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Deficits in Spatial Learning After Exposure of Mice to a 50 Hz Magnetic Field

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A series of four experiments was performed to determine the effect of exposure to a 50 Hz magnetic field on memory-related behaviour of adult, male C57BL/6J mice. Experimental subjects were exposed to a vertical, sinusoidal magnetic field at 0.75 mT (rms), for 45 min immediately before daily testing sessions on a spatial learning task in an eight-arm radial maze. Control subjects were only exposed to a background time-varying field of less than 50 nT and the ambient static field of about 40 μT. In each experiment, exposure significantly reduced the rate of acquisition of the task but did not affect overall accuracy. This finding is consistent with the results of another study that found that prior exposure to 60 Hz magnetic fields affected spatial learning in rats. *Bioelectromagnetics 19:79–84, 1998.* © 1998 Wiley-Liss, Inc.

Key words: memory; radial arm maze; rodents; ELF

INTRODUCTION

Evidence is accumulating that exposure to magnetic fields may affect learning and memory in rodents, although the conditions under which an effect can be seen are not yet well defined. For example, the behaviour of rats performing a spatial memory task in a radial arm maze may be altered using specific combinations of static and time-varying fields [Creim et al., 1990; Lovely et al., 1991, 1992, 1993a, 1994], whereas the performance of voles and deer mice in a water maze may be altered using sinusoidal fields alone [Kavaliers et al., 1993, 1996]. The magnitude of the observed effects in both tasks tend to be rather small, and not all studies have reported significant effects; we found that exposure to a range of 50 Hz magnetic fields from 5 μT to 5 mT did not affect the spatial memory of mice in a radial maze [Sienkiewicz et al., 1996]. In this study mice were exposed to a range of 50 Hz fields for a short time during testing in the maze; no changes in either initial performance, rate of learning, or overall accuracy could be observed.

More recently, however, a much larger effect on learning has been reported. Male rats that had been exposed for 45 min to a 60 Hz field at 0.75 mT before daily training sessions in a 12-arm radial maze showed a highly significant deficit in learning, with exposed animals making consistently more errors in the maze [Lai, 1996]. Pretreatment with the choline agonist physostigmine was able to reverse the learning deficits, suggesting that changes in central cholinergic transmission were responsible for the observed effects.

The present experiment was conducted to determine whether spatial learning in mice would also be reduced after exposure to an intense magnetic field. Here, the animals were exposed to a 50 Hz field at 0.75 mT for 45 min each day immediately before a training session in an eight-arm radial maze. This experiment was not intended as an exact replication of the previous experiment by Lai [1996] using rats, and there are a number of important differences between the studies. These differences include operational differences such as the frequency of the applied field and the number of arms of the maze, and procedural differences such as the number of pretraining sessions given to each animal and the number of arm entries that could be made each day in the maze.

Biological effects of low intensity magnetic fields are notoriously mercurial, and it has not always been possible to replicate reported effects either at a later date or by an independent laboratory. Examples include field-dependent effects on pineal function and changes in gene expression [Cridland et al., 1996]. Therefore

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to check for the consistency and robustness of any memory-related effect here, four separate experiments were performed using different batches of animals.

MATERIALS AND METHODS

Subjects

Male C57BL/6J mice at 12–14 weeks of age were purchased from the Medical Research Council Radiobiology Unit, Chilton. They were housed in individual polypropylene cages (29 cm × 15 cm × 12 cm) in a colony room adjacent to the behavioural laboratory and were given free access to water and standard maintenance diet (SDS RM-1) for 1 week before any procedure. Bedding was provided by commercial sawdust (Litalabo; SPPS, Argenteuil, France). The ambient conditions were maintained within the range 21 to 23 °C and 45 to 60% relative humidity. Lighting was provided from 06:00 to 18:00 h. The background time-varying fields were 0.1–0.4 μT and the static field was 44 μT. All procedures involving animals were carried out in accordance with the Animals (Scientific Procedures) Act of 1986.

Apparatus

The magnetic field exposure system has been described previously [Kowalczuk et al., 1993]. The magnet consists of two sets of horizontal aluminium coils held 25 cm apart in a laminated steel yoke. The yoke and coils were vacuum impregnated with epoxy resin to minimise vibration. The complete structure rests on rubber mounts on top of a free standing iron frame. The air temperature between the coils of the magnet was maintained at 21 to 23 °C by an air conditioning unit and air extract fan within the laboratory. These devices also provided a background masking noise of 55 dB(A) over the range of 20 Hz to 20 kHz.

