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Behavioral effects of static high magnetic fields on unrestrained and restrained mice

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Abstract

High-strength static magnetic fields are common tools in clinical imaging, but the behavioral effects are not well characterized. Previous studies on rats showed that fields of 7 T or above produced locomotor circling, conditioned taste aversion (CTA) and c-Fos in vestibular nuclei. To determine the generality of the behavioral effects on a smaller species, we subjected restrained or unrestrained mice to 30-min exposures in a 14.1-T field. Mice were given saccharin immediately prior to magnet or sham exposure on 3 consecutive days. All mice exposed to the magnet developed a CTA, and a significant number displayed tight circling and suppression of rearing. Unrestrained mice exhibited larger effects than restrained mice. These effects, similar to the effects in rats, may be the result of a vestibular disturbance caused by the magnetic field.

Keywords: Vestibular; Circling; Rearing; Conditioned taste aversion; Locomotor activity

1. Introduction

Although the magnetic fields used in clinical imaging are increasing in strength to produce higher-resolution images, the effects of static high magnetic fields on the subjects are not well characterized. Currently, clinical practice uses fields with 1–2 T strength, although fields of 4–9 T are in use experimentally. Vertigo and nausea were reported after exposure to a 4-T machine in an early safety study [1], suggesting the need for more investigation, but other behavioral effects and their physiological basis are largely unknown.

Additional behavioral and neural symptoms arise in rodents at higher field strengths. Our previous findings have shown that a 5–30 min exposure to a 7, 9.4 or 14.1 T field can induce circling locomotor activity [2,3], conditioned taste aversion (CTA) [2,4] and c-Fos expression in visceral and vestibular nuclei [3] in rats. The mechanism by which the field exposure mediates these effects is unknown. The effects are similar, however, to the responses to vestibular stimulation (e.g., circling and c-Fos in hemilabryinthectomized rats [5]), leading to the hypothesis that the high-strength magnetic field exposure may produce a vestibular disturbance, which might induce secondary effects.

In this study, we have extended our observations from rats to mice. Mice were chosen for several reasons. First, the demonstration of behavioral effects in mice is a critical test of the generality of the phenomenon across species. Second, it removes the potential confound of restraint stress present in all our rat studies to date. Because of the small size of the bores of the superconducting magnets, rats are required to be tightly restrained within Plexiglas tubes for the duration of their exposure. Because of their small size, unrestrained mice can be exposed in small containers that allow more freedom of movement. Finally, the establishment of mice as a model for the behavioral effects of magnetic fields will allow the future use of a variety of mutant and transgenic mouse models. Because there are several lines of mice with well-characterized vestibular deficits, the use of mice would assist the mechanistic exploration of magnetic field effects.

In order to compare the results of this study to our previously published data, we have attempted to replicate the previously used procedures as closely as possible [2]. We measured the acute effects of the magnetic field on locomotor activity immediately after exposure (i.e., rearing and circling) and the delayed effects of magnetic field exposure to induce a saccharin taste aversion. CTA is an

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associative learning paradigm in which an animal receives a novel taste followed by an aversive stimulus, after which the animal persistently avoids the taste. CTA is a sensitive measure to determine the level to which an animal can detect an aversive stimulus by measuring its intake of a palatable solution days after its pairing with the stimulus.

As in previous studies with rats, mice were restrained during their exposure within the bore of the 14.1-T magnet. While the small diameter of the magnet required rats to be restrained, the smaller size of mice allowed to us to add a new manipulation, in which some of the mice were allowed to move more freely within the core. Movement within the field might interact with the proposed vestibular effects of the magnetic field, either amplifying the effects or allowing the mice to counteract the effects through compensatory behavior.

2. Material and methods

2.1. Subjects

Male mice (C57BL/6, 20–30 g, Charles River Laboratories, Wilmington, MA) were housed individually in plastic cages in a temperature- and humidity-controlled room at the National High Magnetic Field Laboratory (NHMFL). The light–dark cycle was 12 h light, 12 h dark, with the lights on at 0700 h. All conditioning trials were conducted in the second half of the light phase, approximately 7 h after lights on. Mice had continuous access to pelleted Purina chow and deionized-distilled water, except where noted below. Six days prior to the conditioning trials, mice were placed on a water deprivation schedule. Water was given in one access period for 3 h/day beginning at 1400 h and gradually decreased to 10 min/day.

