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Physiology & Behavior 86 (2005) 379 – 389

PHYSIOLOGY & BEHAVIOR

Behavioral effects on rats of high strength magnetic fields generated by a resistive electromagnet

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Abstract

It has been reported previously that exposure to static high magnetic fields of 7 T or above in superconducting magnets has behavioral effects on rats. In particular, magnetic field exposure acutely but transiently suppressed rearing and induced walking in tight circles; the direction of circular locomotion was dependent on the rats' orientation within the magnet. Furthermore, when magnet exposure was paired with consumption of a palatable, novel solution, rats acquired a persistent taste aversion. In order to confirm these results under more controlled conditions, we exposed rats to static magnetic fields of 4 to 19.4 T in a 189 mm bore, 20 T resistive magnet. By using a resistive magnet, field strengths could be arbitrary varied from −19.4 to 19.4 T within the same bore. Rearing was suppressed after exposure to 4 T and above; circling was observed after 7 T and above. Conditioned taste aversion was acquired after 14 T and above. The effects of the magnetic fields were dependent on orientation. Exposure to +14 T induced counter-clockwise circling, while exposure to −14 T induced clockwise circling. Exposure with the rostral–caudal axis of the rat perpendicular to the magnetic field produced an attenuated behavioral response compared to exposure with the rostral–caudal axis parallel to the field. These results in a single resistive magnet confirm and extend our earlier findings using multiple superconducting magnets. They demonstrate that the behavioral effects of exposure within large magnets are dependent on the magnetic field, and not on non-magnetic properties of the machinery. Finally, the effects of exposure to 4 T are clinically relevant, as 4 T magnetic fields are commonly used in functional MRI assays.

Keywords: High magnetic fields; Superconducting magnets; Taste aversion

1. Introduction

Magnetic resonance imaging (MRI) machines that are in typical clinical use generate high strength static magnetic fields of 1 to 2 Tesla (T). However, the theoretical capacity for MRI to resolve images down to 0.5 μm^3 is driving the production of MRI machines with higher magnetic fields [1]. Experimental MRI machines with field strengths of up to 8 T are used on humans [2], and 11.4 T MRI is used on animal models [3].

While static high magnetic fields are generally considered to be benign and undetectable to mammals, the physiological effects of very high magnetic fields are unknown. There is, however, some evidence suggesting that high fields can affect humans. A survey of engineers and workers developing 4 T MRI machines reported a significant incidence of vertigo and nausea [4]. Furthermore, there are anecdotal reports of vertigo and nausea associated with exposure to 8 T [2].

Using the large superconducting magnets of nuclear magnetic resonance (NMR) machines at the US National High Magnetic Field laboratory (NHMFL), we have found that exposure to high fields has behavioral and neural effects in rats. Specifically, four pieces of evidence have been gathered: 1) immediately after 30-min exposure to 7, 9.4, or 14 T, rats walk in tight circles for up to 2 min [5]. The direction of these circles is dependent on the orientation of the rat within the magnet, such that rats walked counter-clockwise after facing +14 T but clockwise if upside down

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doi:10.1016/j.physbeh.2005.08.008

(i.e. facing −14 T). 2) If magnet exposure is paired with consumption of a novel sweet solution (glucose + saccharin), rats form a profound conditioned taste aversion such that they refuse to consume the sweet solution for many days after the exposure [6]. 3) When trained to climb towards a food reward at the top of a ladder, rats will refuse to climb the ladder if it traverses the bore of a 14 T magnet [7]. 4) At the neural level, 30-min exposure to high magnetic fields activates vestibular and visceral relays within the brainstem of the rat, as shown by the induction of c-Fos expression [8]. These results are consistent with an aversive or vestibular effect of the high magnetic fields. Also, they are similar to what might be seen after rotation or other vestibular stimuli leading to motion sickness in rodents and humans.

A significant operational problem with our earlier studies on rats was the use of superconducting NMR magnets. The advantages of the NMR machines are that they operate on the same principle as MRI machines, they produce extremely homogeneous fields, and they are available in a variety of field strengths (from 7 to 20 T at the NHMFL). They have several disadvantages, however, when used to provide sensory stimuli to rats in behavioral studies. Because they are superconducting and remain energized for months while drawing little outside current, it is very inconvenient to turn the magnetic field off and on again. Thus, a "sham–magnet" must be used for the 0 T controls (e.g. a PVC tube outside the magnetic field); this sham–magnet, of course, lacks many of the potential non-magnetic characteristics of the NMR machine such as odor, sounds, etc. Furthermore, the superconducting magnets are designed to be energized only to a set field strength, so that different strengths of magnetic field can only be applied within superconducting magnets in different physical locations. Finally, the NMR machines, being used primarily for biochemical studies, have relatively small, vertical bores (89 mm diameter), so that rats can be exposed only one at a time with their rostral–caudal axis perpendicular to the ground.

Resistive magnets do not have these disadvantages of superconducting magnets. Both superconducting and resistive magnets are electromagnets. The electric current in resistive magnets circles the bore through regular copper wiring (which has some resistance), and not through superconductors (without resistance) as in the NMR magnets. The magnetic field generated in a resistive magnet is proportional to the current, and therefore can be varied by changing the applied current. The polarity of the field can easily be reversed by reversing the current. The magnetic field also disappears when the current is stopped. This contrasts with an NMR magnet, in which the current and magnetic field persist long after energizing the superconductor. While superconducting NMR and MRI magnets are fairly common, large resistive magnets are rare due to their size and cost of operation. The major limitations on resistive magnets are the availability of electrical power (up to 20 MW for hours at a time) and the capacity to dissipate heat from the copper wiring during operation.

In order to confirm our findings that used NMR magnets, we employed a resistive magnet at the NHMFL with a vertical bore of 189 mm diameter that can produce fields between 0 and 20 T [9]. In this study we repeated and extended our stimulus response curve derived on NMR magnets [5] across 6 field strengths from 0 to 19.4 T, all within the same physical magnet (Experiment 1). In particular, we explored the effects of a relatively low 4 T exposure that is clinically relevant (Experiment 2). Because the field orientation as well as strength can be varied by the direction of the magnet's current without changing the rat's position, we contrasted the effects of exposure to +14 or −14 T (Experiment 3). Finally, because the resistive magnet had a 189 mm diameter bore, we were able to expose rats with their rostral–caudal axis parallel to the ground (Experiment 4).

The dependent measures were the acute effects of exposure on locomotor circling and rearing, and the acquisition and extinction of conditioned taste aversion after pairing a sweet taste with exposure. We have reported elsewhere the effects of resistive magnet exposure on ladder-climbing and c-Fos induction [in preparation].

2. General methods

2.1. Animals

Male Sprague–Dawley rats (175–200 g; Charles River) were housed individually in plastic cages in a temperature-controlled colony room at the National High Magnetic Field Laboratory at The Florida State University. The light/dark cycle was 12 : 12 with lights on at 0800 h. All conditioning trials were conducted during the light cycle. The rats had free access to pelleted Purina Rat Chow 5001 and deionized-distilled water ad libitum except where specified otherwise.

