Disturbance of the Magnetic Field Did Not Affect Spatial Memory

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Received June 21, 2013
Accepted November 8, 2013
On-line February 24, 2014

Summary

Extremely low-frequency magnetic field (ELF-MF) has been suggested to influence the cognitive capability but this should be dynamically evaluated in a longitudinal study. Previous training can affect performance, but the influence under magnetic field is unclear. This study aims to evaluate the effects of previous training and ELF-MF exposure on learning and memory using the Morris water maze (MWM). Sprague-Dawley rats were subjected to MWM training, ELF-MF exposure (50 Hz, 100 μT), or ELF-MF exposure combined with MWM training for 90 days. Normal rats were used as controls. The MWM was used to test. The data show that the rats exposed to training and ELF-MF with training performed better on spatial acquisition when re-tested. However, during the probe trial the rats showed no change between the training phase and the test phase. Compared with the control group, the ELF-MF group showed no significant differences. These results confirm that previous training can improve the learning and memory capabilities regarding spatial acquisition in the MWM and this effect can last for at least 90 days. However, this improvement in learning and memory capabilities was not observed during the probe trial. Furthermore, ELF-MF exposure did not interfere with the improvement in learning and memory capabilities.

Key words

ELF-MF • Previous training • Morris water maze • Learning and memory

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Introduction

The increased use of electrical equipment brought the biological effects of electromagnetic fields to public attention. The extremely low-frequency magnetic field (ELF-MF) has been controversially suggested to affect cognitive function. For example, Jadidi *et al.* (2007) reported that exposure to a 50 Hz magnetic field at 8 mT for 20 min can impair the consolidation of spatial memory. Zhang *et al.* (2004) found that exposure to hypomagnetic field space causes amnesia in Drosophila. However, the learning of adult male CD1 mice was unaffected after exposure to a vertical, 50 Hz sinusoidal magnetic field at 5 μT, 50 μT, 0.5 mT, or 5.0 mT (Sienkiewicz *et al.* 1996). The reason for the different among findings remains unclear, and more investigations are needed.

A large number of studies on the effects of magnetic field on learning and memory were based on acute exposure. For example, the exposure to a magnetic field (60 Hz, 45 min, 0.75 mT) or to a magnetic field (60 Hz, 1 h, 1 mT) before an experiment caused a deficit in learning and memory (Lai 1996, Lai *et al.* 1998). There are only a few studies about long-term magnetic field exposure. However, to evaluate the long-term effect of occupational exposure or resident exposure, learning and memory capabilities should be dynamically monitored in a longitudinal study. A previous study showed that early training in a spatial task may affect performance during a later re-test (Pitsikas *et al.* 1991). Similar results were found in mice and rats (Li *et al.* 2011, Vicens *et al.* 2002, Vicens *et al.* 2003). These finding gives rise to the following question: Does the effect of early training on learning and memory exist

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under chronic magnetic field exposure? The answer to this question requires data from a longitudinal study that dynamically monitors learning and memory capabilities.

Our present study evaluated the effects of previous training and extremely low-frequency magnetic field on learning and memory using a Morris water maze (MWM).

Materials and Methods

Animals

Forty 10-week-old adult male Sprague-Dawley rats (250 g to 300 g) were used in this study. Five rats were housed in each cage. The rats were divided into the following four groups (n=10 per group): control group without training (control group), experimental group without training exposed to ELF-MF (ELF-MF group), control group with training (training group), and experimental group with training exposed to ELF-MF (ELF-MF with training group). The MWM test was then conducted for the training group and the ELF-MF with training group as the training phase. The ELF-MF group and the ELF-MF with training group were then treated with magnetic field for 90 days continuously, whereas the control group and the training group were treated with sham exposure. After 90 days, all rats were tested using the MWM (Fig. 1). During the experiments, the animals had free access to food and water at constant ambient temperature (23±1 °C) with a 12 h:12 h light-dark cycle (08:00-20:00). The experiment was conducted according to the regulations of the Beijing Laboratory Animal Use and Care Committee and the assessment was made in a double blind way.

