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Acute Exposure to a 60 Hz Magnetic Field Affects Rats' Water-Maze Performance

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Rats were trained in six sessions to locate a submerged platform in a circular water-maze. They were exposed to a 1 mT, 60 Hz magnetic field for one hour in a Helmholtz coil system immediately before each training session. In addition, one hour after the last training session, they were tested in a probe trial during which the platform was removed and the time spent in the quadrant of the maze in which the platform was located during the training sessions was scored. Control animals were sham-exposed using the exposure system operating with the coils activated in an anti-parallel direction to cancel the fields. A group of ''non-exposed'' control animals was also included in the study. There was no significant difference between the magnetic field-exposed and control animals in learning to locate the platform. However, swim speed of the magnetic field-exposed rats was significantly slower than that of the controls. During the probe trial, magnetic field-exposed animals spent significantly less time in the quadrant that contained the platform, and their swim patterns were different from those of the controls. These results indicate that magnetic field exposure causes a deficit in spatial ''reference'' memory in the rat. Rats subjected to magnetic field exposure probably used a different behavioral strategy in learning the maze. *Bioelectromagnetics 19:117–122, 1998.* © 1998 Wiley-Liss, Inc.

Key words: 60 Hz; magnetic field; water-maze; spatial learning; memory

INTRODUCTION

The study of spatial learning and memory functions in rodents has been suggested to be relevant to the understanding of normal and pathological cognitive and memory functions in humans [Anger, 1991; Gallagher and Nicoll, 1993; Gallagher and Pelleymounter, 1988; Gower and Lamberty, 1993; Upchurch and Wehner, 1989]. One of us [Lai, 1996] has recently reported that acute exposure to a 60 Hz magnetic field caused a spatial ''working'' memory deficit in the rat in a radial arm maze. Rats were trained to obtain food reward from a maze with arms protruding from a center hub and to remember and not re-enter the arms from which food has been taken. Thus, the memory involved varies according to the responses of the animal in a particular training session. This is sometimes also referred to as ''short-term'' memory.

In the present research, the effect of magnetic field exposure on spatial learning and memory functions was further studied using the Morris water-maze [Morris, 1984]. In this behavioral model, rats are required to locate a submerged platform in a circular pool containing opaque water. It is assumed that a rat locates the platform by learning spatial cues in the environment to form a reference map. This is generally referred to as the ''reference'' memory.

METHODS AND PROCEDURES

Animals

Male Sprague–Dawley rats (2–3 months old, 250–300 gm), were purchased from B & K Laboratory,

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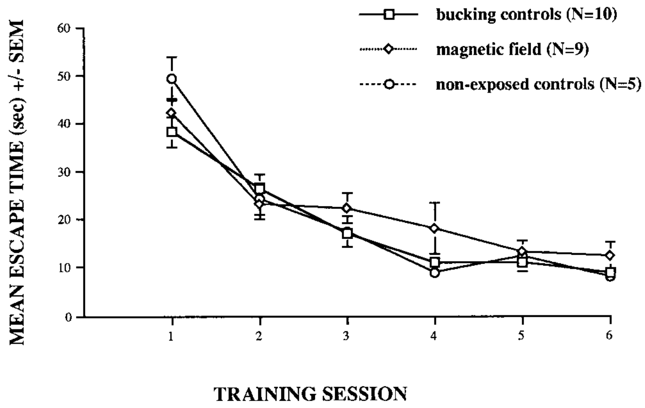

Fig. 1. Average escape time in seconds, i.e., time to reach the platform after release into the water, during the six training sessions of magnetic-field exposed rats, bucking controls, and non-exposed controls. *N* is the number of animals studied in each group.

Bellevue, WA. They were housed in the same room in which they were exposed to magnetic fields and adjacent to the room where the water-maze test was carried out. The rooms were maintained on a 12-h light-dark cycle (light on 0700–1900) with an ambient temperature of 22 °C (range 21–24 °C) and a relative humidity of 65% (range 62–67%). Animals were provided with Purina rat chow and water ad lib during the experiment.

