock. Also, the duration of RL was significantly increased after two weeks in comparison to after 1week duration.

G3 results were similar to that of G2 but with only one difference, RL duration showed no significant difference one week and 24 h after shock.

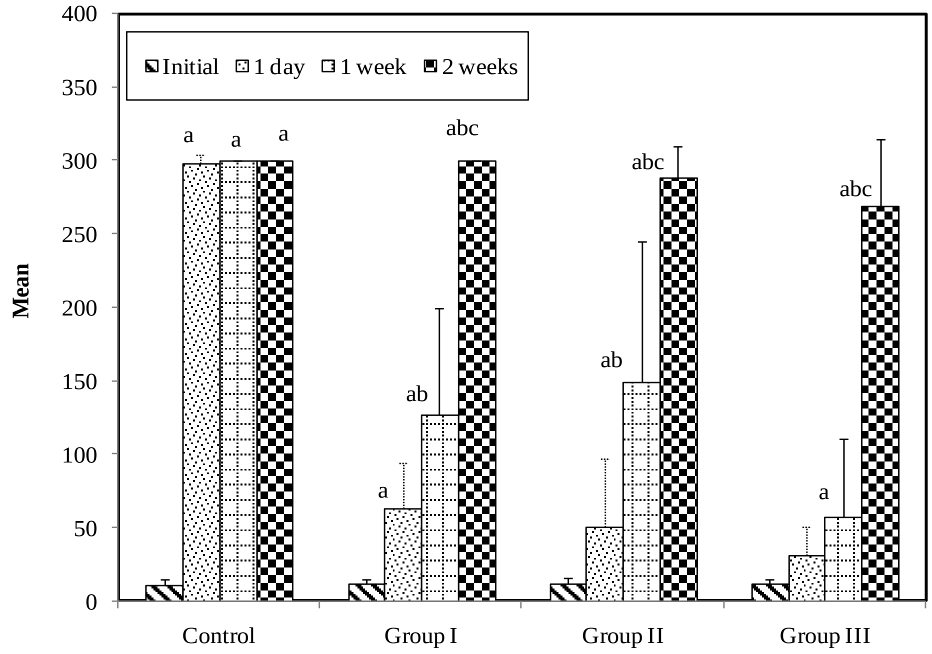

Figure 1. Comparison between the different studied groups according to retention latency.

4. Discussion

The effect of electromagnetic field (EMF) radiation on brain chemistry and function had been a point of interest to many researchers in the past few decades. This is obviously due to the enormous development in technology around us. Many studies were held to emphasize the changes of learning and memory occurring due to ELF MF exposure, but the results were somehow conflicting. In this study, we aimed to reduce this confusion and also to retrieve some new points in the extent of these changes if they ever happened.

The results of this study emphasize temporary impairment on memory function in mice as a result of exposure to ELF MF with different intensities, confirming the results of more recent studies [Ntzouni MP et al., 2011, Narayanan SN et al., 2010, Chen YB et al., 2011] suggesting such effect on rat and mice.

In our study, mice in all groups, behaved normally in the acquisition phase of PAT, as all mice were eager to discover the dark compartment shortly after opening the guillotine door separating the two test compartments. Results showed no significant difference in the initial latency between different groups denoting that the exposure to a magnetic field has no effect on normal exploratory behavior of mice.

In control group, the RL 24 h after the shock was significantly higher than IL. Also RL was significantly increasing after one week and two weeks, in a way that it reached the test ceiling duration after one and two weeks from acquisition, that's to say, all mice never reentered the dark compartment after one and two weeks. This was simply explained by the ability of these mice to learn and memorize this painful stimulus 24 h, one week and two weeks after experiencing it.