2.2. 0–20 T resistive magnet

High strength static magnetic fields were generated in a 20 T magnet constructed and operated at the NHMFL (see Fig. 1A) [9]. The basic design of the magnet is 400 mm thick copper coils of 500 mm outer diameter and 600 mm height wrapped around a 189 mm bore. Direct current of up to 40 kA at 500 V (20 MW) is passed through the copper coils, resulting in static magnetic fields of up to 20 T in the core of the magnet. The field strength falls off rapidly with distance, so that the field is near 0 T at 2 m distance from the center of the magnet (see Fig. 1B). The magnet is cooled by a chilled water system (173 l/s). The current and hence magnetic field could be arbitrary set to any field strength up to 20 T by the experimenters at the magnet, or remotely by the NHMFL control room located approximately 100 m away. Several minutes were required to ramp up the field strength from 0 T to the desired intensity; the field was set and allowed to stabilize before exposing any rats within the magnet.

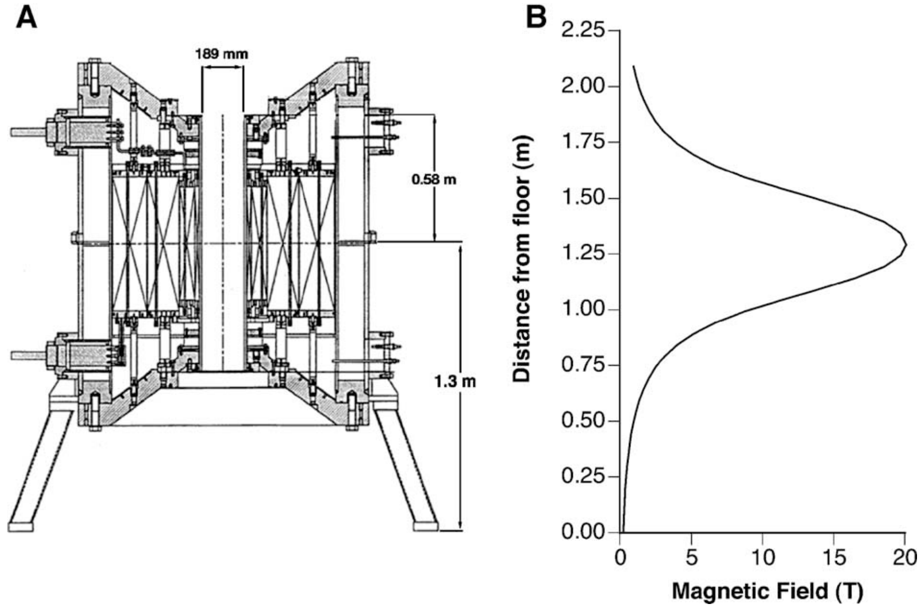

Fig. 1. (A) Side view of the 189 mm bore, 20 T magnet constructed at the NHMFL. Rats were restrained in Plexiglas tubes and lowered to the center of the bore. (B) Intensity of the maximum field generated by the 20 T magnet as a function of vertical distance from the floor beneath the magnet's bore [B. Brandt, personal communication]. Rats were positioned so that they spanned the region of peak intensity.

The resistive magnet was located in the same building as the animal facility, but in a separate wing of the building approximately 500 m away. Rats were transported in a covered stainless steel cart from the animal facility to the resistive magnet on each conditioning day prior to CS access, and returned to the animal facility after the postconditioning test of locomotor activity.

2.3. Conditioning

Eight days prior to the conditioning day, the rats were placed on a water restriction schedule under which they received daily water access in one drinking session, during which a water bottle was presented simultaneously with an empty bottle to accustom the rats to a two-bottle choice. The first daily session was 3 h in length and the session times were diminished each day so that the day before conditioning the rats received water access in a single 10-min session.

On the conditioning day, rats were moved in groups of 6 to the room containing the resistive 20 T magnet. Rats were given access to 3% glucose, 0.125% sodium saccharin solution (G+S) for 10 min. No significant difference between groups in G+S intake on the first conditioning day was observed in any experiment below. Immediately following this drinking period, rats were placed in restraint tubes for sham or magnet exposure. Rats were placed individually into a Plexiglas restraining tube that had an inside diameter of 56 mm and an outside diameter of 64 mm. A plug was inserted into the rostral end of the tube and held in position by nylon screws. The inside of this rostral plug was fabricated in a cone shape to accommodate the head of the rat. A 1-cm hole was bored in this plug at the apex of this cone to allow fresh breathing air. A second plug was inserted into the caudal end of the tube and could be adjusted to restrain the movement of the rat. A hole in the center of this plug accommodated the rat's tail. When in the tube, the rat was almost completely immobilized.

The bore of the resistive magnet was large enough to accommodate four rats at once. In order to position the four rats for equivalent exposures, an insert to the magnet was constructed of PVC plates and polycarbonate tubes. The insert consisted of two circular positioning plates (diameters of 41 and 18.5 cm) that held four polycarbonate guide tubes (1.22 m in length and 76 mm in diameter) equally spaced in a circle around the center of the insert. When lowered into the magnet, the smaller circular plate fitted within the magnet's bore with a clearance of less than 2 mm; the larger plate rested on a flange at the top of the magnet. Thus the four guide tubes were suspended vertically in the magnet, parallel to the magnetic field lines, and equidistant from the vertical walls of the magnet.

A rat placed in a restraint tube could be lowered by string into any of the four polycarbonate guide tubes, so that four rats could be exposed simultaneously. Nylon screw "stops" in the guide tubes ensured that the rats were placed at the core of the magnet and exposed to the maximal magnetic field.

2.4. Behavioral scoring

After 30-min exposure within the bore of the magnet, the rostral plug of the restraining tube was removed and each rat was released into an open polycarbonate cage (37 cm wide by 47 cm long by 20 cm high) with cob bedding. The locomotor behavior of each rat was recorded on videotape for 2 min after release into the cage (most rats exhibited locomotor effects of the magnetic field for less than 1 min;

thus, 2 min of recording captured most of the phenomenon of interest). The rat was then returned to its home cage and ad lib water was returned to them. The videotapes were scored later by an observer blind to the rats' treatment. Instances of tight-circling behavior and rearing behavior (one or both forepaws on the side of the cage) were quantified. Rats were scored as "circling" if they moved continuously around a full circle with diameter less than length of the rat's body. Partial circles or circles interrupted by stationary pauses were not counted.