During exposure, the animals were housed in polycarbonate cages (33 cm × 15 cm × 13 cm). These cages contained no metallic parts. Water was available from a glass bottle fitted with a Melamine spout, and sawdust was provided as bedding. The cages were mechanically isolated from the magnet by resting on a free-standing, rigid Perspex table that straddled the lower coils of the magnet.

The exposure system produced a vertical, sinusoidal magnetic field at 50 Hz. Flux density was measured using a Bell 9200 Gaussmeter equipped with a STB92-0404 transverse probe. The variation in magnetic flux density within the magnet was less than ±5%. The ambient magnetic fields were measured using an EMDEX II magnetic field dose meter. The background time-varying field within the magnet was less than 50 nT, and the static magnetic field was 40 μT. The latter was horizontal (±5°), aligned down the axis of the magnet and orthogonal to the applied field. The axis of the magnet itself was aligned in a NNE direction.

The radial arm maze is identical to that used before [Sienkiewicz et al., 1992]. The maze was constructed from Perspex and consisted of a central, octagonal arena 24 cm in diameter, and eight arms each 32-cm long. Close to the end of each arm was a small, circular food well. Access to and from the arms was controlled by the use of guillotine doors, these doors being remotely operated by the experimenter using a series of solenoid-activated pneumatic cylinders. These small solenoids were powered by 24 V DC and produced a maximum static field of 2 μT within the arena. The equipment within the laboratory provided visual cues to guide behaviour. Illumination was provided by overhead fluorescent lighting.

Behavioural Procedures

All subjects were experimentally naive. They were randomly assigned to either an experimental or sham-exposed control group. Subjects were food deprived to 85% of their free-feeding weight over the 14 days before testing, and they were maintained at that level for the duration of the experiment. Water was always available in the home cages.

For preliminary training for both groups, each arm of the maze was baited with a food reward (45-mg food pellet: BioServ, Frenchtown, NJ), and a subject was placed in the maze for one session lasting 5 min. Once the subject was in the maze, all doors were opened after about 5 s and these remained opened until the end of the session. Each subject was under constant, remote surveillance using a system of video cameras and monitors, but its behaviour during this session was not recorded.

Before each testing trial, a subject was transported in its home cage to a nearby laboratory containing the exposure system, transferred into a separate exposure cage, and placed within the bore of the magnet. After 45 min, the subject and its exposure cage were taken out of the magnet, and the subject was replaced in its own home cage. It was returned to the behavioural laboratory, weighed, and placed within the maze to begin the session. The distance between laboratories was about 100 m, and the maximum interval between the end of exposure and the start of the trial was about 1 min. A maximum of three animals were exposed at one time within the magnet, with the start of exposure being staggered between successive animals by 15 min.

The subjects were required to learn the standard, win-shift version of the task. They were confined in the central arena for 5–10 s between arm choices to stop the use of kinaesthetic or other simple choice strategies. Visiting an arm was defined as the placement of all four feet within that arm. Because food rewards were not replaced during a trial, revisiting an arm during the same session was considered an error. The sequence of arms entered and the time to complete the session were recorded. Times were measured to the nearest second using a hand-held stopwatch from the moment the guillotine doors were first opened until the last pellet was eaten. The subject was removed from the maze once it had visited all eight arms or after the maximum session length of 15 min. It was then returned to its home cage and fed its daily food ration. All surfaces of the maze were cleaned between sessions using alcohol wipes and compressed air.

A series of four experiments was carried out using separate experimental and sham-exposed control groups, each consisting of six subjects. Each experiment consisted of 10 consecutive daily testing sessions. Subjects from each experiment were drawn from different batches of animals. Experimental subjects were exposed at 0.75 mT, rms; control subjects underwent identical procedures but were not exposed to any source-generated field. All experiments were performed between 10:00 and 15:00 hours. In the first two experiments, the experimental subjects were exposed and tested first followed by the control subjects. To control for a possible circadian effect on learning, the timing of exposure and testing was reversed between groups in the third and fourth experiments.