2.2. Materials

Magnetic field exposures resulted from a Bruker Cryo 600-MHz magnet. The magnet has a vertical bore of 89 mm in diameter and a fixed field strength of 14.1 T. The field is oriented vertically with the positive pole at the top of the magnet, and a shim magnet extended along the bore for approximately ±15 cm from the core to stabilize the magnetic field and to give uniform field strength.

2.3. Conditioning procedure

On the first day of conditioning, mice were given 30-min access to a 0.125% saccharin solution (saccharin) in 25 ml sipper tubes as the conditioned stimulus (CS). Consumption was measured by weighing the sipper tubes before and after the 30-min session to 0.1 g. Immediately following saccharin access, the mice were taken from their home cages, carried to the magnet (approximately 50 m) and exposed to the 14.1-T magnetic field for 30 min. This was the unconditioned stimulus (US). The conditioning procedure was repeated for 3 consecutive days.

The mice were divided into two magnet-exposed groups, restrained or unrestrained. The first group, the restrained magnet-exposed group, was restrained by placing them in a plastic tube made from a 50 ml conical cylinder, with the head of the mouse positioned at the cone end. A hole in the tip of the cone allowed for breathing. A plastic plug with a hole to allow for the tail of the mouse at the caudal end of the tube restrained the mouse from moving. Two restrained mice in these tubes were fitted into a plastic collar that was inserted vertically into the bore of the magnet and exposed to the 14.1-T magnetic field (see Fig. 1A).

Unrestrained magnet-exposed mice in the second group were individually placed into plastic cups with lids, approximately 11.5 cm in length and 7.0 cm in diameter. Two cups were then stacked on top of one another to be inserted vertically into the magnet (Fig. 1B). The mice were inserted into the bore so that in the restrained condition the mouse heads were at the center of the magnetic field and in the unrestrained condition the center of the field was placed equidistant from the bottom of each cup where the mouse would sit. Because the magnetic field is homogeneous at 14.1 T within 15 cm of the center, mice in both restrained and unrestrained groups would experience equivalent field exposures.

As sham controls, mice were either restrained or placed in cups then were placed into an opaque PVC tube with the same diameter as the inside of the magnet bore and in the same room to control for light, sound and temperature. The sham–magnet tube was placed beyond the 5 G line, and pairs of either restrained or unrestrained sham control mice were run at the same time as matched restrained or unrestrained magnet-exposed mice.

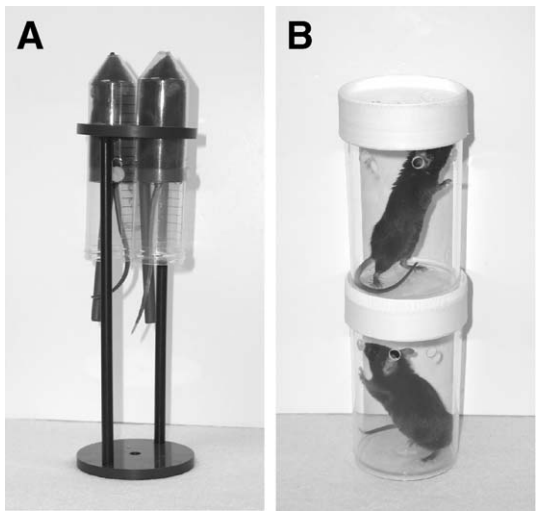

Fig. 1. Apparatus used to contain mice during exposures. Mice were either restrained (A) or allowed to move more freely (unrestrained) (B) during the 30-min magnet or sham exposure. Two mice were exposed at the same time in each treatment condition.

2.4. Locomotor activity

After the 30-min magnet or sham exposure, mice were placed in an open Plexiglas cage ($37 \times 47 \times 20$ cm) with cob bedding in the same room as the magnet and sham–magnet. The locomotor behavior of the mice was videotaped for 2 min and the mice were then returned to their home cages in the animal facility room. Two minutes was chosen because in previous experiments with rats almost all circling behavior ends within 2 min. Behavior was scored for number of rears (with at least one paw touching cage side) or tight circling (complete turns with a diameter no longer than the body of the mouse) by an individual blind to treatment condition.