Magnetic Field Exposure System

A Helmholtz coil pair system was used to expose rats to a 60 Hz magnetic field. This system has been described in detail previously [Lai et al., 1993]. Briefly, a computer program was used to design this Helmholtz coil pair system, which is capable of producing a magnetic field with minimal heating and field variations over the exposure area. Each Helmholtz coil is made of two sets of 40 turns each of #6 copper wire, wound in rectangular loops; minimum internal dimensions were 0.86×0.543 m. During construction, epoxy was layered between loops to glue them together. This minimizes vibration and noise when the coils are activated. The coils are wound on frames fabricated from wood and aluminum and, therefore, are completely shielded against emission of electric fields. They are designed with split windings terminated on multi-terminal blocks so that they may be wired in various series or parallel combinations for impedance matching and connecting to multichannel or multifrequency sources. A switch can be used to put the coils ''in phase'' to generate magnetic fields or in the ''bucking mode.'' Since there are two sets of coils in each Helmholtz coil, in the ''bucking mode'' they are activated in an anti-parallel direction (with the same current as in the ''in phase'' condition) to cancel the fields generated by each other. The ''bucking mode'' was used as a sham-exposed control condition in our research to control for the possible effects of heat and vibration generated by the coils.

By varying the input current to the coils, exposure fields could be set anywhere from the ambient level to the maximum coil-designed magnetic field strength of 5.6 mT. With an exposure level set at 1 mT, the heat dissipation from each of the Helmholtz coils is less than 8 W of power. The heat generated is efficiently dissipated due to the large surface area of the coils and good ventilation in the exposure room. The magnetic field during exposure was monitored by measuring the input current to the Helmholtz coils and by measuring the magnetic flux density with an Enertech EMDEX II magnetic field survey meter. The variation of the magnetic fields within the space between the coils as determined by theoretical calculation and actual measurement was $\pm 3\%$ of the mean. The ambient magnetic field (40–800 Hz) in our laboratory when the power supply to the coils was turned off, was 0.14 μT.

During exposure, rats were housed in a plastic cage (length 45 cm, width 21 cm, height 22 cm) with a styrofoam cover. The cage was placed in the center of the space between the coils. A maximum of three rats were exposed in the system at one time.

Water-Maze Procedure

The water-maze was a plastic circular pool (diameter: 246 cm; height: 39 cm; wall thickness: 1 mm)

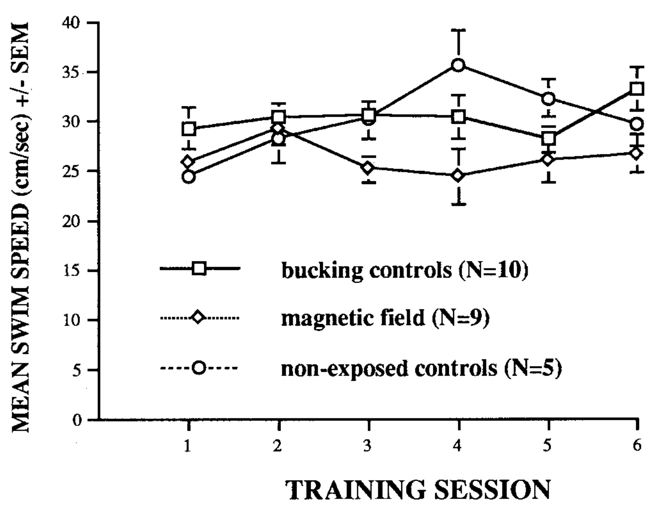

Fig. 2. Average swim speed of the three groups of experimental animal during training sessions.

filled with water (23 °C) to a depth of 27 cm. The water was made opaque by addition of powdered milk. A Plexiglas platform (15 × 20 cm) was placed at the center of the north-east quadrant of the maze and submerged 5 cm below the surface of the water. Each rat was given 2 training sessions daily on 3 consecutive days. The two daily sessions were separated by 3 h. Maze training was carried out between 0900–1500. Up to 3 rats were run at a time by staggering the exposure time between 2 rats by 10 min. The sequence in which the rats were run was the same over the 3 days.

In each training session, an animal was first exposed to the 1 mT, 60 Hz magnetic field or with the ‘‘bucking mode’’ (sham-exposed) for 1 h in the Helmholtz coil system. It was then released into the water from the wall of the maze at arbitrarily defined east, south, west, and north points. Therefore there were four trials per animal per training session. The sequence of points of release into the water followed a random order, but included one release from each of the east, south, west, and north points in each training session. The animal was allowed to swim to the platform. If it could not locate the platform within 1 min, it was picked up and placed on the platform. It remained there for 30 s before another trial and was removed from the maze after four trials. Performance in the maze was videotaped using a closed-circuit television system for detailed analysis later. In addition, 1 h after the last (6th) training session, each animal was given a probe trial, in which the platform was removed from the maze and the animal was released from the south point and allowed to swim in the maze for 1 min.

