Behavioral Effects of High-Strength Static Magnetic Fields on Rats

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Advances in magnetic resonance imaging are driving the development of more powerful and higher-resolution machines with high-strength static magnetic fields. The behavioral effects of high-strength magnetic fields are largely uncharacterized, although restraint within a 9.4 T magnetic field is sufficient to induce a conditioned taste aversion (CTA) and induce brainstem expression of c-Fos in rats. To determine whether the behavioral effects of static magnetic fields are dependent on field strength, duration of exposure, and orientation with the field, rats were restrained within the bore of 7 or 14 T superconducting magnets for variable durations. Behavioral effects were assessed by scoring locomotor activity after release from the magnetic field and measuring CTA acquisition after pairing intake of a palatable glucose and saccharin (G+S) solution with magnetic field exposure. Magnetic field exposure at either 7 or 14 T suppressed rearing and induced tight circling. The direction of the circling was dependent on the rat's orientation within the magnetic field: if exposed head-up, rats circled counterclockwise; if exposed head-down, rats circled clockwise. CTA was induced after three pairings of taste and 30 min of 7 T exposure or after a single pairing of G+S and 1 min of 14 T exposure. These results suggest that magnetic field exposure has graded effects on rat behavior. We hypothesize that restraint with high-strength magnetic fields causes vestibular stimulation resulting in locomotor circling and CTA acquisition.

Key words: **conditioned taste aversion; vestibular; circling; rearing; magnetic resonance imaging; magnet**

Introduction

Advances in magnetic resonance imaging (MRI) are driving the development of more powerful and higher-resolution MRI machines. Although MRI machines with static magnetic fields of 1–2 T and resolutions of 2 mm^3 are standard in clinical use, higher resolution requires stronger magnetic fields of 4–9 T (Narasimhan and Jacobs, 1996).

The effects of high-strength static magnetic fields on mammals are largely unknown, and there is no evidence of long-term toxic effects of magnetic field-exposure in humans and rats. In humans, no acute aversive effects or sensations have been reported after exposure to magnetic fields of ≤1.5 T (Schenck et al., 1992; Winther et al., 1999). In rats, standard MRI protocols conducted from 0.15 to 1.89 T have been reported to have no effect on a variety of behavioral tasks (Innis et al., 1986; Ossenkopp et al., 1986; Messmer et al., 1987).

At higher field strengths, however, there have been reports of vertigo and nausea in workers around large magnets, for example, in a safety study of an early 4 T MRI machine (Schenck et al., 1992). Likewise, acute behavioral and neural effects on rats become apparent at higher field strengths (Weiss et al., 1992). Our laboratories have shown that a 30 min restraint of a rat within a 9.4 T superconducting magnet can induce circling locomotor activity (Snyder et al., 2000), conditioned taste aversion (CTA) (Nolte et al., 1998), and c-Fos expression (Snyder et al., 2000). CTA is a form of associative learning in which an animal learns to avoid the taste of a food previously paired with a toxic postingestive effect or nausea-inducing stimulus such as rotation. After intake of a highly palatable glucose plus saccharin solution (G+S) was paired one to three times with restraint within the 9.4 T magnetic field, rats decreased their intake of G+S relative to water for several days (Nolte et al., 1998). Because the magnetic field induced a CTA, it may have a visceral or vestibular effect that the rat can associate with the novel taste of G+S. Consistent with this hypothesis, 30 min of 9.4 T exposure induced significant c-Fos immunoreactivity in visceral and vestibular nuclei of the brainstem (Snyder et al., 2000).

Here we extend the previous behavioral results by using 7 and 14 T magnets to quantify the effects of different magnetic field strengths on circling behavior and CTA acquisition and to determine the thresholds of magnetic field intensity and duration of exposure sufficient to induce behavioral effects. The magnetic field exposure paired with the taste varied in four ways: field strength (7 vs 14 T), number of pairings (one vs three), duration of exposure (0–30 min), and orientation of the rat (head-up vs head-down within the magnetic field). To compare the results of the current study with our previously published data, we attempted to replicate as closely as possible the procedures used previously (Nolte et al., 1998) and included the data from the previous 9.4 T experiment in our analysis for comparison.