Statistical Analysis

The results were analyzed by estimating the probability that an animal will not re-enter any given arm of the maze using maximum likelihood techniques, and then using these probabilities as the measure of performance of the task [Sienkiewicz et al., 1992]. To determine whether differences existed between treatments, various models were fitted to the performance scores of the two groups for each experiment. These models considered that there were either no differences in performance between the groups; or that there was a constant difference, an initial difference that tended to zero, or a difference in the rate of improvement in performance. Other models considered a combination of these parameters.

The relative fit of these models were examined by comparing the differences in the deviances, taking into account any changes in the degrees of freedom. Such techniques are a generalization of analysis of variance (ANOVA) and may be considered equivalent to a two-way ANOVA, but using a binomial distribution for the underlying random variability.

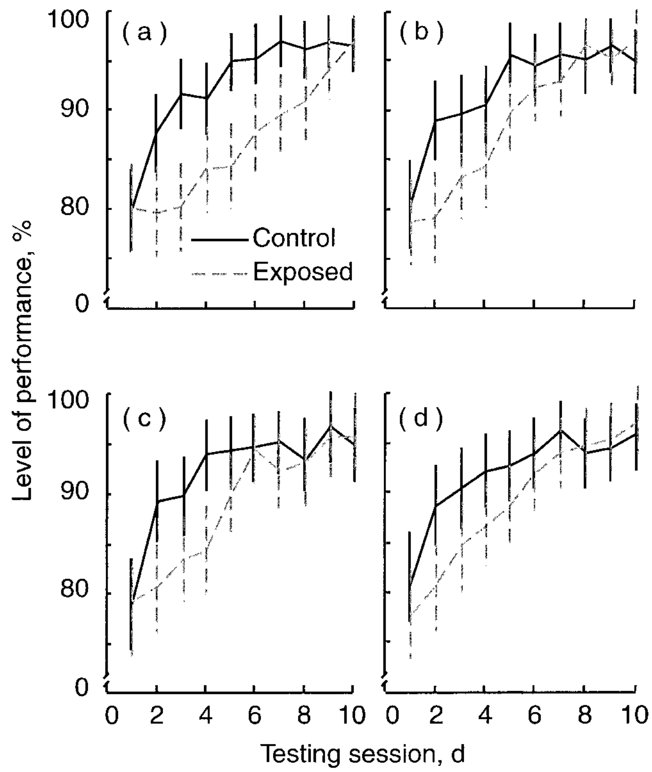

Fig. 1. **(a–d)** Mean performance scores (± standard error) of mice during testing in a radial arm maze. Experimental groups (n = 6) were exposed to a 50 Hz magnetic field at 0.75 mT for 45 min immediately before testing; separate control groups (n = 6) were sham-exposed for each of four experiments. Performance was measured as the probability of not re-entering any given arm of the maze and expressed as a percentage. In all cases, the performances of the experimental groups were significantly different from those of the control groups.

An advantage over some conventional methods of analysis is that this method exploits the full pattern of responses from each session and so is able to differentiate, for example, between an animal that makes a given number of errors evenly dispersed throughout a session and one that makes the same number of errors toward the end of that session.

RESULTS

Figure 1 shows the change in performance with testing for each of the experiments, performance being measured as the probability of not re-entering any arm of the maze. As can be seen, the four experiments gave very similar results. In each case, exposure to the magnetic field impaired overall performance, but the exposed groups finally reached the same level of accuracy as the controls. Statistical analysis showed that for each experiment these data were best described by

TABLE 1. Results of statistical analysis

Experiment	Model	Deviance	Degrees of freedom	χ^2	*P*
a	B	1623.35	1455		
	R	1581.53	1454	41.8	<.05
b	B	1545.64	1393		
	R	1523.74	1392	21.9	<.05
c	B	1522.21	1387		
	R	1499.54	1386	22.67	<.05
d	B	1472.27	1369		
	R	1464.56	1368	7.71	<.05

Eight different models were fitted to the results to compare the performances of the experimental and control groups. In each experiment, the best fitting model to describe the data was provided by the model that assumed that there was only a difference in the rate of learning the task (model R). This provided the greatest improvement in fit compared with the base model, which assumed no differences between treatment groups (model B). Results of comparisons with the other models are not shown.

the model that assumed that the only difference between the treatment groups was in the rate of improvement in performance of the task. In all cases, this model provided the greatest improvement in fit compared with the base model, which assumed no differences between the groups (Table 1).