This wasn't the case in other groups, as the RL in all groups was significantly lower than that of the control group, 24 h and one week after acquisition. While after two weeks RL in G3 only was significantly lower than the control group. These findings suggest that such mice memory was affected in a way that they didn't memorize the painful stimulus of the electric shock they experienced neither 24 h nor one week after stimulation. But after 2 weeks, mice in G1 and G2 were able to regain their memory and thus all mice in G1 and almost all of them in G2 didn't reenter the dark compartment. G3 mice results showed maintained memory impairment even after two weeks as the RL was significantly lower than that of CG, G1 and G2 after the same time interval.

The pattern of memory affection against time was followed and compared in each experimental group. In G1 although some mice entered the dark compartment 24 h and one week after electrical shock, but the mean duration taken was significantly increasing until reaching the test ceiling (300 s) after two weeks. This can be explained in a way that exposure to a magnetic field of 1mT intensity can affect memory only temporary, and it can be fully regained two weeks after stoppage of exposure.

In accordance to our results, Lama Sakhnini et al. [2013], investigated the possible effect of ELF MF (50 Hz, 1 mT) on spatial learning and memory functions in prenatal and neonatal mice. Their results provide convincing evidence that long time MF exposure to immature mice causes appreciable long-term deficit in learning abilities. Furthermore, Elaheh Nooshinfar et al. [2012] investigated the effects of low frequency EMF of 50 and 217 Hz, with variable intensities (0.5 to 2 mT) on learning and memory in adult male mice. Results showed significant deficiencies in learning and memory in the EM exposed mice compared to controls in the 50 Hz group when exposed to 1 and 1.5 mT intensities. On the contrary, Yu Fu et al. [2008] investigated the short-term (7 days) and long-term (25 days) effects of ELF magnetic fields on spatial recognition memory in mice by using a two-trial recognition Y-maze. They found that only long-term exposure to 50 Hz fields reduced recognition of the novel arm. In G2, the results were almost the same as that of G1, the only noticed difference, that there was no significant difference between RL and IL in this group, denoting impairment in memory early after exposure. But, again there was an improvement of memory with time as RL significantly increases one week and 2 weeks after, which accordingly suggest the temporary effect on memory due to chronic exposure to magnetic field of 2mT intensity

Randa M et al. [2002] had examined the effects of chronic exposure (1 and 2 weeks) to an ELF-MF of 2G intensity on memory in rats using an object recognition task. Their results were in accordance to our results in this exposure group (G2), as they reported impairment in discrimination between familiar and novel objects. On the other hand, Lui T et al. [2008] examined the changes in spatial learning and memory by the Morris water maze test after 4 weeks of daily exposure of rats to a 50-Hz magnetic field of 2 mT for either 1 or 4 h. They found that chronic exposure to ELF MF reduced the latency to find the hidden platform and improved long-term memory of former location of platform without affecting the short-term memory and motor activity. These findings being opposite to most previous studies and to ours too, but we suggest that this may be due to the different pattern of exposure.

While G3 results showed the highest deterioration in memory 24 h and one week after shock as the RL in both times were indifferent from that of the IL, meaning that the mice took minimal time to enter the dark compartment, this was due to their impaired memory that made them go to the painful stimulus once again. But 2 weeks after shock the RL significantly increased in comparison to the previous 2 timelines denoting the improvement in memorization but when compared to the RL of other groups at the same time interval, it was found to be significantly lower. This suggests that although the memory affection after exposure to 3mT magnetic field is temporary but still more affected than that due to lower exposures (1 and 2 mT). Also it had never reached normal, as its value was significantly lower than the test ceiling duration.

Conclusion

The present study supports a large number of investigations reporting that exposure to EMF radiation does have a harmful effect on learning and long-term memory. It also reports that these effects are more obvious as the intensity of exposure increases. In addition to that, the memory affection associated with the exposure to EMF is only temporary and restoration of normal memory can take place in two weeks after exposure cassation in exposure intensities of 1mT and 2mT. On the other hand, memory affection due to exposure intensity of 3mT, although declined after 2 weeks but still didn’t reach normal as compared to other groups.

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