The strength of the CTA induced by the magnet was measured with daily 24-h 2-bottle preference tests that were initiated the day following conditioning. Two bottles were placed on the cages, one containing G+S and the other distilled water. Fluid consumption was measured every 24 h and a preference score was calculated as the ratio of saccharin to total fluid consumption:

$$\frac{(saccharin\,consumption)}{(saccharin\,consumption + water\,consumption)}.$$

The preference tests were continued for up to 8 post-conditioning test days. The left/right position of saccharin and water bottles on the rats' cages was reversed each day. Because saccharin access during the preference tests was not paired with any treatment, the preference tests constituted extinction trials. The CTA of an experimental group was considered extinguished when the average saccharin preference was not different from the average preference of rats exposed to 0 T. Preference for saccharin measured during the first 24-h 2-bottle test was analyzed as the magnitude of CTA; changes in preference across repeated 2-bottle tests were analyzed for extinction rate.

2.5. Statistics

Comparisons between groups on single-day data were analyzed with one-way ANOVA or Student's *t*-test (Prism, Graphpad). Results collected over multiple conditioning or extinction days were analyzed with 2-way repeated measures ANOVAs (Statistica). Post hoc comparisons were made with Newman–Keuls multiple comparison test.

3. Experimental methods and results

3.1. Experiment 1: Effects of magnetic field strength

Male Sprague–Dawley rats ($n=48$) were placed on a water restriction schedule as above. On the day of conditioning, rats were moved to the room containing the 20 T magnet. The magnetic field was ramped up to 0 (no current), 4, 7, 14, 17, or 19.4 T (a maximum strength of 19.4 T was used because the cooling capacity of the magnet was not sufficient to maintain 20 T for more than an hour). In groups of 4, rats were given access to G+S for 10 min, and then placed in restraint tubes and lowered to the center of the magnetic field for 30 min ($n=8$ at each field strength). Rats were then removed from the magnet, released into the locomotor test cage, and videotaped for 2 min. Rats were returned to their home cages, transported back to the NHMFL animal facility, and given ad lib access to water.

Beginning the day after conditioning, two-bottle 24-h preference tests were begun to measure CTA strength and extinction. Preference tests continued for 7 days.

3.2. Results of Experiment 1

3.2.1. Circling and rearing

Magnetic field strength had a significant effect on locomotor behavior, such that higher field strengths induced more circling ($F[5,42]=4.37$, $p<0.005$) and suppressed rearing more ($F[5,42]=19.19$, $p<0.0001$). Tight counter-clockwise circling was seen after exposure to 7 T or above; the average number of circles expressed in 2 min was maximal at 17 and 19.4 T (see Fig. 2A). The number of rears was significantly reduced after 4 T exposure, and was maximally suppressed after exposure to 14 T and above (see Fig. 2B).

3.2.2. Extinction of conditioned taste aversion

On conditioning day, rats drank a mean of 11.1 ± 0.6 g of G–S prior to sham or magnet exposure; there was no significant difference in intake among groups. After the first day of 2-bottle preference testing, significant taste aversions were observed in rats exposed to 14 or 17 T, but not 19.4 T ($F[5,42]=4.84$, $p<0.005$, Fig. 3A). A 2-way ANOVA comparing all 6 groups on days 1–7 showed a significant effect of field strength ($F[5,252]=3.3$, $p<0.01$) and day ($F[6,252]=10.2$, $p<0.0001$), but no interaction ($F[30,252]=1.47$, $p=0.06$).

To more accurately determine the differences between groups, data were combined from groups with similar magnitude of taste aversions. Post hoc tests showed no significant difference between the 0, 4 and 7 T groups; therefore, the data of those three groups were combined. Likewise, no significant difference was seen between the 14 and 19.4 T group, so the data of those two groups were combined. When the 0+4+7 T group, the 14+19.4 T group, and the 17 T group were compared by 2-way ANOVA, a significant interaction of group and day was found ($F[12,252]=3.3$, $p<0.005$; see Fig. 3B). No taste aversion was seen after exposure to 0, 4 or 7 T. A significant reduction in saccharin preference was seen after exposure to higher fields (14, 17, or 19.4 T), with the maximal CTA observed after 17 T exposure. Taste aversions were extinguished after 6 or 7 days of two-bottle testing.

3.3. Experiment 2: Repeated exposures to 4 T

We have previously found that exposure to a 7 T superconducting magnet had a weak behavioral effect [5];

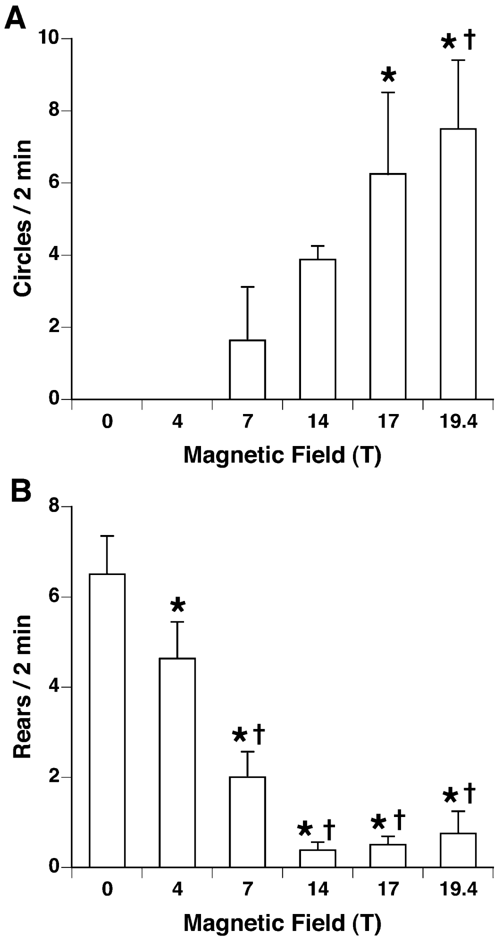

Fig. 2. Number (mean ± s.e.m.) of circles (A) or rears (B) exhibited by rats during 2 min in a locomotor test cage after 30-min exposure to static magnetic fields. (A) Rats were observed to walk in tight, counter-clockwise circles after exposure to 7 T or above, but not after sham (0 T) or 4 T exposure; *$p<0.05$ vs 0 T, †$p<0.05$ vs 7 T. (B) Compared to sham exposure at 0 T, rearing was significantly suppressed after exposure to 4 T or above; *$p<0.05$ vs 0 T, †$p<0.05$ vs 4 T. Thus acute behavioral effects of magnetic field exposure were observed after a minimum magnetic field of 4 T, and behavioral effects appeared proportional to field strength.

Experiment 1 of this study confirmed this result and showed that a single exposure to 4 T also had no observable effect on circling or CTA, and only a small effect on rearing. Weakly aversive stimuli that fail to induce a CTA after a single pairing may still be capable of inducing a CTA after multiple pairings, however. Therefore, rats were given repeated pairings of G+S with 4 T exposure to determine if exposure to 4 T was weakly aversive.

