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Effects of single, brief exposure to an 8 mT electromagnetic field on avoidance learning in male and female mice

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Abstract

The present study investigated the effect of extremely low frequency (8 mT, 50 Hz) electromagnetic fields (ELF-EMF) on avoidance learning in mice and compared the effect of an ELF-EMF in adult male and female mice. Learning was evaluated using a passive avoidance learning procedure in a standard wooden box, in which, despite their instinctive tendencies, mice learn to stay on a small platform to avoidant an electric shock. Before each learning session, the animals were exposed to an 8 mT, 50 Hz ELF created by a round coil. Immediately after 60 min exposure to the ELF-EMF, the mice were subjected to avoidance learning. The animals in the sham-exposed control group were placed in the coil for 60 min but were not exposed to the EMF and were subjected to the same behavioral procedures as the experimental group. The comparison of learned behaviors in the experimental and control groups showed that exposure to an 8 mT, 50 Hz ELF for 60 min significantly affected passive avoidance learning in both male ($p < .023$) and female ($p < .015$) mice. **Keywords:** electromagnetic field, passive avoidance learning, mice.

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Introduction

The advancement of new technologies has been associated with the creation of communication services and various electrical devices with electromagnetic fields of varying intensities. However, alongside the benefits of these new technologies are potential worries about the influence of electromagnetic fields (EMFs) on metabolism, biological processes, molecular mechanisms, and cellular organisms. The first report in 1979 about the possible damaging effects of exposure to EMFs by Whertteimer and Leeper related electrical fields to cancer in children (Manikonda et al., 2007).

In 1980, researchers investigated the increased risk of leukemia and brain tumors in people who were exposed to extremely low frequency EMFs (ELF EMFs). Such evidence led to increased attention to the risk of EMFs (Ahlbom, 2001). Occupational exposure to the same fields increased the risk of cardiac disorders, cardiac arrhythmia-related conditions, and acute myocardial infarction (Savitz, Liao, Sastre, Kleckner, & Kavet, 1999). Further research focused on the risk of central nervous system disorders, including Alzheimer's disease and Parkinson's disease, in people who were exposed to occupational EMFs and electric shock (Ahlbom, Green, Kheifets, Savitz, & Swerdlow, 2004).

Animal models and clinical studies have shown that ELF EMFs can change peripheral and central nervous system activity (Prato, Kavaliers, & Thomas, 2000). These changes include increased activity of hypothalamic and intracerebral nuclei (Sieron et al., 2001), changes in neurotransmitter synthesis in synapses and ganglia (Massot et al., 2000), changes in the activity of neuronal receptors, including dopamine and 5-hydroxytryptamine-1B (5-HT_{1B}; Chance et al., 1995), and changes that ultimately may affect learning and memory (Trimmel & Schweiger, 1998).

Behavioral and psychological studies have shown that exposure to ELFs can affect human cognitive function and animal behavior (Lai, 1996; Lai & Carino,1999; Lai, Carino, & Ushijima,1998; Fu, Wang, Wang, Lei, & Ma, 2008). For example, rats were exposed to 25 or 50 Hz fields for 7 days or 25 days and examined in the Y-maze. The results showed that neither short-term nor long-term exposure altered

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locomotor activity, but 50 Hz field exposure decreased the recognition of the new arm of the maze (Fu et al., 2008). In another experimental study, Jadidi et al. (2007) confirmed that 20 minutes exposure to an 8 mT, 50 Hz field impaired spatial memory consolidation, but such impairments were not induced by a 2 mT field. The researchers concluded that ELF EMFs can alter calcium ion homeostasis in neuronal tissue. Hippocampal regions of the mouse brain exposed to a 50 Hz field for 90 days at 50 and 100 mT were isolated and compared with a control group. Exposure to the ELF EMF increased Ca^{2+} ion levels in cells (Manikonda et al., 2007).