In addition to the magnetic field ($N = 9$) and bucking-exposed ($N = 10$) animals, a group of non-exposed ($N = 5$) rats was also included in this experiment. These animals were housed in cages in the laboratory and subjected to the same water-maze protocol, but without the exposure procedure of the other two groups of animals. The housing area was sufficiently far away from the Helmholtz coil that there was no significant change in ambient magnetic field intensity at the cages when the coil was turned on.

Data Analysis

From the video recording, escape time (the time between release into the water to landing on the platform) was measured using a stop-watch. Trials with no successful escape were given a score of 60 s. The average escape time of the four trials in each training session of each rat was used in data analysis. The swimming pattern of each trial was traced on transparencies. The distance swum was measured from the tracing, scaled to the dimension of the pool. Swim speed (cm/s) was calculated for each rat for each training trial by dividing the distance swum (cm) by the time of escape (s). For the probe trial, swim speed and the time spent in the quadrant (N-E) of the maze where the platform was previously located were scored. These analyses were done by an experimenter unaware of the treatment conditions of the rats being scored.

Escape time and swim speed data from training sessions were analyzed by the repeated measurement analysis of variance (ANOVA) and response curves were compared by the method of Krauth [1980]. Data from the probe trial were analyzed by one-way ANOVA and the Newman–Keuls test. A difference at $P \leq 0.05$ was considered statistically significant.

RESULTS

Results of escape time during the six training sessions are shown in Figure 1. Data analysis showed a significant session effect ($F[5, 105] = 73.94$, $P < 0.005$, i.e., a significant decrease in escape time with training) but no significant treatment effect ($F[2, 21] = 0.79$, $P > 0.05$) nor (treatment × session) interaction effect ($F[10, 105] = 1.67$, $P > 0.05$). Therefore, there was no significant difference in performance among the three groups of animals during training. Figure 2 shows the average swim speed of the three groups of animals during the training sessions. Statistical analysis showed a significant treatment effect ($F[2, 21] = 3.77$, $P < 0.05$) and (treatment × session) effect ($F[10, 105] = 2.14$, $P < 0.05$), but no significant session effect ($F[5, 105] = 1.303$, $P > 0.05$). Magnetic field-exposed rats had significantly slower swim speed during training sessions ($P < 0.05$, comparing the response curves of magnetic field-exposed vs sham-exposed by the method of Krauth [1980]).

Data of the probe trial are presented in Figure 3, showing the mean time (in seconds) the three groups of rats spent during the 1 min probe trial period in the quadrant where the platform was located during training sessions. One way ANOVA of the data showed a significant treatment effect ($F[2, 21] = 3.75$, $P < 0.05$). Magnetic field exposed rats spent significantly less time in the quadrant than the bucking (sham-exposed) and non-exposed rats ($P < 0.05$, Newman–Keuls test). Figure 4 shows representative samples of tracing of swim patterns of bucking (Figure 4 a, b), magnetic field (Figure 4 c, d), and non-exposed (Figure 4 e, f) rats during the probe trial. Swim pattern of magnetic field-exposed rats was different from that of the sham-exposed and non-exposed rats. It appears that they did not search for the ‘‘missing’’ platform in the N-E quad-

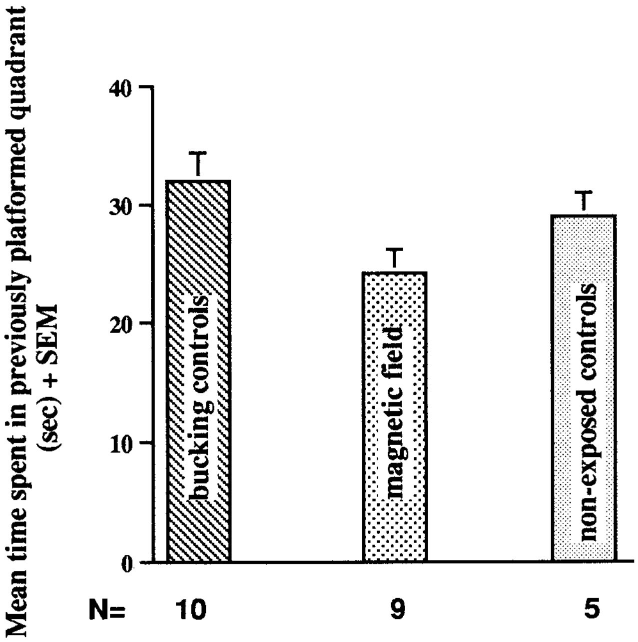

Fig. 3. Average time (s) spent during the probe trial in the quadrant where the platform was located during training sessions.