Male Sprague–Dawley rats ($n=8$) were placed on a water restriction schedule as above. On the day of conditioning, rats were moved to the room containing the 20 T magnet. The magnetic field was ramped up to 4 T. In groups of 4, rats were given access to G+S for 10 min, and then placed in restraint tubes and lowered to the center of the magnetic field for 30 min. Rats were then removed from the magnet, released into the locomotor test cage, and videotaped for 2 min. Rats were returned to their home cages, and transported back to the NHMFL animal facility. This procedure was repeated on the next two days, for a total of 3 consecutive pairings of G+S with exposure to 4 T.

After the third pairing, rats were given ad lib access to water. On the next day, two-bottle 24-h preference tests were begun to measure CTA strength and extinction. Preference tests continued for 2 days.

3.4. Results of Experiment 2

Immediately after exposure to 4 T for 30 min, rats showed no locomotor circling and an average of 3.3 ± 0.5 rears in 2 min. Compared to the number of rears seen in sham-exposed rats in Experiment 1 above, the number of

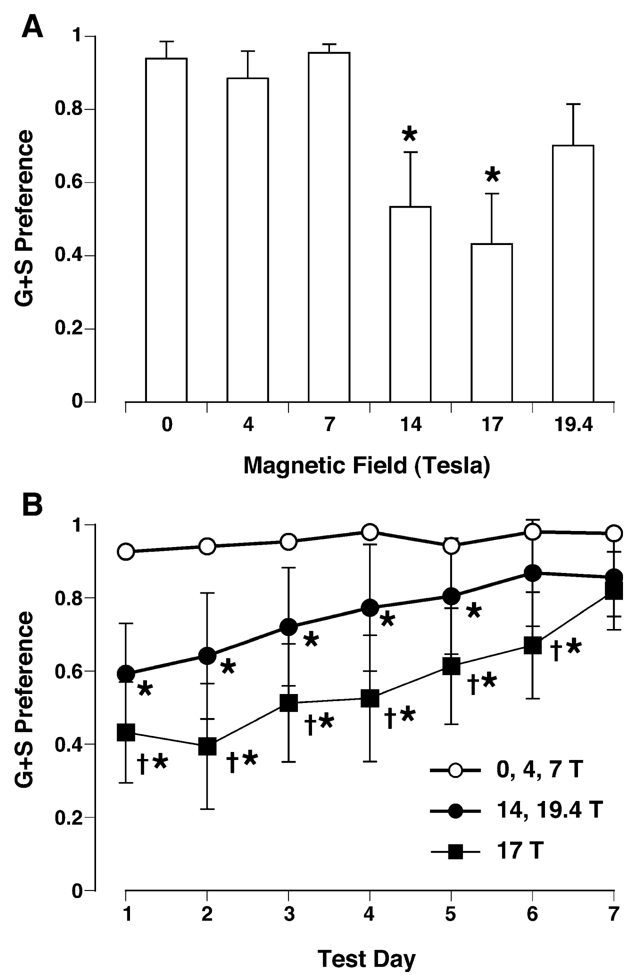

Fig. 3. (A) Expression of a CTA against G+S measured in the first 24-h 2-bottle preference test after a single pairing of G+S and 30-min magnetic field exposure. Compared to the effects of pairing G+S with sham exposure (0 T), pairing with 4 and 7 T did not decrease G+S preference, while 14 and 17 T induced a significant taste aversion; pairing with 19.4 T produced an intermediate preference. *$p<0.05$ vs 0 T. (B) Extinction of CTA against G+S induced by magnetic field exposure. During one week of 2-bottle preference tests, the strongest aversion after 17 T exposure extinguished in 7 days, an intermediate aversion after 14 or 19.4 T exposure extinguished after 6 days, and no aversion was seen after 0, 4, and 7 T exposure (groups combined as described in the text). *$p<0.05$ vs 0+4+7 T group; †$p<0.05$ vs 14+19.4 T group.

rears was significantly reduced only on the third conditioning day ($F[3,25]=5.10$, $p<0.01$; see Fig. 4).

G+S access was also paired with exposure to the 4 T magnetic field. One-way ANOVA showed that there was a significant increase in G+S intake over the 3 conditioning days ($F[2,21]=4.52$, $p<0.05$), rising from an average intake of 10±1.0 to 14.3±0.9 ml ($p<0.05$ by Newman–Keuls). During the two days of post-conditioning preference tests, rats showed an average preference of 0.97±0.01 for G+S over water. Thus there was no evidence that rats acquired a CTA after repeated conditioning with a 4 T magnetic field.

3.5. Experiment 3: Polarity of magnetic field

Previously we have found that rats circled counter-clockwise after they were positioned vertically in a high magnetic field with their heads toward the positive pole of the superconducting NMR magnet (+14 T). After the rats were exposed while inverted with their heads facing down and towards the negative pole (−14 T), they circled clockwise [5]. While these results suggest that the orientation (or polarity) of the magnetic field relative to the rat determines the direction of circling, it is possible that restraining the rats upside-down within the magnet also affected their response.

Unlike the superconducting NMR magnet, the polarity of the 20 T resistive magnet can be reversed simply by reversing the polarity of the electrical current that energizes the electromagnet. Thus rats could be exposed to either +14 or −14 T while restrained in the same vertical, heads-up position. Therefore, we examined the acute locomotor effects and CTA expression after pairing G+S intake with +14 or − 14 T exposure.

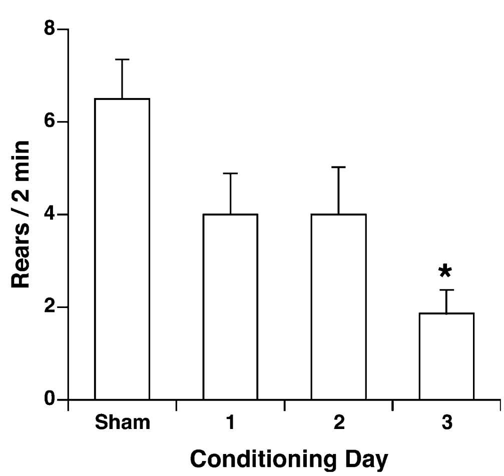

Fig. 4. Number (mean±s.e.m.) of rears exhibited by rats during 2 min after 30-min exposure to a 0 T (sham) or a 4 T magnetic field on three consecutive conditioning days. Although the average number of rears was decreased after 4 T, this difference was only significant on the third day. No circling was observed in any of the rats. Sham-exposure data taken from Experiment 1 (Fig. 1B). $p<0.05$ vs sham.

3.5.1. Experiment 3A: Single pairing of G+S with −14, 0, or +14 T

Male Sprague–Dawley rats ($n=21$) were placed on a water restriction schedule as above. On the day of conditioning, rats were moved to the room containing the 20 T magnet. The magnetic field was ramped up to either −14, 0, or +14 T. Control room staff of the NHMFL set the magnetic fields in randomized order, so that the experimenters were blind to the order of magnetic field exposure. In groups of 3 or 4, rats were given access to G+S for 10 min, and then placed in restraint tubes and lowered to the center of the magnetic field for 30 min ($n=7$ each at −14, 0, and +14 T). Rats were then removed from the magnet, released into the locomotor test cage, and videotaped for 2 min. Rats were returned to their home cages, transported back to the NHMFL animal facility, and given ad lib access to water. On the next day, two-bottle 24-h preference tests were begun to measure CTA strength and extinction. Preference tests continued for 8 days.