Some research has reported that ELF fields have positive effects on cognitive function. Liu, Wang, He, and Ye (2008) examined spatial learning and memory changes using the Morris water maze after 4 weeks of exposure to an ELF EMF (2 mT, 50 Hz ELF, 4 h daily). They reported that such exposure led to a reduction in the long-term delay to find the hidden platform in the maze and improved long-term memory without affecting short-term memory or locomotor activity. Kavaliers et al. (1996) observed behavioral improvement in water maze responses in mice associated with the opioid system.

The above findings do not absolutely confirm that ELF fields can either improve learning and memory or impair cognitive function. The present study investigated the effect of an ELF EMF (8 mT, 50 Hz) on passive avoidance learning in male and female mice.

Methods

Subjects

Adult male and female mice (25-30 g) were separately housed five per cage in a room with a natural light cycle and constant temperature (24 ± 2ºC). Food and water were available *ad libitum*. All procedures and experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Apparatus

Inhibitory (passive) avoidance apparatus

This study examined one of the most stable forms of learning: avoidance learning. In the process of avoidance learning, the animal not only does not receive reinforcement, but also is subjected to stimuli or situations that may threaten its survival. Because of the perceived serious threat of these situations, only one experimental trial is necessary for long-term, stable learning.

The mice were evaluated in an inhibitory (passive) avoidance task to measure learning or the acquisition of information. The passive avoidance learning box was a wooden box (30 x 30 x 40 cm) with 29 steel bars (0.3 cm diameter) on the floor, spaced 1 cm apart. A wooden platform (4 x 4 x 4 cm) was placed in the middle of the floor of the box. The aversive stimulus was an experiment-controlled electric shock (Grass S44, Quincy, MA, USA). In the learning session, the animal was gently placed on the small wooden platform in the middle of the box. The instinctive tendency of the mouse is to immediately step down from the platform and move to the open space of the larger wooden box. A 15 s electric shock was administered as soon as the mouse stepped down from the platform and contacted the steel bars on the floor. Despite its innate tendency, the mouse learns to remain on the platform, which is referred to as the step-down latency, an index of learning. Twenty-four hours after the test session, avoidance learning, reflected by the step-down latency, was calculated using a chronometer. The experimental procedure was similar to Hiramatsu, Sasaki, and Kameyama (1995).

Electromagnetic field exposure system.

An EMF was applied in a room adjacent to that used for the behavioral experiments. A sinusoidal magnetic field was created with a round electromagnet coil made from 1000-turn copper wire (0.50 mm). The electromagnet power was supplied by a sinusoidal waveform signal generator (GFG-8019G, Good Will Instrument Co., Taiwan). The amplifier output drove the coil, producing an ELF of 8 mT at the center of the coil. The desired intensity of the ELF (8 mT) was calibrated using a Gauss meter (Lakeshore 410 meter, Magwerks, Indianapolis, Indiana, USA) at the center of the coil. The heat generated by the coil dissipated because of good ventilation in the exposure area. The electrical apparatus and exposure system was adjusted on a non-metallic laboratory table. The temperature inside the coil was kept constant (24 ± 2ºC) during the learning tests using a fan and aluminum water pipes (Fig. 1).

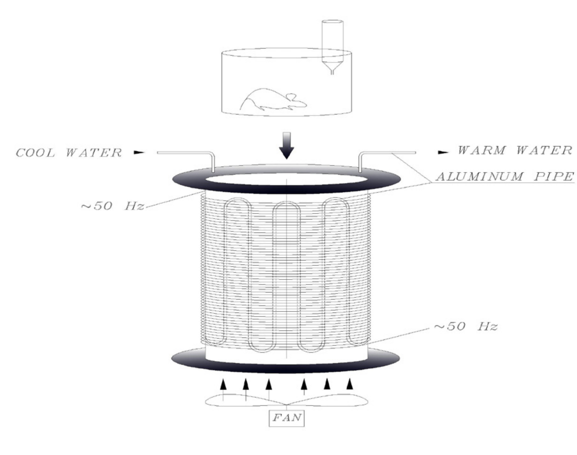

Figure 1. Electromagnetic field exposure system.