All mice were conditioned using either magnet or sham control conditions for 3 consecutive days in the same manner. After the magnet or control exposure and videotaping on the third day, water was returned to mice overnight.

2.5. Preference testing

Strength of conditioning was measured by 24-h two-bottle extinction testing. On the fourth day of the experiment, mice were given ad lib 24-h access to both water and 0.125% saccharin solutions in two 25 ml sipper tubes, with the bottles placed side by side. Each day, the placement of the bottles was alternated to observe possible position bias. Consumption of both water and saccharin was measured daily by weight (0.1 g accuracy). The preference score was calculated for each mouse for each day by dividing the saccharin consumed by the total fluid consumed (saccharin/(water + saccharin)). A score of 1.0 would indicate that all fluid intake was saccharin; the lower the preference score, the stronger the aversion, with aversion operationally defined as a significant decrease in preference compared with sham controls. The 24-h preference tests continued for 6 days.

2.6. Statistical analysis

Significance of rearing and intake data during conditioning days and preference test days were determined using same subjects repeated-measures trend analysis and analysis of variance (ANOVA) with a two-factor design for repeated measures [6]. Post hoc comparisons were made using Student's t tests or orthogonal comparisons. Because locomotor behavior (circling) was measured nonparametrically, data were analyzed for significance by χ^2 test.

3. Results

3.1. Conditioning days

Mice were given 30-min access to saccharin immediately prior to 30-min magnet exposure or sham–magnet control exposure on 3 consecutive days. All mice consumed close to 2 g of saccharin on the first conditioning day. Same subjects repeated-measures trend analysis revealed a significant effect of treatment group (magnet restrained, magnet unrestrained, sham restrained or sham unrestrained) on consumption of saccharin [$F(3,20) = 5.98$, $P < .01$]. Restrained magnet-exposed mice consumed significantly less than sham-exposed mice on the second conditioning day (Fig. 2A), while unrestrained magnet-exposed mice consumed less than sham-exposed mice on both second and third conditioning days ($P < .05$ by t test) (see Fig. 2B).

3.2. Rearing

Same subjects repeated-measures trend analysis revealed a significant effect of treatment group (magnet restrained, magnet unrestrained, sham restrained or sham unrestrained) on rearing [$F(3,20) = 4.5$, $P < .05$] (see Fig. 3), such that magnet exposure suppressed rearing in both restrained and unrestrained mice. In separate two-way ANOVA of restrained or unrestrained mice, restrained mice showed significant interaction of treatment group (magnet vs. sham exposed) and conditioning day [$F(2,20) = 3.42$, $P < .05$], while unrestrained mice revealed a significant effect of treatment group only [magnet vs. sham exposed $F(1,10) = 5.10$, $P < .05$]. In restrained animals, there was a significant

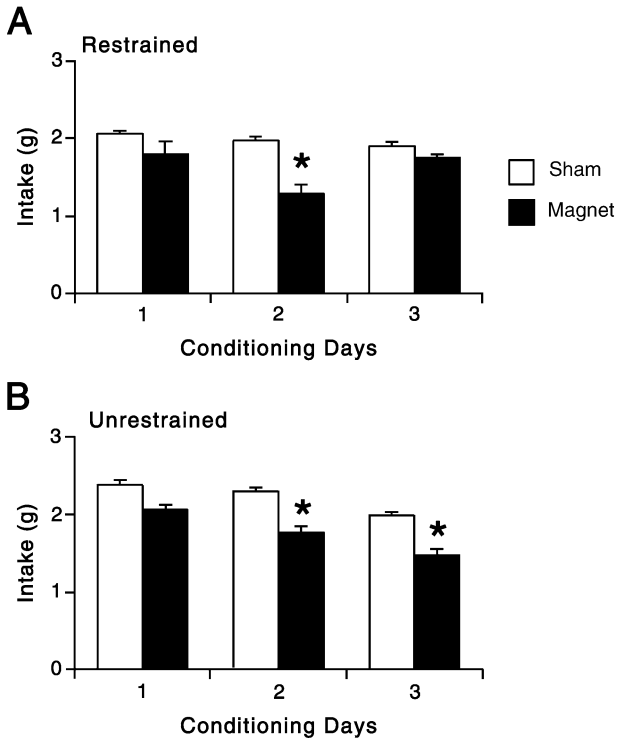

Fig. 2. Acquisition of CTA across three pairings of saccharin with 30-min exposure to 14.1-T magnetic field. (A) Intake was less on the second day in restrained magnet-exposed mice (black bars) compared with sham-exposed mice (white bars). (B) Intake was less in unrestrained magnet-exposed mice (black bars) on the second and third days compared with the sham-exposed mice (white bars). * $P < .05$ vs. sham-exposed mice.