3.5.2. Experiment 3B: Three pairings of G+S with −14, 0, or +14 T

Male Sprague–Dawley rats ($n=21$) were conditioned as in Experiment 3A above, except that the rats received 10-min access to G+S and 30-min exposure to −14 ($n=7$), 0 ($n=6$), or +14 T ($n=8$) on three consecutive days for a total of three pairings. On the day after the third pairing, two-bottle 24-h preference tests were begun to measure CTA strength and extinction. Preference tests continued for 8 days.

3.6. Results of Experiment 3

3.6.1. Experiment 3A

3.6.1.1. Circling and rearing. There was a significant difference in the number of circles observed in the groups ($F[2,18]=6.3$, $p<0.01$) such that the −14 and +14 T groups circled more than the 0 T group (none of which circled). Furthermore, all rats that circled after −14 T exposure walked clockwise, while all rats that circled after +14 T exposure walked counter-clockwise (see Fig. 5A). There was also a significant effect of treatment on rearing, such that sham-exposed rats reared significantly more than either magnet-exposed group ($F[2,18]=14.4$, $p<0.001$; see Fig. 5B).

3.6.1.2. Conditioned taste aversion. On conditioning day, there was no significant difference between groups in intake of G+S (mean intake: 13.4±1.0 g). On the first day of 2-bottle preference testing, a 1-way ANOVA showed significant differences between groups ($F[2,18]=4.26$, $p<0.03$), indicating that rats exposed to +14 or −14 T acquired a CTA (see Fig. 6A). Some of these rats continued to show decreased G+S preference during subsequent 2-bottle preference tests. However, a 2-way ANOVA comparing

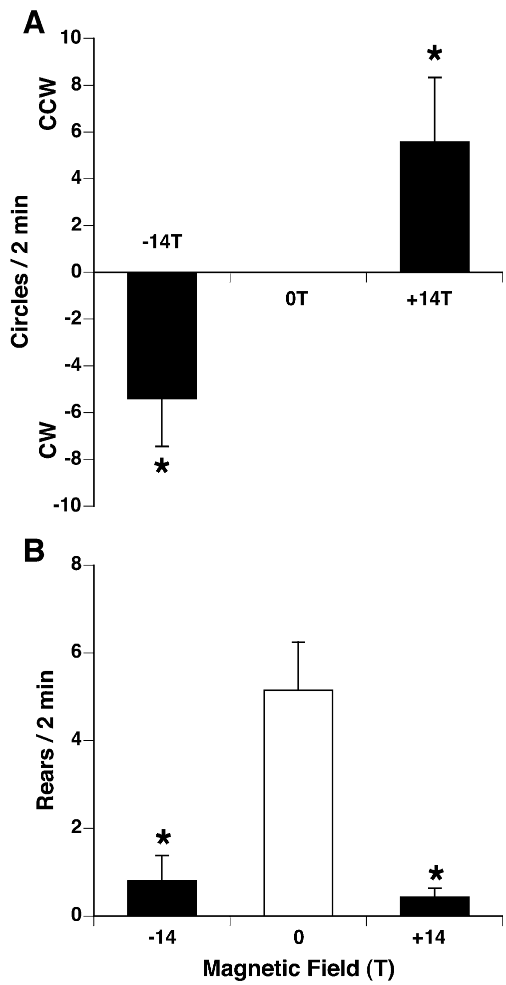

Fig. 5. Number (mean ± s.e.m.) of circles (A) or rears (B) exhibited by rats during 2 min in a locomotor test cage after 30-min exposure to either − 14, 0, or + 14 T magnetic fields. (A) Rats were observed to walk only in clockwise circles after exposure to − 14 T but only counter-clockwise circles after + 14 T exposure; no circling was observed after sham exposure (0 T). *$p<0.05$ vs 0 T, †$p<0.05$ vs − 14 T. (B) Compared to sham exposure at 0 T, rearing was significantly suppressed after exposure to both − 14 and + 14 T. *$p<0.05$ vs 0 T.

the 0 T exposed group with a combined + 14/− 14 T group showed no significant effect of either group ($p=0.06$) or extinction day ($p=0.56$) across all 8 days (see Fig. 6B).

3.6.2. Experiment 3B

3.6.2.1. Circling and rearing. There was a significant effect of magnet exposure (but not conditioning day) on locomotor circling ($F[2,4]=4.98$, $p<0.02$). Compared to sham exposure (0 T), exposure to 14 T magnetic field induced significantly more circling on the first 2 conditioning days of 14 T, while − 14 T exposure induced significant circling on all 3 days (see Fig. 7A).

There was also a significant interaction between magnet exposure and conditioning day on rearing across the 3 conditioning days ($F[2,4]=4.27$, $p<0.01$). Rearing was significantly suppressed after all 3 exposures to 14 or − 14 T, and within the − 14 T group the number of rears increased significantly after repeated exposures (see Fig. 7B).

3.6.2.2. Conditioned taste aversion. There was a significant interaction of magnet exposure and the 3 conditioning days on G + S intake ($F[2,4]=3.22$, $p<0.05$). Rats exposed to − 14 and + 14 T magnetic fields showed decreased intake on 2nd day compared to sham-exposed rats (see Fig. 8). Intake of the − 14 T exposed rats was significantly lower on the third day than on the first day. This suppression of intake reveals the presence of a CTA in the − 14 T group after the first two pairings.

On the first day of 2-bottle preference testing, a 1-way ANOVA showed a significant decrease in preference by the − 14 T group ($F[2,18]=10.35$, $p<0.001$; see Fig. 9A). Across all 7 days of 2-bottle testing, there was a significant