Procedure

Animals were selected randomly, and four groups were used: male sham-exposed (n = 10), male exposed to 8 mT (n = 10), female sham-exposed (n = 10), female exposed to 8 mT (n = 10). Before each learning session, the animals that were selected for the experimental groups were exposed to an 8 mT, 50 Hz EMF in the coil. After 60 minutes exposure, they were subjected to the avoidance learning task in the learning session. The animals in the sham-exposed control groups were placed in the coil for 60 min. without an EMF. Twenty-four hours after the test session, each animal was placed on the platform of the inhibitory avoidance apparatus, and the step-down latency for each animal was measured and recorded as an index of passive avoidance learning.

Statistical analysis

Because of significant differences in the innate ability of behavioral learning and differences in behavior in the test session, nonparametric statistical methods were used for the data analysis. Using nonparametric methods is common in biopsychological research in which passive learning or other cognitive processes in mice are examined using an inhibitory avoidance apparatus (Ukai & Lin, 2002; Rezayat, Niasari, Ahmadi, Parsaei, & Zarrindast, 2010).

Results

No behavioral differences or differences in locomotor activity were observed between the four groups during exposure to the ELF system, regardless of whether is was turned on or off. Gender differences in the learning session were first compared. Using SPSS software and the nonparametric Mann-Whitney U- test, the male and female groups were compared. The results showed no significant differences between the two groups (n = 20 male, n = 20 female) in the learning session (Mann-Whitney U = 47.500; $p < .843$).

The comparison between the male control group and male experimental group (n = 10 per group) in the learning session was not significant (Mann-Whitney U = 43.00; $p < .577$). Similar results were found in the female groups (Mann-Whitney U = 35.00; $p < .243$; Fig. 2 and 3).

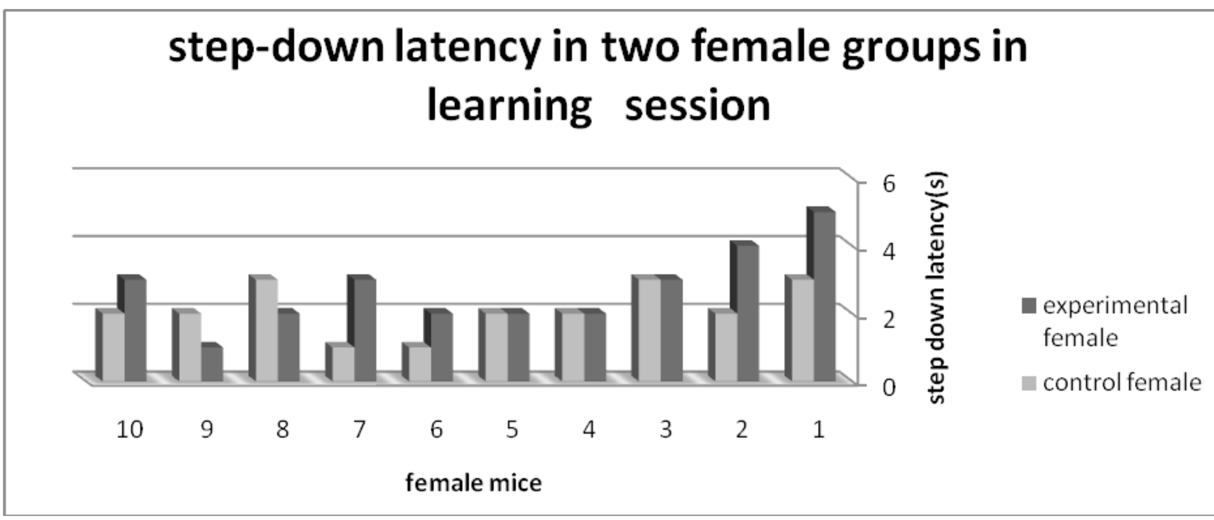

Figure 2. Step-down latency in the learning session in female mice.