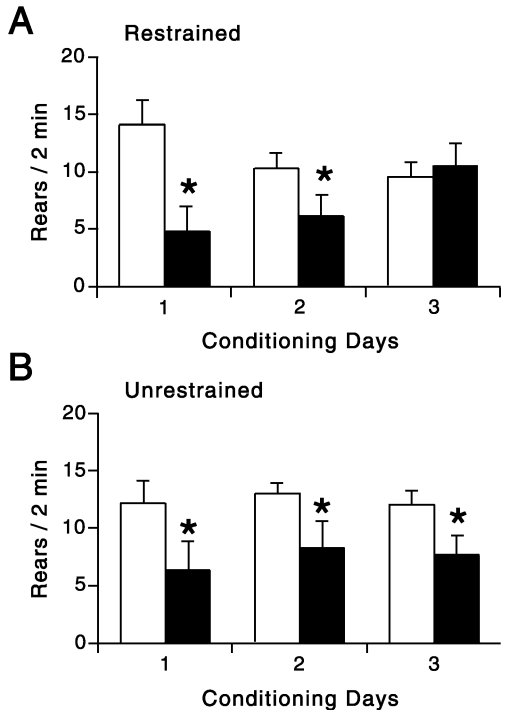

Fig. 3. Suppression of rearing across three pairings of saccharin with 30-min exposure to 14.1-T magnetic field. (A) Rearing was significantly lower on the first and second days in restrained magnet-exposed mice (black bars) compared with sham-exposed mice (white bars). (B) Rearing was significantly lower on all 3 days in unrestrained magnet-exposed mice (black bars) on the second and third days compared with the sham-exposed mice (white bars). $^{*}P<.05$ vs. sham-exposed mice.

difference between magnet- and sham-exposed animals on day 1 ($P<.01$) and day 2 ($P<.05$) but not day 3. In unrestrained mice, there was a significant difference between magnet- and sham-exposed mice on all 3 days ($P<.05$).

3.3. Circling

The effects of magnet exposure on circling locomotor activity are summarized in Table 1.

No sham-exposed mice circled. Exposure to the 14.1-T magnetic field, however, induced circling locomotor activity in some of the restrained and unrestrained mice. The circling induced in the magnet-exposed restrained mice was significantly different from the sham-exposed restrained mice on day 2 ($\chi^2=14.4$, $P<.01$). All mice that circled turned in a counterclockwise direction.

Table 1
Number of mice that circled in the first 2 min following magnet or sham exposure

Treatment group ($n=6$ per group)	Day 1	Day 2	Day 3
Restrained sham	0	0	0
Restrained magnet	2	4	1
Unrestrained sham	0	0	0
Unrestrained magnet	2	0	0

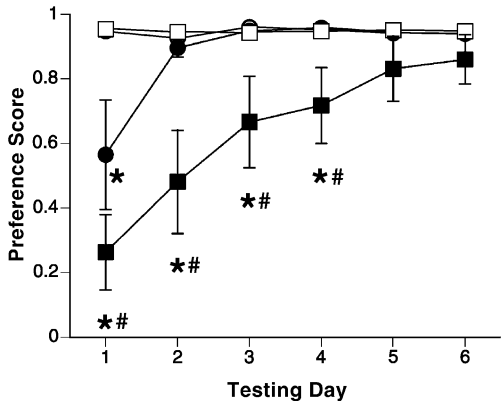

Fig. 4. CTA induced in restrained (circles) and unrestrained (squares) mice after magnet (black symbols) or sham (white symbols) exposure. Magnet-exposed groups showed significantly different saccharin preference scores from sham controls and each other on the first testing day. The taste aversions of restrained magnet-exposed mice extinguished quickly, while those of unrestrained mice extinguished more slowly. $^{*}P<.05$ vs. sham-exposed mice. $^{\#}P<.05$ vs. restrained mice.