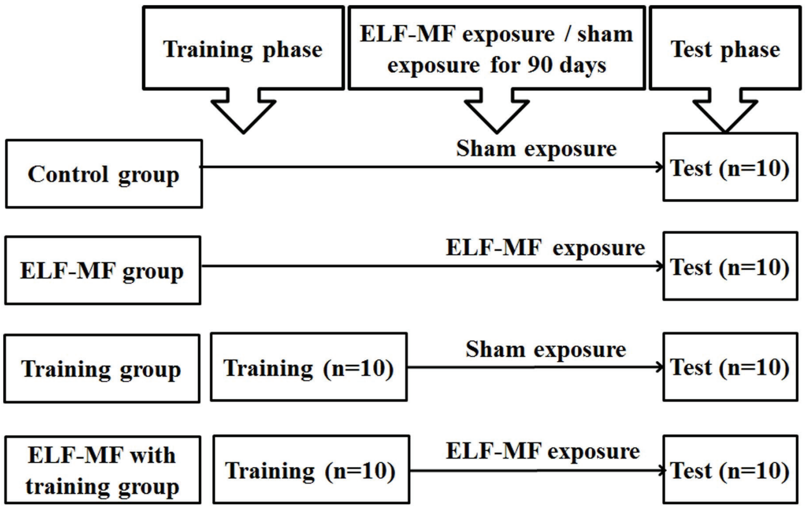


Fig. 1. Chart of experimental procedure.

Magnetic field expose

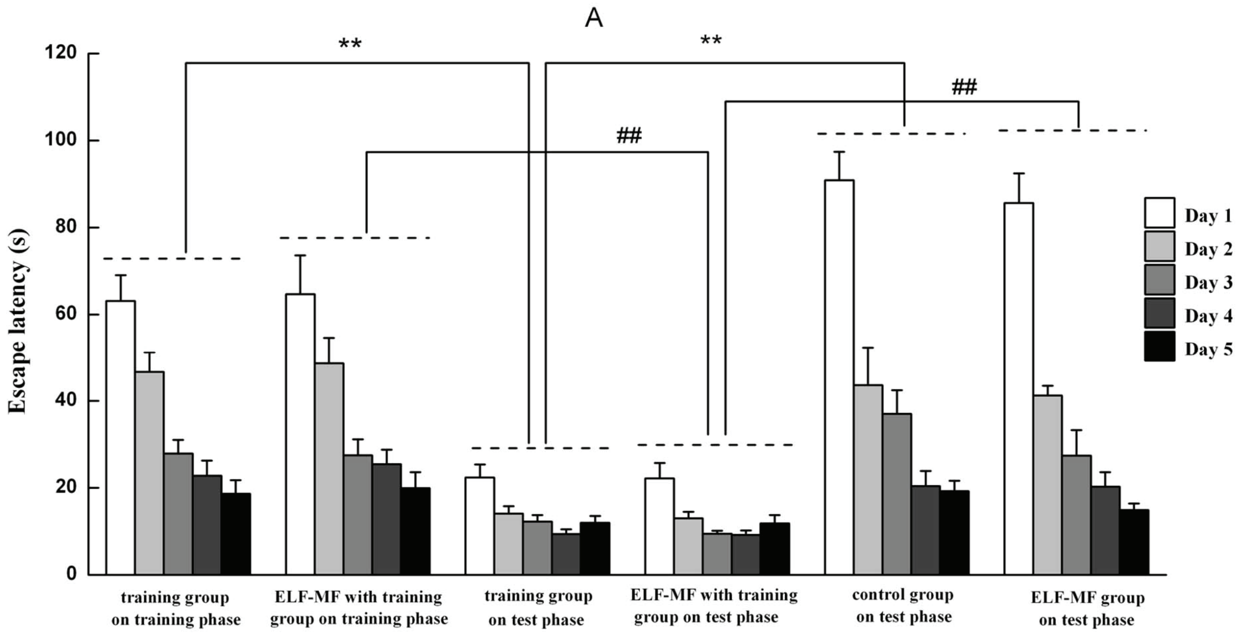
The ELF-MF exposure apparatus was developed by our laboratory. The ELF-MF group and the ELF-MF with training group were exposed to the 100 µT (rms), 50 Hz alternating magnetic field produced by Helmholtz coils (1.4 m in diameter), whereas the control group and the training group were exposed to the geomagnetic environment (sham exposure). A control system comprising a controller, two temperature sensors, and two heaters was used to ensure that temperature differences between the exposure and sham region were less than 0.2 °C. To prevent the stainless steel material of the cages from interfering with the magnetic field, the cover of cages and the spout of the water bottles were replaced by epoxy resin and glass.