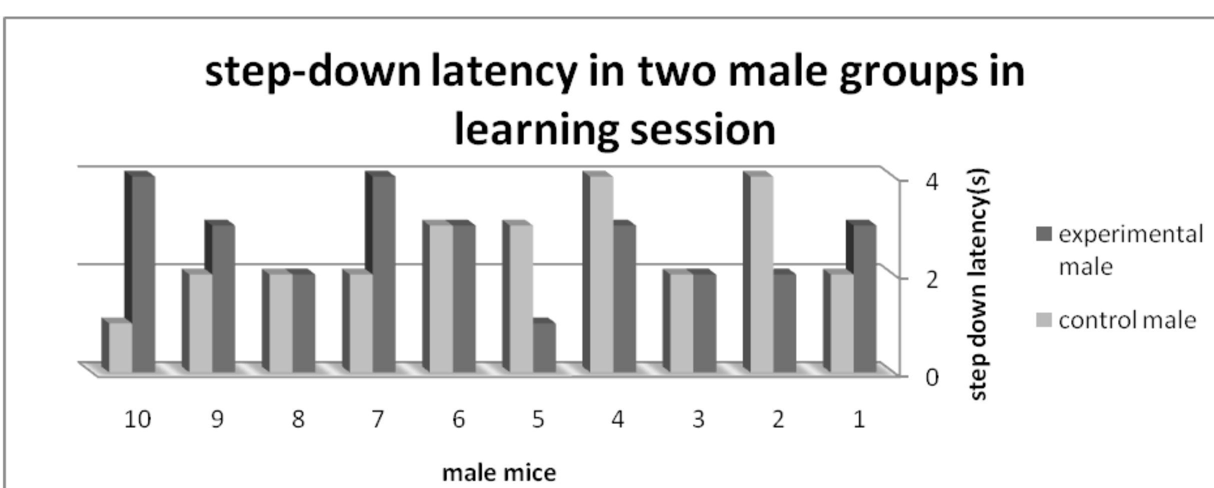

Figure 3. Step-down latency in the learning session in male mice.

The test session analysis of the passive avoidance learning index showed that the step-down latency of the groups exposed to an 8 mT, 50 Hz EMF was significantly lower than the control groups (Mann-Whitney U = 20.00; $p < .023$). Significant differences were also found between control and experimental female mice (Mann-Whitney U = 18.00; $p < .015$; Fig. 4 and 5).

Discussion

Based on these results, exposure to an 8 mT, 50 Hz EMF before animals were subjected to an avoidance learning task impaired learned behavior. The electric shock and aversive situation were not learned by the experimental group or the learned behaviors may not