Figure 2 shows the mean times to complete the daily trials for each experiment. All animals on every day managed to visit all eight arms within the 15 min period allowed. For each experiment, the data were best described by a model that assumed no differences between treatment groups; any improvements in fit provided by the other models were not significant ($P > .05$ in all cases).

DISCUSSION

These results show that repeated exposure to an intense magnetic field may affect the performance of a radial arm maze task in adult mice. This finding indicates that exposure impairs spatial memory function in some fashion. However, this impairment is subtle and exposure does not cause either a constant deficit in performance or an initial difference that increases with testing. Rather, exposure causes the animals to learn the task more slowly, but they eventually learn the task as well as controls. This finding may suggest that either the initial stages of learning are more susceptible to disruption, which is consistent with ideas formulated by Lovely et al. [1991], or that the magnitude of any effect reduces with repeated exposure. The present study provides little insight to the mechanism(s) whereby magnetic fields could influence learning and modify behaviour, although several possibilities have been suggested elsewhere [Kavaliers and Ossenkopp, 1993; Lovely et al., 1993b; Lai, 1996]. The lack of any significant effect on the times to complete the trials suggests that the differences in learning between treatments was not due to some deficit in motivation or impairment in motor activity by the exposed animals.

The consistency and robustness of the effect is illustrated by the similarity of the results obtained in each experiment. These experiments were performed using different batches of naive animals over a 5 month period. This would seem to indicate that any differences attributable to performing the experiments at different times of the year were not detectable. However, it is worth remembering that all animals were maintained on a constant-length day cycle and held within the same temperature and humidity ranges for all experiments, and this strategy may have blunted any potential seasonal variation.

Similarly, to compensate for the slight differences in the time of day during which exposure and testing occurred between experimental and control animals, the order of exposure and testing was reversed in two of the experiments. It was reasoned that even small circadian differences might explain these results; for example, if animals were more motivated in the early afternoon than in the morning, some effect on performance might be detected. However, it would appear that circadian influences alone cannot account for these results: there were no significant differences between the experimental outcomes when the animals were exposed in the late morning compared with those when the animals were exposed in the early afternoon.

Taken together, these results argue that any circadian, seasonal, or other influences both within and between experiments did not significantly contribute toward the observed effect on learning, strengthening the belief that the deficit in learning was caused by exposure to the magnetic field.

Lai [1996] first reported that rats were impaired in learning to run a 12-arm radial maze for food after

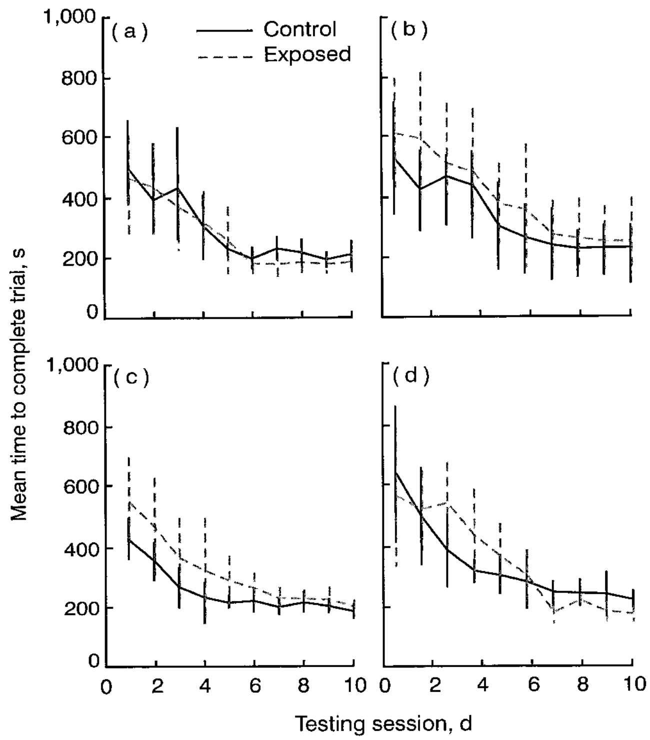

Fig. 2. **(a–d)** Mean trial times (± standard error) of mice during testing in a radial arm maze. Times were measured from the initial opening of the guillotine doors until the last food pellet was eaten. In all cases, there are no significant differences between the times of the experimental and control groups.