Materials and Methods

Subjects. Male Sprague Dawley rats (175–200 gm; Charles River Laboratories, Wilmington, MA) 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 rats had a 12 hr light/dark cycle with lights on at 8:00 A.M. All conditioning trials were

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conducted during the light cycle. The rats had access to pelleted Purina (St. Louis, MO) Rat Chow 5001 and deionized–distilled water *ad libitum* except where specified otherwise. Four days before the conditioning day, the rats were placed on a water-deprivation schedule under which they received daily water access in one drinking session. The initial session was 1 hr, and the session times were diminished each day so that the day before conditioning the rats received their water in a 10 min session.

Conditioning procedure. The procedure described previously (Nolte et al., 1998) was replicated in the present experiments. The conditioned stimulus (CS) was a solution of 30 gm of glucose and 1.25 gm of sodium saccharin mixed in 1 l of deionized–distilled water (G+S). The rats were allowed 10 min for access to the G+S. The unconditioned stimulus (US), which followed immediately after the CS, was a 30 min exposure in one of the two superconducting magnets. To expose the rats in the vertical bores of the magnets, rats were placed individually in 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. Individual restrained rats were carried from the animal facility to the superconducting magnets (~100 m distance). The restrained rat was inserted into the bottom of the vertical bore of the magnet and raised until the head of the rat was in the center of the magnetic field. Rats remained in the bore of the magnet for 1–30 min.

To control restraint and handling, sham-exposed rats were allowed 10 min to access G+S and were then inserted in identical restraining tubes. The sham-exposed rats were vertically inserted into an opaque polyvinylchloride (PVC) pipe with dimensions and conditions (sound, light, and temperature) similar to those of the magnet bore. One magnet-exposed rat and one sham-exposed rat were conditioned in parallel time.

Magnets. The magnet exposures were done in one of two superconducting magnets with vertical bores designed for biochemical nuclear magnetic resonance (NMR) studies. The 7 T magnet was an Oxford Instruments (Concord, MA) D 15,000/19/19 300 MHz magnet with a fixed field strength of 7 T and a 89 mm bore. The 14 T magnet was a 600 MHz Bruker Cryo magnet with an 89 mm bore and a fixed field strength of 14.1 T. Both magnets contained shim magnets extending along the magnet bore for approximately ±15 cm from the magnet core, which were used to stabilize the magnetic field and give a central core field of uniform strength. The magnetic fields in both magnets were orientated vertically so that the positive pole was at the top of the magnet. The magnets were operated without radiofrequency pulses, so rats were exposed to only static magnetic fields.

Locomotor activity. At the conclusion of magnet or sham exposure, each rat was carried back to the animal facility while still restrained. The rostral plug of the restraining tube was removed, and the rat was allowed to emerge from the tube into an open Plexiglas cage (37 cm wide × 47 cm long × 20 cm high). The floor of this open-field cage was covered 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 <1 min; only one rat in the present study showed an effect for >2 min. Thus, 2 min of recording captured most of the phenomenon of interest.) The rat was then returned to its home cage. 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 a diameter less than length of the rats body (i.e., with nose almost touching the end of the tail) Partial circles or circles interrupted by stationary pauses were not counted.

Preference tests. To test for magnetic field-induced CTA, a series of 24 hr, two bottle preference tests was initiated on the day after the last conditioning trial. Two bottles were placed on the cages, one containing the G+S solution and the other containing distilled water. Fluid consumption was measured every 24 hr and a preference score was calculated as a ratio of G+S solution to total fluid consumption.

The preference tests were continued for 5–9 postconditioning days. Because G+S access during the preference tests was not paired with magnet exposure, the preference tests constituted extinction trials. A CTA was considered extinguished when the average G+S preference of magnet-exposed rats was not different from the average preference of sham-exposed rats.