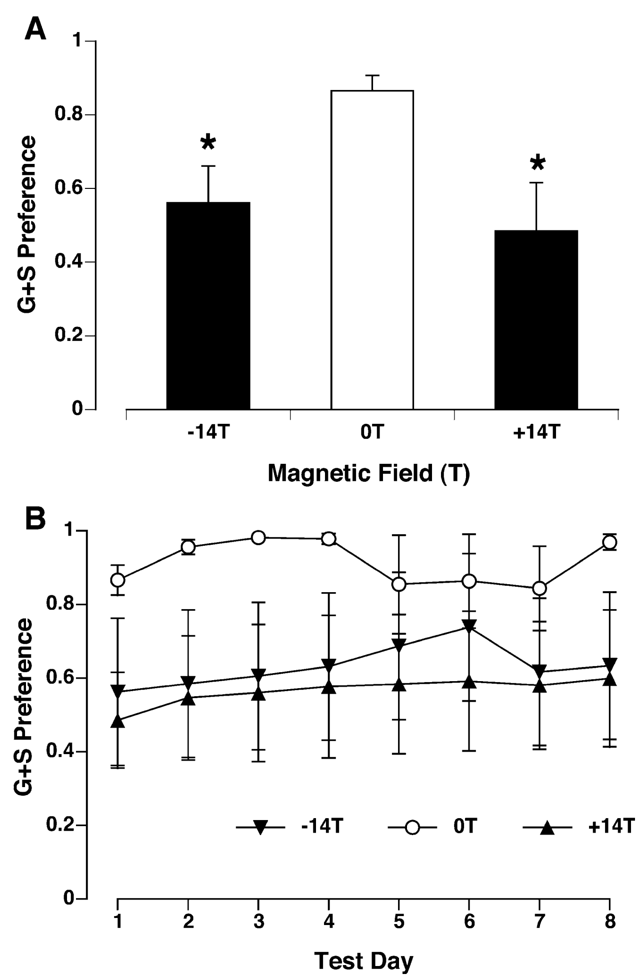

Fig. 6. (A) Expression of CTA to G + S on the first day of 2-bottle preference testing after a single pairing with − 14, 0, or + 14 T. Compared to sham-exposed rats, G + S preference was significantly decreased after pairing with either − 14 or + 14 T. *$p<0.05$ vs 0 T. B. G + S preference over 8 days of 2-bottle preference testing. Two-way repeated measures ANOVA found no significant difference between rats exposed to − 14, 0, or + 14 T ($p=0.06$).

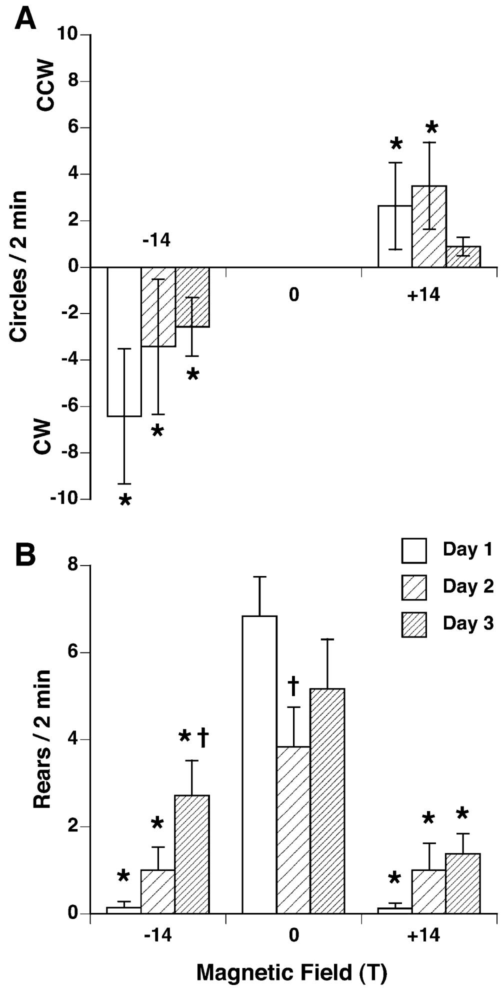

Fig. 7. Number (mean ± s.e.m.) of circles (A) or rears (B) exhibited by rats during 2 min in a locomotor test cage after 30-min exposure to either −14, 0, or +14 T magnetic fields on 3 consecutive conditioning days. (A) Counter-clockwise circles were observed in rats exposed to −14 T while clockwise circles were seen after +14 T exposure; $*p<0.05$ vs 0 T. (B) Compared to sham-exposed rats (0 T), rearing was suppressed in rats exposed to −14 or +14 T. $*p=0.05$ vs 0 T, $\dagger p<0.05$ vs day 1 of same group.

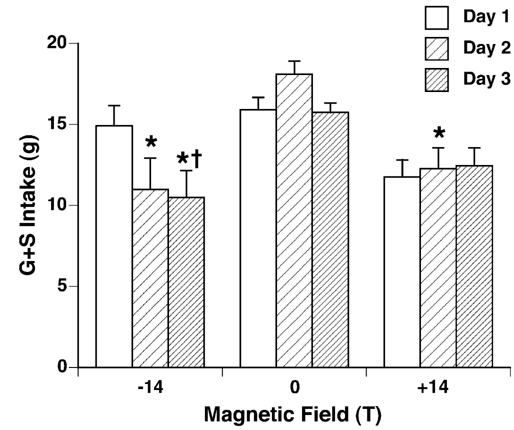

Fig. 8. Intake of G+S on 3 conditioning days prior to 30-min exposure to −14, 0, or +14 T. After one pairing, G+S intake decreased in rats exposed to −14 or +14 T, but not 0 T. $*p<0.05$ vs 0 T on same day; $\dagger p<0.05$ vs −14 T intake on day 1.

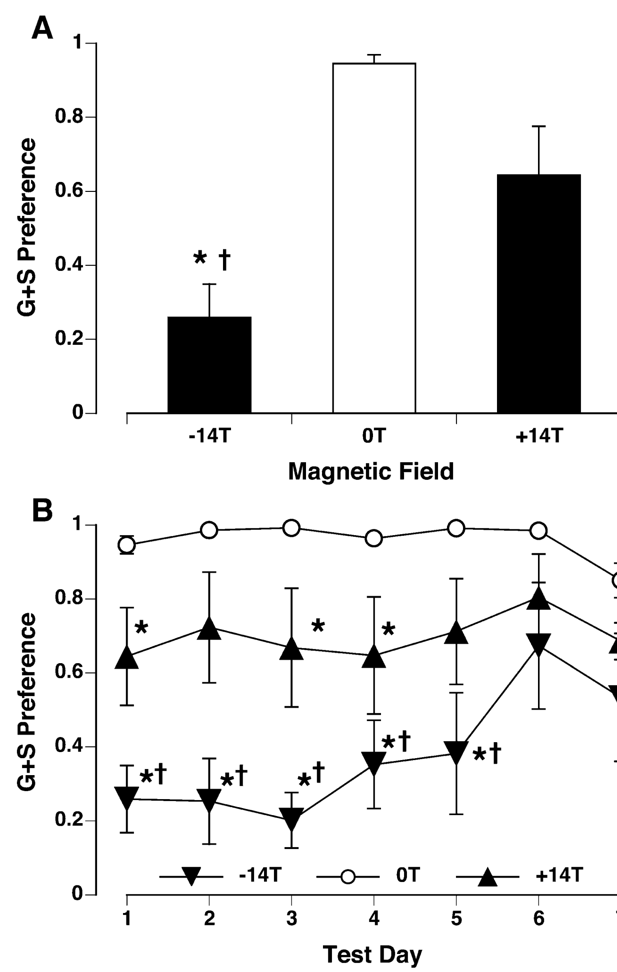

Fig. 9. (A) Expression of CTA against G+S after the first 2-bottle preference test. Preference for G+S was significantly reduced in rats after pairing with −14 T compared to 0 or 14 T exposure. $*p<0.05$ vs 0 T, $\dagger p<0.05$ vs +14 T. (B) Extinction of CTA against G+S induced by −14 or +14 T exposure. Two-way repeated measures ANOVA revealed that pairing G+S with +14 T induced an aversion that extinguished in 6 days, while pairing with −14 T exposure induced a stronger aversion that extinguished after 7 days. $*p<0.05$ vs 0 T; $\dagger p<0.05$ vs +14 T.

interaction of magnet exposure and test day ($F[2,12]=2.47$, $p<0.01$), such that the rats exposed to −14 and +14 T showed significantly lower preference than sham-exposed rats on almost all days until day 6. Furthermore, the rats exposed to −14 T also showed significantly lower preference than 14 T-exposed rats until day 6 (see Fig. 9B).