MWM

The MWM consisted of a round pool, an escape platform and a video camera. The pool (150 cm diameter, 50 cm deep) was filled with opaque water, and the water level was maintained at 1.5 cm above the platform's surface. Four equal points around the edge of the pool were designated to divide the pool into four imaginary quadrants (east (E), south (S), west (W), and north (N)). The hidden platform was located in the southwest (SW) quadrant. The video camera was used to record the MWM test process.

In this study, the training and test phases in the MWM involved the same processes of spatial acquisition and probe trial. During the spatial acquisition, rats had one training session per day for five consecutive days to locate the platform. During a training session, rats were allowed four trials at different starting positions that were N, E, southeast (SE), and northwest (NW) and selected semi-randomly. A trial was terminated when the rat had climbed onto the platform or when 120 s had elapsed. Each rat was allowed to stay on the platform for 15 s between two trials. The escape latency and the swimming distance were recorded.

A probe test was performed on the sixth day. During this test, the platform was removed and swimming paths were recorded for 30 s. The probe test only included one trial. The following indices were recorded: (1) initial time of crossing the platform; (2) the number of times a rat crossed the platform; and (3) percent time in each quadrant.



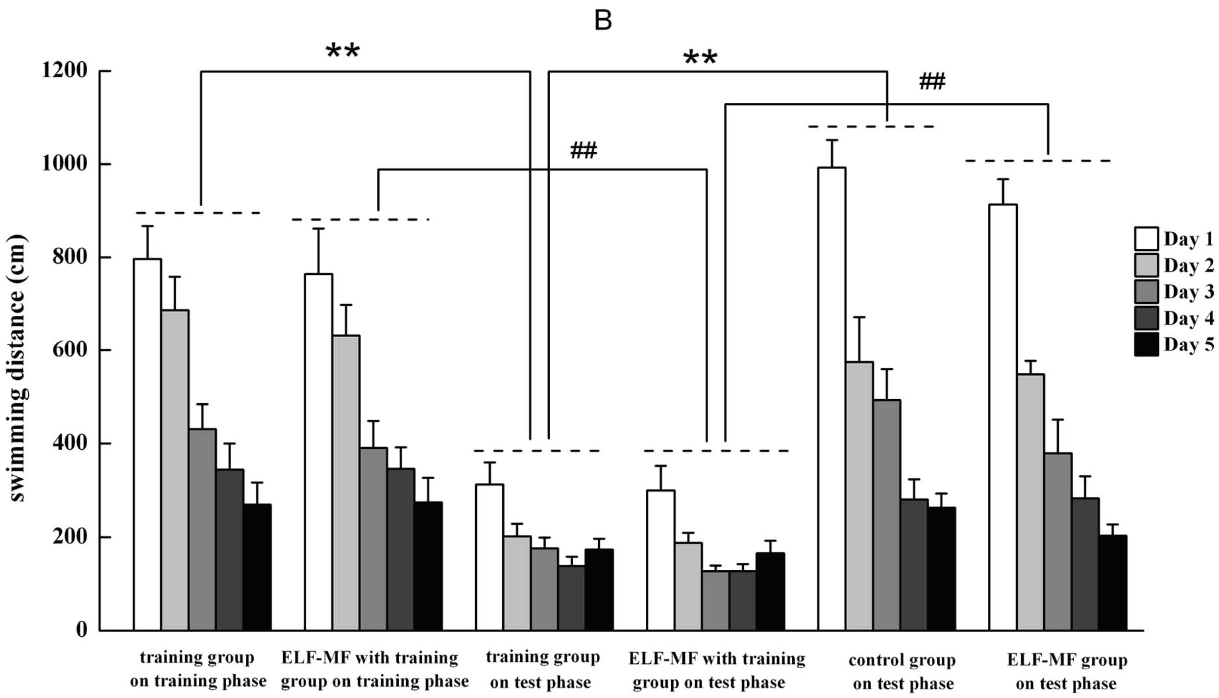


Fig. 2. Effect of training and ELF-MF on the rats during spatial acquisition. **A**: escape latencies. **B**: swimming distance. ** p<0.01, compared with the training group during the test phase. ## p<0.01, compared with the ELF-MF with training group during the test phase.