In summary, measurements were made of four dependent variables: (1) visual scoring of the locomotor behavior of the rats immediately after the first magnet or sham exposure, as a test of the immediate effects on activity; (2) consumption of the G+S solution during the 10 min CS period on the second and third conditioning days as a one bottle test of CTA during acquisition (experiment 2); (3) preference score for G+S versus water on the first day of postconditioning preference testing as a two bottle test of CTA expression; and (4) the number of days of preference testing required for extinction of the CTA.

Statistical analysis. Significant effects were determined using one- or two-way ANOVA. *Post hoc* comparisons were made using Fisher's least significant difference (FLSD) test, *t* test, or orthogonal comparison as indicated. As binomial variables, the significant presence or absence of circling and rearing was tested using the χ^2 test.

Experiment 1: single pairing of G+S and magnet exposure. A total of 65 rats were housed and placed on the water-deprivation schedule as described above. On the first conditioning day, all rats had access to the G+S solution for 10 min. Immediately after this drinking period, rats were placed in the restraining tubes and individually inserted head-up into either the core of the 7 T magnet for 30 min (experiment 1a; $n = 9$) or the core of the 14 T magnet for a varying duration of exposure (experiment 1b). One group of rats ($n = 6$) was inserted and immediately removed from the core of the 14 T magnet and thus received minimal exposure to the magnetic field (0 min). Additional rats were exposed to the 14 T magnetic field for 1, 5, 10, 20, or 30 min ($n = 6$–14/group). Control, sham-exposed rats were inserted into the sham PVC tube for 30 min ($n = 5$).

After being removed from the magnet (or sham magnet), the rostral plug was removed from the restraining tube and the rat was allowed to emerge into the open-field testing chamber. Locomotor behavior was recorded on videotape for 2 min. The rats were then returned to their home cage and given *ad libitum* access to water. On the following day, the first 24 hr, two bottle preference test between G+S and water began. The 24 hr preference tests were continued for 7–9 d.

Experiment 2: three pairings of G+S and magnet exposure. A total of 35 rats were housed and placed on the water-deprivation schedule as described above. On the first conditioning day, all of the rats had access to the G+S solution for 10 min. Immediately after this drinking period, half of the rats were inserted into either the 7 T magnet ($n = 8$) or the 14 T magnet ($n = 10$) and remained at the magnet core for 30 min. The remaining rats were inserted into the sham PVC tube for 30 min. After being removed from the magnet (or sham magnet), the rostral plug was removed from the exposure chamber and the rat was allowed to emerge from the restraining chamber into the open-field testing chamber. Locomotor behavior was recorded on videotape for 2 min. The rats were then returned to their home cage and remained on water deprivation until the following day, when the second conditioning trial was given. This procedure was repeated for a third day of conditioning. After the third pairing of G+S and magnet (or sham) exposure, the locomotion behavior was measured and the animals were returned to their home cages and given *ad libitum* access to water. On the following day, the first 24 hr, two bottle preference test between G+S and water began. These 24 hr preference tests continued for 10 d.

Experiment 3: orientation within the magnet. In the course of experiments 1 and 2, it was found that rats exposed to the14 T magnetic field for 30 min reliably exhibited circling behavior when released from restraint, whereas approximately half of the rats exposed to the 7 T magnet or exposed for shorter periods in the 14 T magnet exhibited circling behavior. If a rat did circle, however, the rotation of the rat was always in the counterclockwise direction. All of these rats had been placed in the vertical bore of the magnets head-up, facing the positive pole of the magnetic

Table 1. Effects of magnetic field exposure on locomotor activity

Field	Duration	Number of rats	% Circling	Mean circles	% Rearing	Mean rears
Experiments 1a and 2						
0 T	30 min	22	5	1	91	3.8 ± 0.5
7 T	30 min	17	47	2.4 ± 0.6*	59	2.8 ± 0.9*
14 T	30 min	17	71	2.6 ± 0.4*	0	0*†
Experiment 1b						
14 T	0 min	6	0	0 ± 0	100	4.8 ± 0.8
14 T	1 min	6	0	0 ± 0	100	1.8 ± 0.7*
14 T	5 min	8	50	5.0 ± 2.5	67	2.3 ± 0.5*
14 T	10 min	14	57	2.9 ± 0.7	43	2.2 ± 0.5*
14 T	20 min	8	63	2.8 ± 0.5	13	1*
14 T	30 min	17	71	2.6 ± 0.4	0	0*
Experiment 3						
0 T Down	30 min	12	0	0	83	4.9 ± 1.1
14 T Down	30 min	15	73	6.1 ± 2.7	33	2.0 ± 0.3*
14 T Up	30 min	13	77	8.4 ± 2.5	54	1.6 ± 0.3*