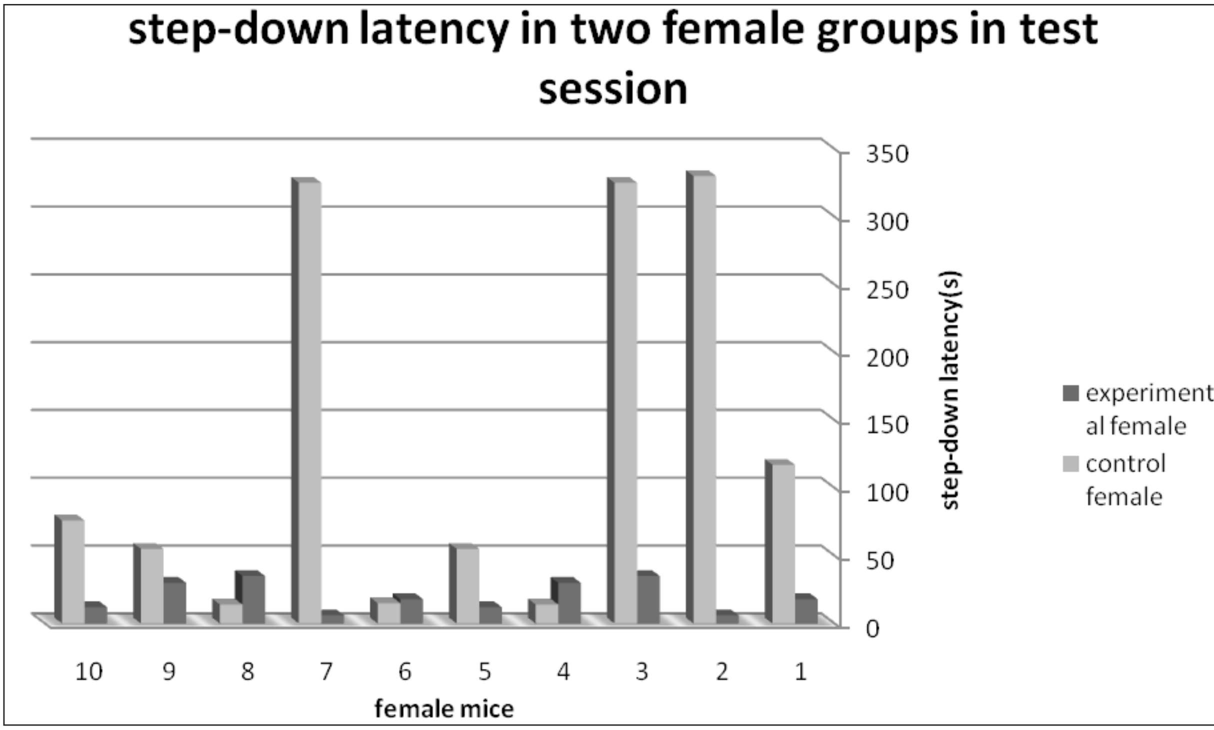

Figure 4. Step-down latency in the test session in female mice.