Data analysis

The data were expressed as means ± SEM. The data of escape latency and swimming distance were analyzed with two-way analysis of variance (ANOVA) of repeated measures. Differences in the number of times the platform was crossed were analyzed using the rank sum test. The data on the initial time of crossing the platform and the percent time in the quadrants were analyzed using a paired t-test (compare the difference in two phases) and a two-tail Student's t-test (compare the difference among groups), respectively.

A level of $P<0.05$ was considered significant in all statistical tests.

Results

Spatial acquisition

In the spatial acquisition test, the results on escape latency and swimming distance were coherent.

With regards to the effects of previous training on spatial memory, the data during the test phase for the training group ($F(1,18)=48.66$, $P<0.01$, escape latency) ($F(1,18)=38.119$, $P<0.01$, swimming distance) and the ELF-MF with training group ($F(1,18)=76.545$, $P<0.01$, escape latency) ($F(1,18)=83.681$, $P<0.01$, swimming distance) was obviously shorter than that during the training phase (Fig. 2).

With regards to the effect of magnetic field exposure on spatial memory, no significant difference was observed between the training phase ($F(1,18)=0.131$, $P>0.05$, escape latency) ($F(1,18)=0.195$, $P>0.05$, swimming distance) and the test phase ($F(1,18)=0.339$, $P>0.05$, escape latency) ($F(1,18)=0.629$, $P>0.05$, swimming distance) for the training group and the ELF-MF with training group. Moreover, no significant difference was observed between the control group and the ELF-MF group ($F(1,18)=1.228$, $P>0.05$, escape latency) ($F(1,18)=1.551$, $P>0.05$, swimming distance) (Fig. 2).

The data on escape latency and swimming distance were also compared between the training group during the test phase and the control group during the test phase, as well as between the ELF-MF group during the test phase and the ELF-MF with training group during the test phase. Significant differences were observed between the control group during the test phase and the training group during the test phase ($F(1,18)=59.87$, $P<0.01$, escape latency) ($F(1,18)=63.127$, $P<0.01$, swimming distance), as well as between the ELF-MF group during the test phase and the ELF-MF with training group during the test phase ($F(1,18)=138.96$, $P<0.01$, escape latency) ($F(1,18)=87.742$, $P<0.01$, swimming distance) (Fig. 2).

Probe trial

Statistical analysis of the probe trial revealed that rats in all groups spent more time in the target quadrant (SW) than in the northeast (NE) and northwest (NW) quadrants ($P<0.05$). Percent time in the SW quadrant was also longer than in the southeast (SE) quadrant, and this result was significantly different for the ELF-MF with training group for both phases and for the training group during the test phase ($P<0.05$). However, no statistical difference was observed in the control, ELF-MF, and the training groups during the training phase ($P>0.05$) (Fig. 3A).