Locomotor activity of rats after magnetic field exposure was scored for tight circling and rearing by a blind observer from videotape records. Rats were sham exposed (0 T) or exposed to 7 or 14 T magnetic fields for 0–30 min. Shown are the number of rats tested, the percentage of rats displaying circling or rearing, and the mean ± SEM number of circles or rears counted for those rats that showed any circling or rearing (i.e. non-zero means). In experiments 1a and 2, rats were exposed head-up to a 7 or 14 T magnetic field for 30 min; both exposures induced circling and suppressed rearing. In experiment 1b, rats were exposed head-up to 14 T for 0–30 min; significant circling was induced by ≥5 min of exposure, and rearing was significantly suppressed after ≥10 min of exposure. In experiment 3, rats were restrained in either the head-up or head-down orientation within a 14 T magnetic field for 30 min. Circling was induced and rearing was suppressed in both groups, but the direction of circling depended on the rats' orientation within the field (Fig. 1).

field. The purpose of this experiment was to test the hypothesis that the circling behavior was determined by the rat's orientation within the magnet.

Acute behavioral effects. Eighteen male rats were housed as described above but allowed *ad libitum* access to water. Four rats were exposed within the vertical bore of the 14 T magnet head-up; eight were exposed head-down, pointing toward the negative pole of the magnet. The other six rats were sham-exposed head-down in the vertical PVC tube. Locomotor activity in the open-field test was recorded immediately after exposure.

CTA expression. To compare the magnitudes of CTAs induced by head-up or head-down exposure to the 14 T magnet, an additional 24 rats were housed and maintained on a water deprivation schedule as above. On conditioning day, rats were allowed 10 min of access to G+S and then restrained and exposed within the vertical bore of the magnet in either the head-up orientation ($n = 6$) or the head-down orientation ($n = 8$) for 30 min. The remaining rats were sham-exposed head-down for 30 min. Locomotor activity in the open-field test was recorded immediately after exposure. The rats were then returned to their home cage, and their water bottle was returned. On the following day, the first two bottle preference test between G+S and water was initiated. These 24 hr preference tests were continued for 10 d.

Results

Locomotor effects

The effects of exposure to magnetic fields on the locomotor behaviors of tight circling and rearing are summarized in Table 1.

Experiments 1a and 2: exposure to 7 or 14 T magnetic field for 30 min

Open-field locomotor activity was scored for tight circling and rearing after the first 30 min exposure to 0 (sham), 7, or 14 T in experiments 1 and 2. Significantly more magnet-exposed rats exhibited circling locomotor behavior; only one of the sham-exposed rats exhibited any circling behavior (χ^2 test; see Table 1). All rats that circled moved in a counterclockwise direction (Fig. 1*A*). Rearing was also significantly reduced in magnet-exposed rats (χ^2 test).

Experiment 1b: duration of exposure to 14 T magnetic field

Locomotor activity was scored after 0–30 min restraint within the 14 T magnet. There was a significant effect of the duration of exposure to the 14 T magnetic field on the number of rats circling