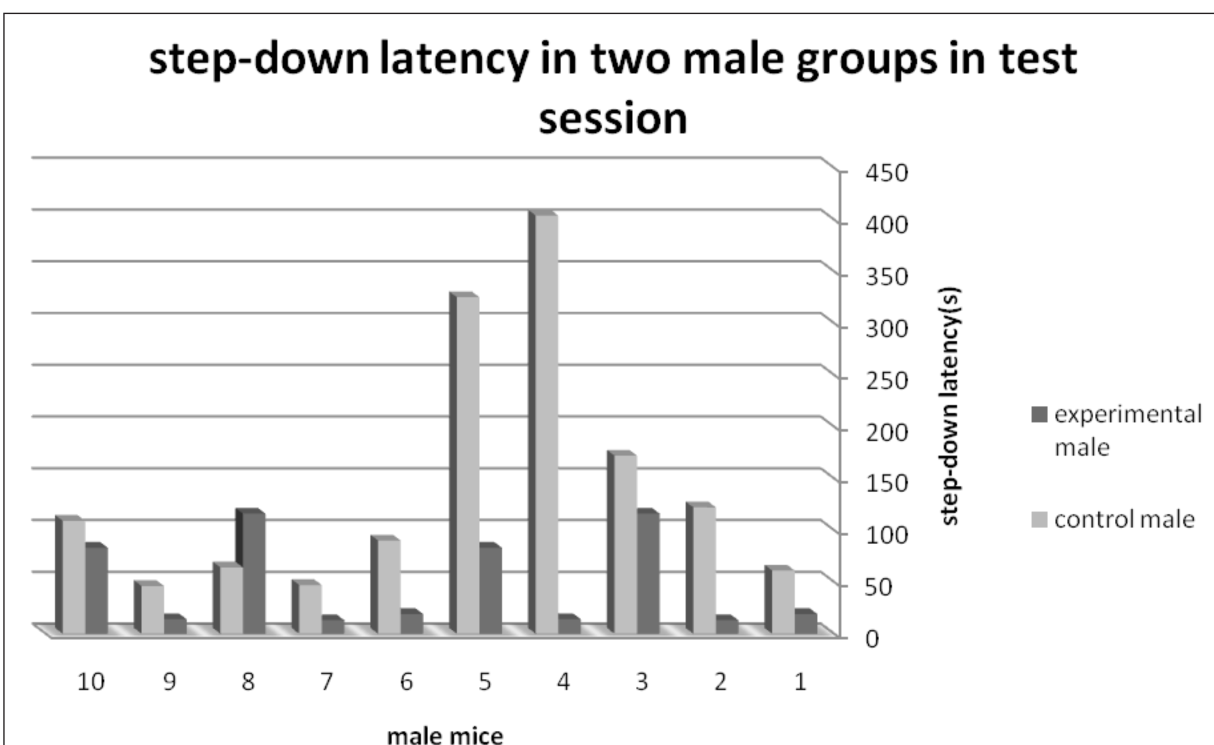

Figure 5. Step-down latency in the test session in male mice.

have been used, reducing the animal's step-down latency and causing a failure to avoid the electric shock. The difference between the step-down latency in the learning session and test session is an index of the rate of learning and information acquisition in inhibitory (passive) avoidance tasks. Staying on the platform in the test session in male and female control mice indicated that information acquisition was retained. The two experimental groups performed significantly more poorly than the control groups. Therefore, exposure to an EMF, even one with a single, brief duration of 60 min, may impair learning and cognitive processing in mice. These findings are consistent with results that showed the impairing effects of an ELF on cognitive function (Jadidi et al., 2007; Trimmel & Schweiger, 1998; Lai, 1996; Lai & Carino, 1999).

Jadidi et al. (2007) provided evidence that exposure to an 8 mT, 50 Hz magnetic field for 20 min impaired the consolidation of spatial memory using a water maze but not the retrieval of learned information. No effect was found with a 2 mT magnetic field (Jadidi et al., 2007). McKay and Persinger (2000) also found that 60 min exposure to a 200-500 nT ELF before the training phase impaired spatial memory in the radial maze, whereas exposure before the testing phase decreased the response times of rats in this task. Lai et al. (1998) showed that 60 min exposure to a 1 mT, 60 Hz ELF before training impaired spatial memory in a water maze. Decreased perception, memory, and cognition function were found with 60 min exposure to a 1 mT, 50 Hz magnetic field in a human study (Trimmel & Schweiger, 1998).

However, evidence has shown no significant effects or even a positive effect of ELFs on learning and memory (Vázquez-García et al., 2004). For example, Kurokawa, Nitta, Imai, and Kabuto (2003) found no significant effects of a 50 ml T, 50 Hz magnetic field on the human brain. No harmful effects were found with 45 min exposure to a 0.75 mT magnetic field on memory in mice (Sienkiewicz, Bartram, Haylock, & Saunders, 2001). This inconsistency may be attributable to differences in the protocols among the different studies, including type of task, intensity of the applied ELF, and exposure duration. Previous studies mainly focused on cognitive function, especially learning and memory, in different tasks and with different exposure durations, so inconsistent results may be expected.

The mechanisms that underlie the possible harmful effects of EMFs on learning and memory are unknown. The brain cholinergic system plays a crucial role in learning and memory (Whishaw, 1989; Whishaw & Tomie, 1987). Lai and Carino (1999) showed that exposure to an ELF decreased the activity of the cholinergic system in the frontal cortex and hippocampus, two regions involved in memory processing. Thus, one possibility is that the impairment of cognitive processing may reflect a decrease in cholinergic transmission. Exposure to an ELF can also change calcium ion conductance (Manikonda et al., 2007). Additionally, changes in γ-aminobutyric acid and calcium ions in the brain may affect cognition (Blackman, 2009).

In summary, the present findings showed that 60 min exposure to an 8 mT, 50 Hz EMF impaired learning and information acquisition in a passive avoidance learning task. Consistent with other studies, our data indicate that exposure to an ELF has an impairing effect on learning and memory. Further studies are required to determine the biopsychological and chemical mechanisms.

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