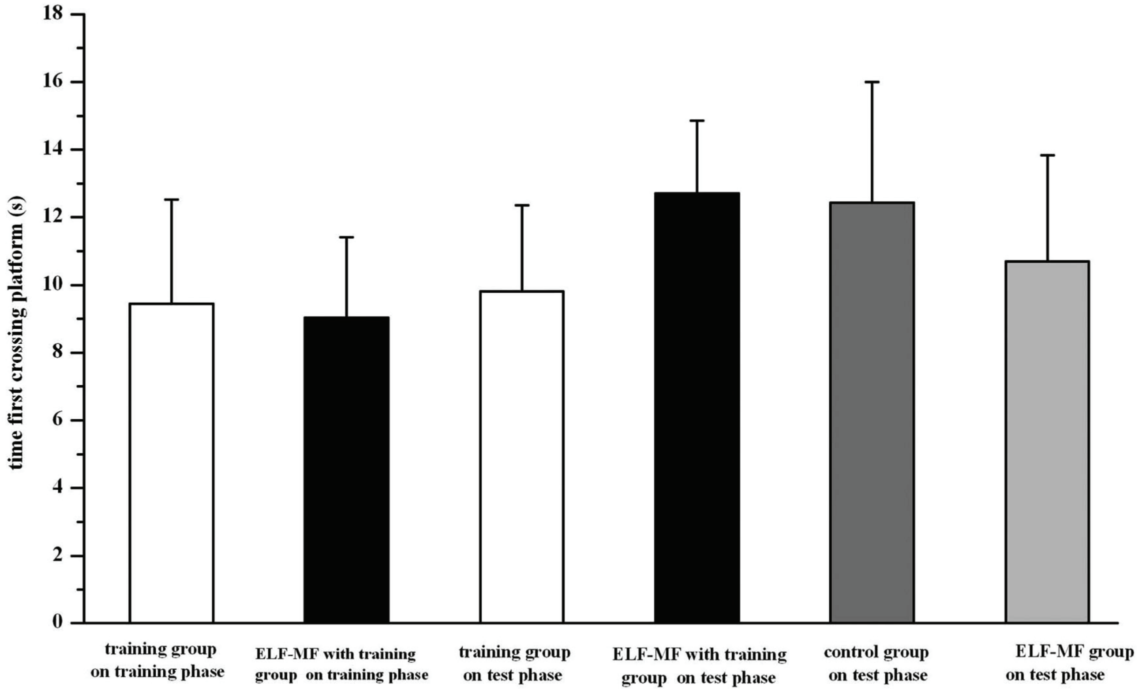
Compared with the training phase, the initial time of crossing the platform was not significantly different during the test phase for the training ($t(9)=0.1$, $t(9)=1.121$, $P>0.05$) and the ELF-MF with training groups ($t(9)=1.121$, $P>0.05$) (Fig. 3B). Percent time in the target quadrant (SW) during the test phase increased compared with that during the training phase in training group and in the ELF-MF with training group, but there was no significant difference between the training phase and the test phase ($t(18)=2.002$, $P>0.05$, $t(18)=1.361$, $P>0.05$, respectively, for training group and ELF-MF with training group) (Fig. 3A). The rank sum test showed no significant difference in term of the number of times the platform was crossed between the training phase and the test phase in the training and the ELF-MF with training groups ($P>0.05$) (Fig. 3C).

To study the effects of the magnetic field, different groups with and without exposure to the ELF-MF were compared. The t-test showed no difference between the control group and the ELF-MF group for the initial time of crossing the platform ($t(18)=0.367$, $P>0.05$) and the percent time in the four quadrants ($t(18)=0.687$, $P>0.05$) (Fig. 3). Moreover, no difference was observed in the initial time needed to cross the platform and percent time in the four quadrants between the training and the ELF-MF with training groups, neither during the training phase ($t(18)=0.104$, $P>0.05$, $t(18)=1.859$, $P>0.05$, respectively, for first crossing time and percent time in target quadrant) nor the test phase (($t(18)=0.865$, $P>0.05$, $t(18)=0.468$, $P>0.05$, respectively, for first crossing time and percent time in target quadrant) (Fig. 3). Data on the number of times the platform was crossed were analyzed using a rank sum test. The result displayed no difference between different groups or phases ($P>0.05$) (Fig. 3).