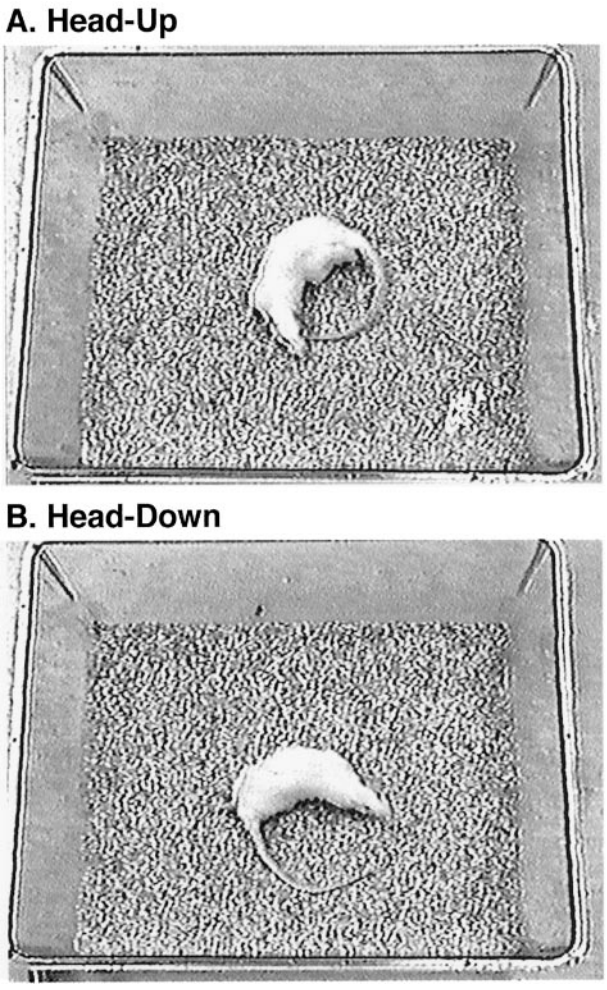

Figure 1. Tight-circling activity induced by magnetic field exposure. Rats were restrained for 30 min within the bore of a 14 T magnet in either head-up orientation (*A*) or head-down orientation (*B*). On release from restraint, rats oriented head-up circled counterclockwise, whereas rats oriented head-down circled clockwise.

and rearing (as determined by χ^2 test). Counterclockwise circling was induced by exposures of ≥5 min; rearing was significantly reduced after only 1 min of exposure.

Experiment 3: orientation within the magnetic field

Magnetic field exposure significantly induced circling in rats regardless of orientation (χ^2 test), but the direction of rotation after 14 T exposure was dependent on the orientation of rats within the magnetic field. Most 14 T magnet-exposed rats showed circling: circling rats exposed head-up turned exclusively counterclockwise (Fig. 1*A*); circling rats exposed head-down turned exclusively clockwise (Fig. 1*B*). Sham-exposed rats did not circle. There was some rearing behavior in all three groups, but the average number of rears was significantly decreased in the two magnet-exposed groups compared with the sham-exposed group ($F_{(2,37)} = 3.8$; $p < 0.05$).

Conditioned taste aversion effects

Experiment 1a: single pairing of G+S with 7, 9.4, or 14 T

Rats were given a single pairing of G+S intake with 30 min of exposure to 0 (sham), 7, or 14 T (rats from the 30 min group of experiment 1b). For purposes of comparison, data collected in the previous study after a single pairing of G+S with 30 min of exposure to 9.4 T were also included in the analysis (Nolte et al., 1998). Magnetic field strength had a significant effect on G+S preference on the first test day after pairing ($F_{(3,25)} = 3.0$; $p < 0.05$) (Fig. 2*A*). *Post hoc* tests showed that rats exposed to14 T after G+S intake had a significantly lower preference for G+S compared with sham-exposed rats, but the preferences of rats exposed to 7 or 9.4 T were not significantly different from either sham-exposed rats or 14 T-exposed rats. Therefore, a single pairing of G+S with a 30 min exposure to 14 T was sufficient to induce a CTA to G+S, but a single pairing with 7 or 9.4 T was at or below threshold for inducing a CTA. The G+S preference of rats exposed to 14 T was not significantly different from sham-exposed rats by the third testing day (Fig. 2*B*).