3.7. Experiment 4: Exposure perpendicular to magnetic field

In prior studies, we exposed animals positioned vertically within superconducting NMR magnets with ~90-mm diameter bores, such that their rostral–caudal axis was perpendicular to the ground and parallel to the magnetic field. The 189 mm bore of the 20 T resistive magnet was large enough to allow rats to be positioned horizontally in

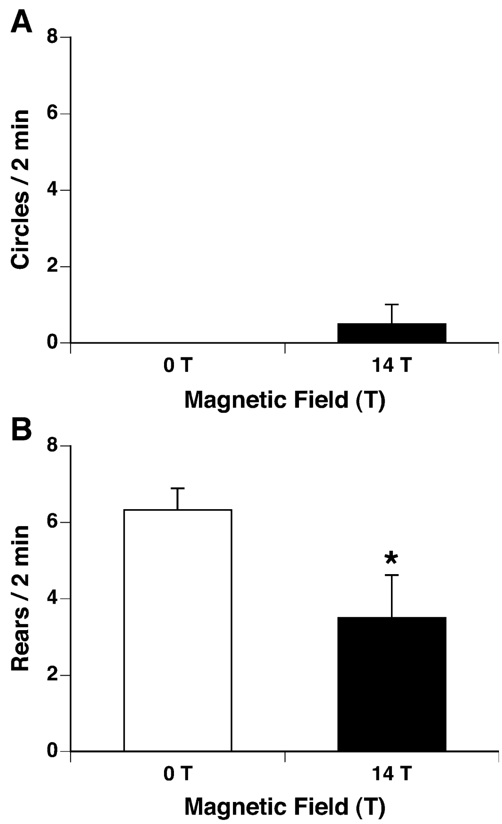

Fig. 10. Number (mean ± s.e.m.) of circles (A) or rears (B) exhibited by rats during 2 min after 30-min exposure while horizontal and perpendicular to a 14 T magnetic field or sham exposure (0 T). (A) Although two rats circled after 14 T exposure, there was no significant difference in circling between groups. (B) Perpendicular exposure to 14 T did significantly reduce rearing compared to sham exposure. $*p < 0.05$ vs 0 T.

the magnet (i.e. with their rostral–caudal axis parallel to the ground and perpendicular to the magnetic field). This allowed us to test the effects of applying magnetic fields parallel to the dorsal–ventral axis.

Male Sprague–Dawley rats ($n = 12$) were placed on a water restriction schedule as above. On the day of conditioning, rats were moved to the room containing the 20 T magnet. The magnetic field was ramped up to 0 or 14 T. In groups of 2, rats were given access to G+S for 10 min.

In order to restrain rats in a horizontal position in the resistive magnet, the rat was restrained in one of our standard restraining tubes. Two restraint tubes with rats were fixed in a horizontal orientation at the top of a pedestal 1.3 m in height. The rats and pedestal were lowered into the magnet until the pedestal rested on the floor, thus positioning the rats in the core of the magnet with the magnetic field lines running parallel to their dorsal–ventral axis. Rats were exposed at the center of the magnetic field for 30 min ($n = 6$ at 0 or 14 T).

Rats were then removed from the magnet, released into the locomotor test cage, and videotaped for 2 min. Rats were returned to their home cages, transported back to the NHMFL animal facility, and given ad lib access to water. On the next day, two-bottle 24-h preference tests were begun to measure CTA strength and extinction. Preference tests continued for 5 days.

3.8. Results of Experiment 4

3.8.1. Circling and rearing

Only 1 rat circled after magnetic field exposure, and no sham-exposed rats circled; there was no significant difference between the two groups (see Fig. 10A). While all rats reared, however, the number of rears was significantly reduced after 14 T exposure ($p < 0.05$; see Fig. 10B).

There was no difference in G+S intake between groups on conditioning day (mean intake: 12.2 g). On the first day of 2-bottle preference testing, t-test showed no significant difference in G+S preference between sham-exposed and magnet-exposed rats ($p = 0.07$; see Fig. 11A). Two-way ANOVA of magnet exposure and test days showed no significant effect across the remaining 2-bottle tests as well (see Fig. 11B). Half the rats in the magnet-exposed group, however, had a preference below 0.5 (and an average saccharin intake of 3±2 ml) for the first two days of preference testing, while all the sham-exposed rats showed preferences higher than 0.5 and an average saccharin intake of 98±20 ml. Therefore, exposure to a 14 T magnetic field

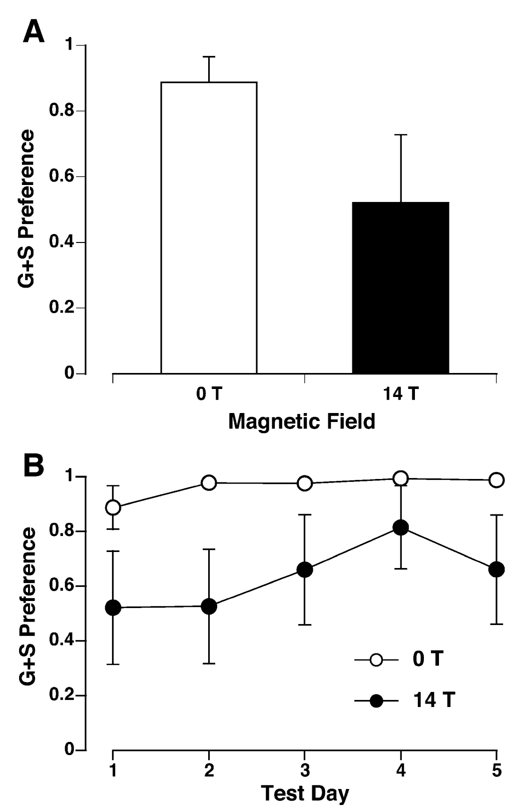

Fig. 11. (A) G+S in the first 2-bottle preference test after pairing with horizontal exposure to 14 T or sham exposure. Although half the rats exposed to 14 T had a G+S preference of 0.5 or less, there was no significant mean difference from the sham-exposed group ($p = 0.07$). (B) Across 5 days of 2-bottle preference tests, no significant difference was seen in G+S preference between 0 and 14 T groups.

parallel to the dorsal–ventral axis may be just at threshold for observing effects on CTA.