A

	Group	NE	NW	SW	SE
Training phase	training group	21.92±2.78*	21.05±3.23*	31.56±3.46	25.30±4.26
	ELF-MF with training group	16.68±2.00**	19.08±2.37**	39.07±2.08	25.05±2.76**
Test phase	training group	15.61±2.90**	14.26±2.66**	43.53±4.88	26.48±3.19**
	ELF-MF with training group	15.51±3.28**	17.91±3.79**	46.94±5.39	19.52±4.24**
	Control group	19.29±3.23*	16.23±3.60**	33.68±4.90	30.56±3.93
	ELF-MF group	18.53±2.76**	14.75±3.28**	38.59±5.20	27.99±5.35

B



C

	Group	Platform crossings									
Training phase	training group	0	3	0	0	1	2	2	2	2	0
	ELF-MF with training group	2	0	1	1	1	0	2	0	2	1
Test phase	training group	0	3	1	5	1	0	1	2	2	5
	ELF-MF with training group	1	0	1	3	1	2	1	4	1	1
	Control group	1	1	0	2	1	1	1	1	0	0
	ELF-MF group	1	0	0	0	4	6	1	1	2	3

Fig. 3. Effect of training and ELF-MF on the rats during the probe trial. **A**: Percent of time the rat spent in the four quadrants. The mean percentage of searching time in each quadrant by all rats during the probe trial was obtained. No difference was observed among groups. The SW quadrant was the target quadrant. * $p<0.05$, compared with the target quadrant (SW), time in the other three quadrants were obviously less in every group. ** $p<0.01$ compared with the target quadrant (SW), time in the other three quadrants were obviously less in every group. **B**: Time that a rat initially crosses the platform. No difference was observed among groups and between phases. **C**: The number of times the platform was crossed for every rat during the experiment. Data were analyzed using a rank sum test. No difference was observed among groups and between phases; n=10 per group.

Discussion

The MWM was first described by Richard Morris 30 years ago (Morris 1984) and subsequently became one of the most frequently used laboratory tools in behavioral neuroscience. Spatial memory is complex and involves non-declarative memory and declarative memory, as well as of short-term and long-term memory (Paul *et al.* 2009). Numerous methodological variations of the MWM task have been used by researchers to enhance the assessment of spatial navigation or to test for related types of learning (Vorhees and Williams 2006).

Previous training has been shown to affect subsequent memory performances. For example, Pitsikas *et al.* (1991) compared aged and young rats and found that previous experience can facilitate the preservation of spatial reference memory for 12 months. More recently, van der Staay and de Jonge (1993) used the repeated acquisition paradigm to test the effects of previous experience on spatial memory. Results show that young rats acquired the task within the first few sessions. However, 24-month-old animals did not acquire the task even after 12 daily training sessions. In mice, prior experience also showed a beneficial effect on the spatial memory even after eight months (Vicens *et al.* 2002).

Our data show that previous training has a beneficial effect on spatial memory and that preservation of spatial memory can last for at least 90 days. However, previous experience has a relatively weaker effect on the probe trial than on spatial acquisition. Although time in the target quadrant was believed to be more useful in the probe trial (Vorhees and Williams 2006), only an increasing trend in percent time in the target quadrant was observed, and no significant difference was evident. This finding is consistent with previous research (Li *et al.* 2011, Vicens *et al.* 2002, 2003). The reason for the weaker effect during the probe trial remains unclear. Scholars believe that rats improve their hidden-platform performance, reaching the maximal level on day 5 of the training phase. Thus, no difference was found between different phases in the probe test, which reflects the existence of a reference memory from previous learning trials (Li *et al.* 2011).

The biological effects of ELF-MF have previously been reported (Mostafa *et al.* 2002, Sienkiewicz *et al.* 1998, St-Pierre and Persinger 2008). Sienkiewicz *et al.* (1998) investigated the effects of a 50 Hz magnetic field on C57BL/6J mice using an eight-arm radial maze. Results indicate that magnetic field exposure at 7.5 and 75 μT for 45 min significantly impaired the performance of mice, and these effects may depend on field strength and tend to be transient and reversible. Cui *et al.* (2012) found that ELF-MF exposure (1 mT, 50 Hz) induced serious oxidative stress in the hippocampus and striatum and impaired hippocampus-dependent spatial learning and striatum-dependent habit learning. In addition, the effects of ELF-MF on oxidative stress depend on the time of animal exposure to the magnetic field (Ciejka *et al.* 2011). In contrast, another research showed that exposure to ELF-MF with 1 mT intensity for 2 h over 9 days increased the duration of short-term memory for up to 300 min and suggested that ELF-MF improved social recognition memory in rats (Vazquez-Garcia *et al.* 2004). Akdag *et al.* (2013) reported that long-term exposure to 100 μT and 500 μT ELF-MF (2 h/day, 7 days/week, for 10 months) did not affect oxidative or antioxidative processes, lipid peroxidation, or reproductive components such as sperm count and morphology in rat testes. However, long-term exposure to 500 μT ELF-MF did affect active-caspase-3 activity, which is a well-known apoptotic indicator. These inconsistencies may be attributed to differences in magnetic field parameters (such as intensity and duration of the applied magnetic field) in the different studies.