Experiment 1b: duration of exposure to magnetic field

Rats were given a single pairing of G+S intake with 0–30 min of exposure to the 14 T magnetic field. On the first day of two bottle testing, there was a significant effect of duration of exposure on G+S preference ($F_{(5,42)} = 4.97$; $p < 0.005$) (Fig. 3*A*). *Post hoc* analysis (FLSD) showed that rats that received ≥1 min of exposure had a significantly lower preference for G+S compared with rats that received no exposure (p values of <0.01). With subsequent 24 hr, two bottle tests of G+S preference, there was a significant interaction of extinction day and duration of exposure ($F_{(5,30)} = 1.67$; $p < 0.05$) (Fig. 3*B*). Thus, a single pairing of G+S with ≥1 min of exposure to 14 T was sufficient to induce a CTA.

Experiment 2: three pairings of G+S with 7 or 14 T

Rats were given three pairings of G+S intake with 30 min of exposure to 0 (sham), 7, or 14 T. For purposes of comparison, data collected in the previous study after three pairings of G+S with 30 min of exposure to 9.4 T were included in the analysis (Nolte et al., 1998).

Intake during conditioning

Because rats had only 10 min access to fluid during the 3 conditioning days, differences in intake were not large during conditioning. Some significant decreases were detected by *t* test in some magnet-exposed groups, however. There was no significant difference in intake of G+S between 7 T-exposed and the sham-exposed rats on any of the 3 conditioning days (Fig. 4*A*). G+S intake was not significantly different between the 9.4 T-exposed and sham-exposed rats on the first and second conditioning days, but intake was significantly lower in 9.4 T-exposed rats on the third conditioning day (Fig. 4*B*). In contrast, the G+S intake was significantly lower in 14 T-exposed than sham-exposed rats on the second and third conditioning days (Fig. 4*C*).

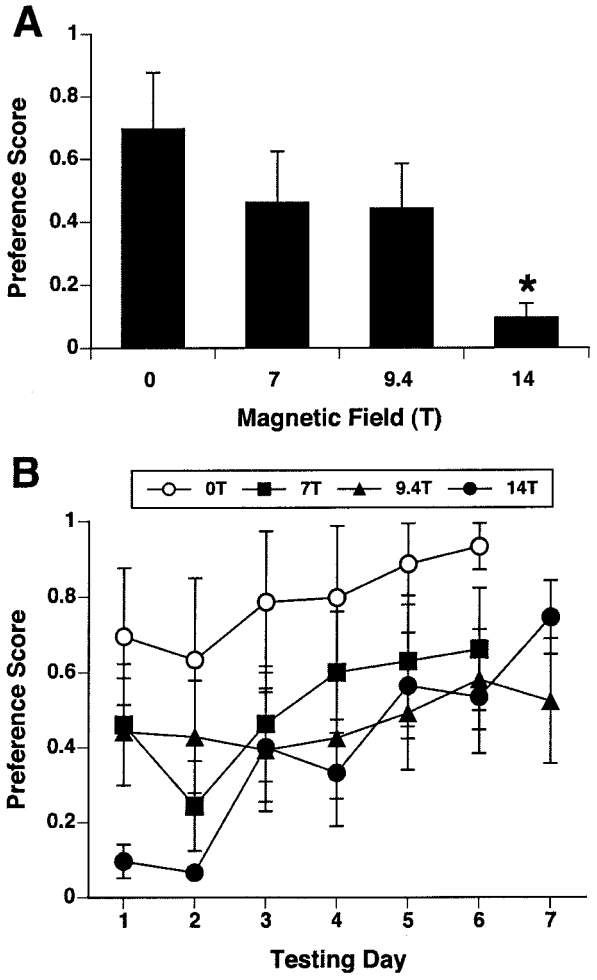

Figure 2. CTAs induced by a single pairing of G+S intake with a 30 min restraint within magnetic fields of different strengths. For the 24 hr two bottle preference test after the pairing of G+S with magnetic exposure (*A*), a significant CTA against G+S was observed only after pairing with 14 T exposure. The CTA extinguished after 3 d of two bottle preference tests (*B*). *$p < 0.05$ versus 0 T (sham) exposure. Data for 9.4 T exposure are replotted from Nolte et al. (1998).