4. Discussion

Earlier studies demonstrated that exposure to static magnetic fields within 7, 9.4, and 14.1 T superconducting NMR magnets had behavioral effects on rats, such that the rats walked in circles, their rearing was suppressed, and a conditioned taste aversion was acquired [5,6,8]. In this study we have confirmed and extended the stimulus–response curve from 0 through 19.4 T using a single, variable strength resistive electromagnet DRC by testing 4 to 19.4 T. Behavioral effects were seen at field strengths as low as 4 T (suppression of rearing), although locomotor circling and conditioned taste aversion were only observed at or above 7 and 9 T, respectively.

Previous experiments on the effects of different field strengths required using different magnets in different locations; sham exposure to 0 T used merely a length of PVC pipe [5,6,8]. In the present study, all treatments at all field strengths (including sham 0 T exposure) were conducted within the bore of the same physical magnet. Importantly, this allowed the non-magnetic features of the magnet, such as odor, light, sound, etc. to be controlled and constant across treatments. Because significant effects on rearing, circling and taste aversion were found only when the magnet was energized, we conclude that the magnet's magnetic field, and not its non-magnetic features, are responsible for its effects.

The ability of a resistive magnet to produce variable strength magnetic fields allowed rats to be exposed to relatively low magnetic fields. A significant behavioral effect on rearing was found after 30-min exposure to 4 T. Rearing is also suppressed in rats after off-center rotation [10], so this finding is consistent with a potential stimulation of the vestibular system by magnetic fields as low as 4 T. Furthermore, this result is of clinical relevance, as 4 T MRI machines are commonly used for functional MRI studies. Indeed, a survey of magnet workers found a significant incidence of dizziness and nausea during the testing of a clinical 4 T MRI magnet [4].

Aside from the suppression of rearing, no other behavioral effect of 4 T was observed. Exposure to 4 T did not cause locomotor circling, and a single pairing of G+S with 4 T was not sufficient to induce a conditioned taste aversion. Because some weak aversive stimuli require multiple exposures to produce a measurable CTA, rats were given 3 pairings of G+S with 4 T to determine if 4 T was only weakly aversive. No CTA was observed even after 3 pairings with 4 T. Although no CTA was observed after 1 pairing with 7 T, we have previously observed CTA after 1 or 3 pairings of G+S with 7 T. We conclude, therefore, that the threshold for circling and for aversive effects sufficient to produce a CTA lies between 4 and 7 T.

At the upper end of magnetic field strengths, a decreased response to 19.4 T exposure was observed relative to the peak response at 14 T. Compared to other exposures, rats were often immobile after exposure to 19.4 T, with a long latency prior to circling. This suggests an inverted-U shape for the stimulus–response curve, such that the effects of extremely high magnetic fields become debilitating and interfere with locomotion and CTA acquisition. Indeed, it has been suggested that within static fields of 25 T or higher, the movement of the blood would be able to induce electrical currents within surrounding tissue sufficient to non-specifically stimulate nerves or muscle [11]. Thus, the response to 19.4 T might reflect the influence of non-specific effects.

An intriguing effect of high magnetic fields is the transient induction of locomotor circling. Previously we had found that rats exposed parallel to the magnetic field with their heads up and towards the positive pole (+14 T) turned counter-clockwise, while rats confined with heads down and facing the negative pole (−14 T) turned clockwise. Because they were exposed in a superconducting NMR magnet with a fixed field orientation, the rats had to be inverted to a heads-down position to face the negative pole. Thus orientation within the field was confounded with the rat's physical orientation. In this study, we confirmed that the direction of circling depends on the orientation of the rat relative to the magnetic field. In the resistive magnet, the orientation of the field relative to the rat was reversed simply by reversing the direction of the applied current. Rats exposed with heads up towards +14 T subsequently walked in counter-clockwise circles, and rats exposed with heads up towards −14 T walked in clockwise circles.

The direction of circling relative to magnetic field orientation has remained absolutely consistent in all our experiments in rats as well as in mice [12]. The reasons for this asymmetry in the direction of magnetically induced circling are unknown. It might reflect asymmetries in 1) the magnetic field interaction with the rat, 2) the vestibular apparatus of the rat or 3) the locomotor circuitry of the rat (e.g., the basal ganglia).

Aside from the direction of locomotor circling, the effects of +14 and −14 T exposure on rearing and CTA were qualitatively similar. Rearing was suppressed acutely after the first exposure, and CTA was acquired after 1 or 3 pairings with G+S. Exposure to −14 T appeared to induce a stronger CTA than +14 T. Whether this was due to experimental variability or points to a quantitative asymmetry in the rats' response to field orientation remains to be determined.

The large 189 mm bore of the resistive magnet also allowed us to experiment with the rat's orientation relative to the magnetic field lines. Earlier studies, constrained by small bore size of NMR magnets, exposed the rats with their rostral–caudal axis parallel to the magnetic field lines running vertically within the bore. There is no a priori reason to believe, however, that this configuration results in a maximal effect of the magnet. In fact, when rats were exposed with their rostral–caudal axis perpendicular to the magnetic field, the effects of the magnet were attenuated compared to exposure parallel to the magnetic field. This suggests that the effects of the field are dependent on the orientation of the rat; it may be worthwhile to probe the effects of exposure at intermediate angles of orientation. Given the three-dimensional structure of the vestibular apparatus of the inner ear, it is possible that particular semicircular canals or a specific otolith organ is more or less stimulated in a particular head orientation.

In conclusion, these studies further characterize the parameters under which magnetic fields have behavioral effects on rodents. They confirm our earlier findings with superconducting NMR magnets under the more controlled conditions afforded by a resistive electromagnet. Furthermore, by demonstrating a behavioral effect after 4 T exposure, we extend our findings to a magnetic field strength that is clinically relevant. It is worth noting, however, that experimental MRI machines with field strengths up to 8 T are in use with humans, with anecdotal reports of induced vertigo [2].

The effects of exposure to high magnetic fields are consistent with stimulation of the vestibular apparatus. Rearing is suppressed after both magnetic field exposure and whole-body rotation [10]. Rats exhibit tight locomotor circling, similar to the circling seen in rats with unilateral vestibular deficits [13]. Both rotation and magnetic field exposure can induce conditioned taste aversions when paired with a palatable solution [6,14,15]. In addition, we have demonstrated that magnetic field exposure induces c-Fos expression in the vestibular relays of the rat brainstem comparable to the induction of c-Fos by rotation [8,16]. The mechanism by which the magnetic field interacts with the vestibular system is unknown. Preliminary experiments with labryinthecomized rats, however, indicate that the inner ear is essential for all of the observed effects of high strength magnetic fields [7].

Acknowledgments

Supported by NIDCD 04607 and an FSU Research Foundation Program Enhancement grant. We thank Dr. Bruce Brandt and staff of the US National High Magnetic Field Laboratory for coordinating access to the magnets.

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