rant and swam over most of the area of the maze. There was no significant difference in swim speed among the three groups of animals during the probe trial (mean speed in cm/s ± SEM): bucking-exposed = 29.5 ± 1.2 (N = 10); magnetic field exposed = 26.1 ± 2.45 (N = 9); non-exposed = 29.5 ± 1.5 (N = 5).

DISCUSSION

Data from this experiment show that acute exposure to a 1.0 mT, 60 Hz magnetic field did not significantly affect the rat's rate of learning to locate a submerged platform in a water-maze, when compared with sham-exposed and non-exposed controls. However, swim speed of the rat was retarded by magnetic field exposure. In addition, magnetic field-exposed rats showed a different swim pattern during the probe trial.

Learning in the water-maze can be achieved by different behavioral strategies [Chapillon and Roullet, 1996; Noonan et al., 1996]: ''place learning,'' learning of a set of cues in the environment which form a ''reference'' map for the rat to locate its position in space; ''cue learning,'' learning to use a particular cue in the environment for the animal to guide itself to a certain location; and ''praxis learning,'' learning a certain sequence of movements in the environment to reach a certain location. The latter two types are generally not considered true ''spatial'' learning. The observation that during the probe trial magnetic field-exposed rats did not show a swim pattern indicative of searching for the ''missing'' platform in the proper quadrant, suggests that no ''place'' learning had occurred in these animals. They probably located the platform during the learning trials using the other learning strategies. This suggests that magnetic field exposure affects the formation of spatial ''reference'' memory. Together, results from our previous [Lai, 1996] and present research show that acute exposure to a 60 Hz magnetic field affects both ''working'' and ''reference'' spatial memory functions in the rat in different tasks.

The mechanism by which magnetic field exposure affects ''place'' learning is not known. However, neuroanatomical and neurochemical processes involved in water-maze performance are very well studied [Brandeis et al., 1989; McNamara and Skelton, 1993; Tsien et al., 1996]. Cholinergic systems in the brain play a major role. Deficits in water-maze performance are seen in animals with decreased cholinergic activity in the brain. We have previously found that acute magnetic field exposure decreases cholinergic activity in the frontal cortex and hippocampus of the rat [Lai et al., 1993]. In addition, central cholinergic systems have been shown to be involved in ''place'' learning, but not ''cue'' or ''praxis'' learning in the water-maze [Whishaw, 1985, 1989; Whishaw and Tomie, 1987]. A magnetic field-induced decrease in central cholinergic activity may partially account for the behavioral deficits observed in the present experiment.

Results from our previous studies [Lai, 1996; Lai et al., 1993] also indicate that magnetic field activates endogenous opioids in the brain, which in turn leads to a decrease in central cholinergic activity and spatial learning deficits. Activation of opioid systems in the brain has generally been shown to cause detrimental effects on water-maze ''place'' learning [Decker et al., 1989; McNamara and Skelton, 1991]. The effect is generally ascribed to a decrease in motivation to escape rather than learning. This may explain the significant decrease in swim speed observed in the magnetic field-exposed animals in our experiment. Kavaliers et al. [1996] have recently reported that brief exposure (less than 10 min) to a 60 Hz magnetic field at a flux density of 0.1 mT could enhance water-maze learning in female deer mice. The facilitating effect was shown to be related to an increase in endogenous opioid activity. The researchers also pointed out that motivational factor could play a part in their observation.