First two bottle test after three pairings

Magnetic field strength had a significant effect on G+S preference on the first two bottle test day after pairing ($F_{(3,28)} = 9.4$; $p < 0.0005$) (Fig. 5*A*). A graded response was found by orthogonal comparison, such that G+S preference was significantly decreased compared with sham-exposed rats after three pairings with 7 T and significantly decreased compared with 7 T after pairings with 9.4 or 14 T.

Extinction after three pairings

Extinction of the magnet-induced CTAs across 9 d of two bottle preference tests was analyzed at each field strength by repeated, two-way ANOVAs with time and treatment as factors, and by *post hoc* analysis by FSLD. After three pairings at 7 T, an extinction

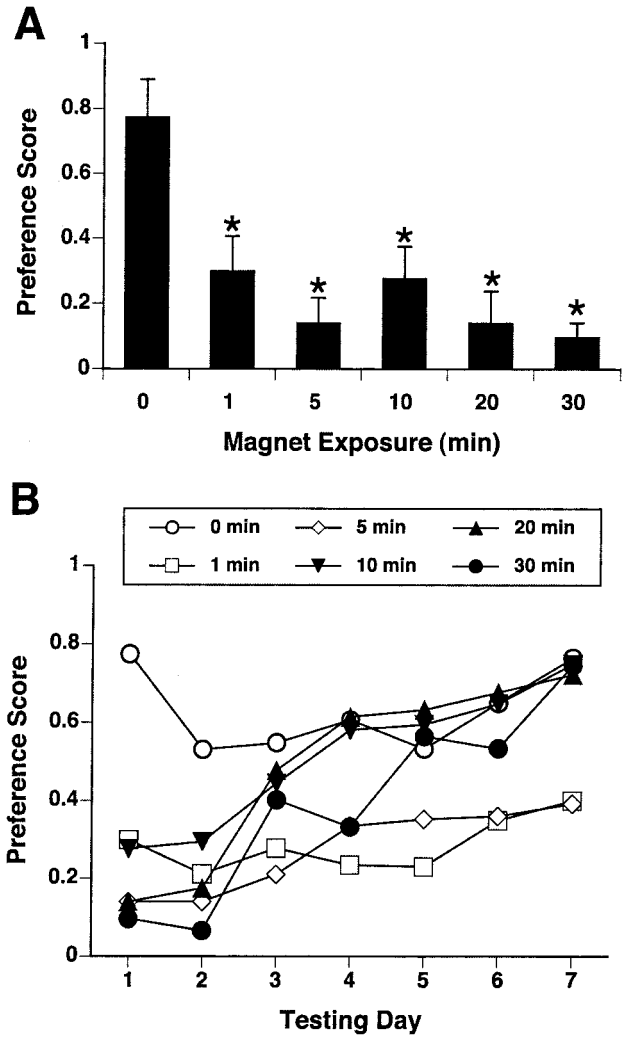

Figure 3. CTAs induced by a single pairing of G+S intake with restraint within 14 T magnetic fields for 0–30 min. Significant CTA was observed after ≥1 min of exposure to the magnetic field on the first 24 hr two bottle preference test (*A*); the CTAs persisted for several days of preference testing (*B*). *$p < 0.05$ versus 0 min exposure.