3.4. Conditioned taste aversion

The aversive effects of pairing saccharin with 30-min exposure to the 14.1-T magnetic field was assessed by measuring saccharin versus water preference using two-bottle preference tests conducted over 6 days. Same subjects repeated-measures trend analysis revealed a significant interaction of treatment group (magnet restrained, magnet unrestrained, sham restrained or sham unrestrained) and two-bottle test day [$F(15,100)=6.4$, $P<.01$] (see Fig. 4). By orthogonal comparison, there was no difference in saccharin preference between the two sham-exposed groups. Both magnet-exposed groups had a significantly reduced saccharin preference on the first two-bottle test day. The unrestrained magnet-exposed group, however, had a significantly lower saccharin preference than the restrained group. CTA extinction was defined as the time point when preference scores are no longer significantly different from control animals (sham-exposed). The CTA of the restrained magnet-exposed group extinguished after 1 day of two-bottle testing, while the unrestrained magnet-exposed group extinguished after 5 days of testing.

4. Discussion

Exposure to the 14.1-T static magnetic field had significant effects on locomotor behavior and the acquisition of a CTA in mice. Magnetic field exposure significantly reduced rearing and induced tight counterclockwise locomotor circling in both groups. When palatable saccharin

solution was paired with the magnetic field exposure, both restrained and unrestrained groups developed a CTA, as determined by decreased consumption of the saccharin on the second and third conditioning days as well as decreased preference for saccharin during two-bottle tests. With the exception of circling locomotor activity, the unrestrained group exhibited larger behavioral effects than the restrained group after a 30-min 14.1-T magnetic exposure, suggesting that movement during exposure enhances the effects of the magnetic field.

These results are strikingly similar to our previous results in rats. Magnetic field exposure at 7, 9.4 or 14.1 T induced counterclockwise circling, suppressed rearing and supported CTA acquisition in restrained rats oriented in the same direction (heads-up) as the restrained mice in this study [2]. The locomotor and CTA effects of the field are consistent with activation of the vestibular system: circling is seen after unilateral hemilabyrinthectomy [5] and whole-body rotation suppresses rearing [7] and can induce CTA in rodents [8–10]. Consistent with a vestibular effect, 9.4-T exposure induces c-Fos protein, a marker of neuronal activity, in the vestibular nuclei of the rat brainstem [3]. This and other types of histological examination should be replicated in mice to examine neural activation after magnetic field exposure. Based on these comparisons, it may be that the magnetic exposure produces the behavioral effects seen here via a vestibular disturbance, as does rotation.

A model proposed by Schenck [11] may explain how the magnetic exposure results in a vestibular stimulation: any motion of the head may lead to a change in the magnetic flux through the semicircular canals of the inner ear, thus producing a small magnetohydrodynamic force on the endolymph and triggering vestibular nerve responses. These signals would conflict with unperturbed visual and proprioceptive inputs and result in nausea. This model may also explain the greater susceptibility of the unrestrained mice to the effects of the magnet, as they can move their heads through the magnetic field more than restrained mice and hence generate stronger conflicting vestibular signals. (This is based on the assumption that the mice are actually moving in the larger containers. Although there is certainly enough room for the mice to move freely, the design of the magnet prevents observation of the mice during exposure. This could be eliminated in the future by installing a camera small enough to enter the bore of the magnet to record the activity during exposure).

5. Conclusion

The observation of behavioral effects in mice demonstrates the generality of the effects of magnetic field exposure across species and removes the potential confound of restraint present in our early studies with rats. It also creates the opportunity to employ available mouse models. For example, there are many mutant and transgenic mouse models, including knockouts such as the Ames waltzer and het mice, which can be used to probe the function of the vestibular system. The Ames waltzer model is caused by a recessive mutation found in mice that causes deafness and a balance disorder associated with the degeneration of inner ear [12]. The behavioral effects are similar to those seen in human mutations, which affect the hair cells within the neuroepithelia of the inner ear that function as mechanoreceptors to transduce sound and motion signals. The het or ‘‘head tilt’’ model is due to a recessive mutation that causes vestibular dysfunction but not deafness. The defect in het mutants is limited to the utricle and saccule of the inner ear, which completely lack otoliths. Homozygotes display abnormal responses to position change and linear acceleration [13]. Exploring these and other related models may provide more information regarding how the magnetic field is producing the behavioral and neural effects in rodents.

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