In our experiment, although the ELF-MF with training group improved the learning and memory capabilities after 90 days of magnetic field exposure, no significant difference was observed compared with the training group. These results indicate that the improvement in learning capability can be attributed to previous training not magnetic field exposure and that magnetic field exposure has no positive or negative effect on spatial memory.

After memory is initially acquired, it is stored and subjected to modification by a variety of treatments (Broadbent *et al.* 2010). Liu *et al.* (2008) reported that chronic exposure to ELF-MF (50 Hz, 2 mT, 4 weeks) improved long-term memory without affecting short-term memory and motor activity, thus suggesting that a magnetic field may have an effect on the maintenance of memory. However, their research was conducted after magnetic field exposure, and the maintenance time for memory was only 24 h. As far as we know, our study is the first experiment in which memory was re-tested after magnetic field exposure following MWM training.

A number of studies found that a magnetic field affects learning and memory (Daniels *et al.* 2009, Fu *et al.* 2008, He *et al.* 2011, McKay and Persinger 2000, Sun *et al.* 2010). Given that an electric signal is a neuronal signal transduction pathway, a magnetic field can induce an electric field, which in turn will drive a current in the conducting body and may cause a biological effect (WHO 2007). Ahmed *et al.* examined the effects of a pulsed magnetic field (PELF-MF) on hippocampal evoked potentials. Results show that exposure to PELF-MF (0.16 Hz, 15 mT) applied for 30 min amplified the population spikes and the slopes of excitatory postsynaptic potential (EPSP) recorded from stratum pyramidale and stratum radiatum, respectively, and this amplification was additive to previously induced long-term potentiation (LTP). The increase in the activity of electrical synapses accompanied PELF-MF-induced amplification of evoked potentials. PELF-MF exposure modified paired-pulse facilitation and paired-pulse inhibition; therefore, it was concluded that it modifies excitatory and inhibitory processes in the hippocampus, which play an important role in memory acquisition and spatial orientation (Ahmed and Wieraszko 2008). However, this effect may only be effective above a certain strength (Sienkiewicz 1998). The strength of a 50 Hz, 100 μT, magnetic field in our study was weak, and the electric field it induced may be below the threshold. Hence, no performance change was found in the MWM test.

In most cases other authors tested the effects of ELF-MF by exposing the animals before training, during training or after training. Thomas *et al.* exposed rats to pulsed (burst firing pattern for 1 s every 3 s) magnetic fields (14 μT) for either 5 min or 30 min immediately or after a 30 min delay following 8 daily training sessions in a maze. They found that the strongest effect occurred when the rats were exposed for 30 min immediately after the training session and the effect was not apparent if a 30-min delay occurred before the exposure or if the exposure occurred for only 5 min immediately after the daily trials (Thomas and Persinger 1997). This effect may be related to opioid and cholinergic systems (Kavaliers and Ossenkopp 1993, Kavaliers *et al.* 1996, Lai *et al.* 1998). These data suggested that the effects of a magnetic field depend on the timing of the exposure. In our study, the exposure was during the break between training and the final test. Therefore, it may be a possible reason that ELF-MF exposure has no effect on memory.

In conclusion, results showed that prior training has a positive effect on spatial acquisition and that chronic magnetic field exposure did not alter the effect induced by previous training. Our study demonstrated that 50 Hz, 100 μT, 90 days ELF-MF exposure has no effect on repeated MWM tests.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the State Key Program of National Natural Science of China (51037006) and by the National Natural Science Foundation of China (31070755 and 81200996). We are grateful to our technical staff for their assistance with the exposure equipment.

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