The role played by endogenous opioid systems in water-maze performance is complicated. Apparently, activity of endogenous opioid systems in the brain can both facilitate and impair performance. For example,

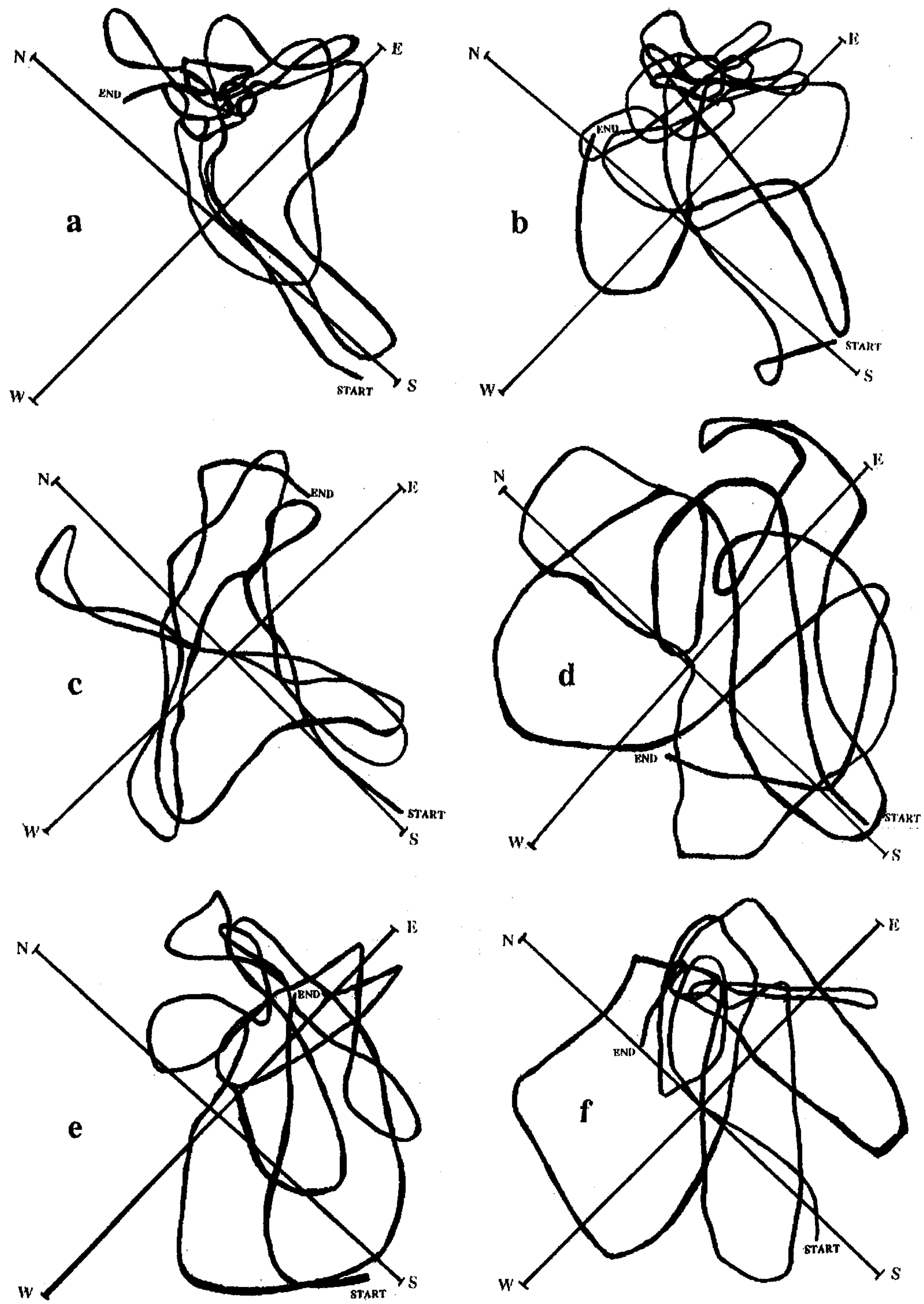

Fig. 4. Representative samples of swim patterns during the probe trial of bucking-controls (**a** & **b**), magnetic field-exposed rats (**c** & **d**), and non-exposed controls (**e** & **f**). Platform was located in the center of the north-east (N-E) quadrant during the training sessions. Rats were released at the south (S) point.

treatment with naloxone, a drug that blocks the action of endogenous opioids, has been reported to enhance, impair, or have no significant effect on water-maze performance, depending on the dose, time of administration, and the protocol of training [Decker et al., 1989; McDaniel et al., 1990; McNamara and Skelton, 1992].

Thus, much work has to be done to further explore the effects of magnetic fields on spatial learning. Due to the relevance of spatial learning in rodents to human health conditions and the fact that much is known about the neural mechanisms of spatial learning, performance in the water-maze provides a powerful mean for the investigation of the effects of magnetic field exposure on functions of the central nervous system.

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