exposure to a magnetic field. He found that exposure to a 60 Hz field at 0.75 mT resulted in large and significant deficits. In his study exposure resulted in a sustained impairment, and after 10 days of training the exposed animals remained inferior to the controls, although his data suggest that the animals were learning the task. We did not try to reproduce the exact conditions under which these deficits were seen in rats, and there are a multitude of potential differences with our study. These differences include the species of animal used, the exposure systems and frequency of the field, and possible differences in geomagnetic flux density. We also adopted a confinement procedure to stop the mice from using nonspatial strategies to run the maze, and in our study testing each day was only limited by time and not by number of arm entries. In addition, there was no attempt to replicate the housing conditions or maintenance diet of the animals. It is possible that any or all of these could have been responsible for the differences found between studies.

However, we consider that a more likely explanation of the difference in results between studies may stem from the number of arms used in either maze. The 12-arm maze is generally considered a more difficult task than the eight-arm maze for animals to learn. Casual inspection of the results using rats would suggest a trend for a very gradual improvement over time. We speculate that had the training of the rats continued, the improvement in performance may have continued and exposed animals may have eventually shown the same level of performance as those sham-exposed. So here too, the (main) effect of the magnetic field could be to cause a decrease in the rate of learning without affecting the final level of performance. This explanation would be in agreement with our study using mice.

The length of time the animals are exposed to the magnetic field and also the intensity of the field appear to be important variables that may interact. In a previous study, we found that exposure of adult mice for 3–15 min to a range of 50 Hz magnetic fields during learning had no effect on performance in a radial arm maze [Sienkiewicz et al., 1996]. This would seem to argue that short-term exposure to even intense fields (of up to 5 mT) produces no significant effects, but there are some conflicting data to this suggestion [Kavaliers et al., 1993]. Recently Lai et al. [1996] investigated the effects of varying the intensity and duration of a 60 Hz magnetic field on cholinergic activity in the hippocampus and frontal cortex of rats. Significant decreases were found when animals were exposed at 1 mT for 90 min or at 2 mT for 60 min; exposure at 1 mT for 60 min produced no significant effects. Behaviour appeared a more sensitive indicator of magnetic field insult than measuring cholinergic activity, and deficits in the retention of a spatial task in a Morris water maze were observed after exposure at 1 mT for 45 min. Additional studies are warranted to investigate these possibilities further.

In summary, the results of this study provide additional evidence to indicate that exposure to intense power frequency magnetic fields may affect spatial learning and memory functions in rodents. Exposure appears to engender a deficit in the rate of learning without having any effect on overall accuracy. Thus the effect appears transient and limited to the initial stages of learning. Although it is too early to draw definite conclusions, the consistency of the results seen with mice in this study and previously with rats [Lai, 1996] argues for a robust effect that appears to generalise at least across two species and frequencies.

The magnitude of the field necessary to cause these effects is much greater than the fields generally encountered in the public environment. In the UK for example, the background flux density in most homes range from 10 nT to a few hundred nT, although fields of a magnitude equivalent to that used in this study may be encountered in a number of occupational situations, for example when working with some types of induction furnace. Even so, in many situations exposure may be of short duration and possibly limited to a part of the body that is not necessarily the head and neck.

The behavioural changes we have reported in mice are suggestive for some similar effect in humans. However, given the inherent difficulty in extrapolating data obtained with animals to humans, it would be useful for similar learning or behavioural changes to be confirmed in volunteer studies before using these data in assessing risk. In addition, the behavioural changes are of a modest magnitude, and they do not appear to represent a lasting effect. This finding suggests that these changes do not pose an obvious risk to health.

Further research would help to resolve more fully the exposure conditions under which the behavioural changes can be produced, because these are not well defined at present. The identification of the mechanism whereby the magnetic field causes the changes in behaviour is also important. A number of further experiments are underway, and these experiments are investigating the relationship between the magnitude of the behavioural impairment and intensity of the field and the effect of varying the length of exposure period and the interval between exposure and testing.

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