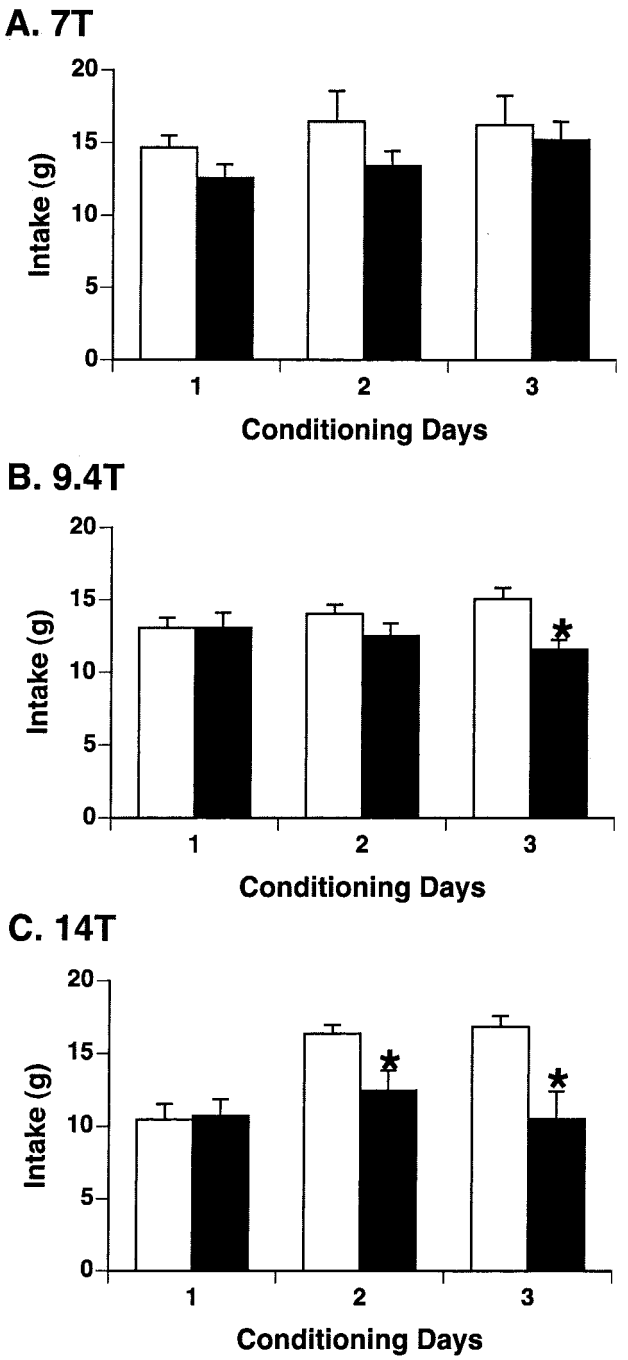

Figure 4. Acquisition of CTAs across three pairings of G+S with 30 min restraint within 7, 9.4, or 14 T magnetic fields. Intake during 10 min access to G+S was not different between sham- and 7 T-exposed rats across the 3 conditioning days (*A*). Rats exposed to 9.4 T decreased intake compared with sham-exposed rats on the third day of conditioning (i.e., after two pairings; *B*). Rats exposed to 14 T decreased intake on the second day of conditioning (i.e., after one pairing; *C*). *$p < 0.05$ versus sham-exposed rats. Data for 9.4 T exposure are replotted from Nolte et al. (1998).

effect was found ($F_{(8,112)} = 3.19$; $p < 0.005$) (Fig. 5*B*). G+S preference was significantly decreased in 7 T-exposed rats compared with sham-exposed rats on days 1 and 2.

After three pairings at 9.4 T, there was a significant interaction of extinction day and treatment ($F_{(8,112)} = 5.96$; $p < 0.001$) (Fig. 5*C*). G+S preference was significantly decreased in 9.4 T-exposed rats compared with sham-exposed rats for days 1–8, but not on day 9. Similarly, after three pairings at 14 T, there was a significant interaction of extinction and treatment ($F_{(8,112)} = 4.06$; $p < 0.001$) (Fig. 5*D*). G+S preference was significantly decreased in 14 T-exposed rats compared with sham-exposed rats for days 1–8, but not on day 9.

Experiment 3: orientation within magnetic field

Both groups of rats exposed to the 14 T magnet in head-up or head-down orientation after G+S access formed significant CTAs that extinguished by the third postconditioning test day ($F_{(5,90)} = 9.49$; $p < 0.001$) (Fig. 6). The G+S preferences were not different between the two magnet-exposed groups on the first day, but both magnet-exposed groups showed significantly lower G+S preference than head-down, sham-exposed rats. Thus, restraint within the 14 T magnetic field produced equivalent CTAs regardless of the rat's orientation within the magnet.

Discussion

In this study, exposure to high-strength static magnetic fields had acute effects on the locomotor behavior of rats, and a CTA was induced when a novel palatable solution was paired with exposure to the magnetic fields. Both 7 and 14 T magnets induced

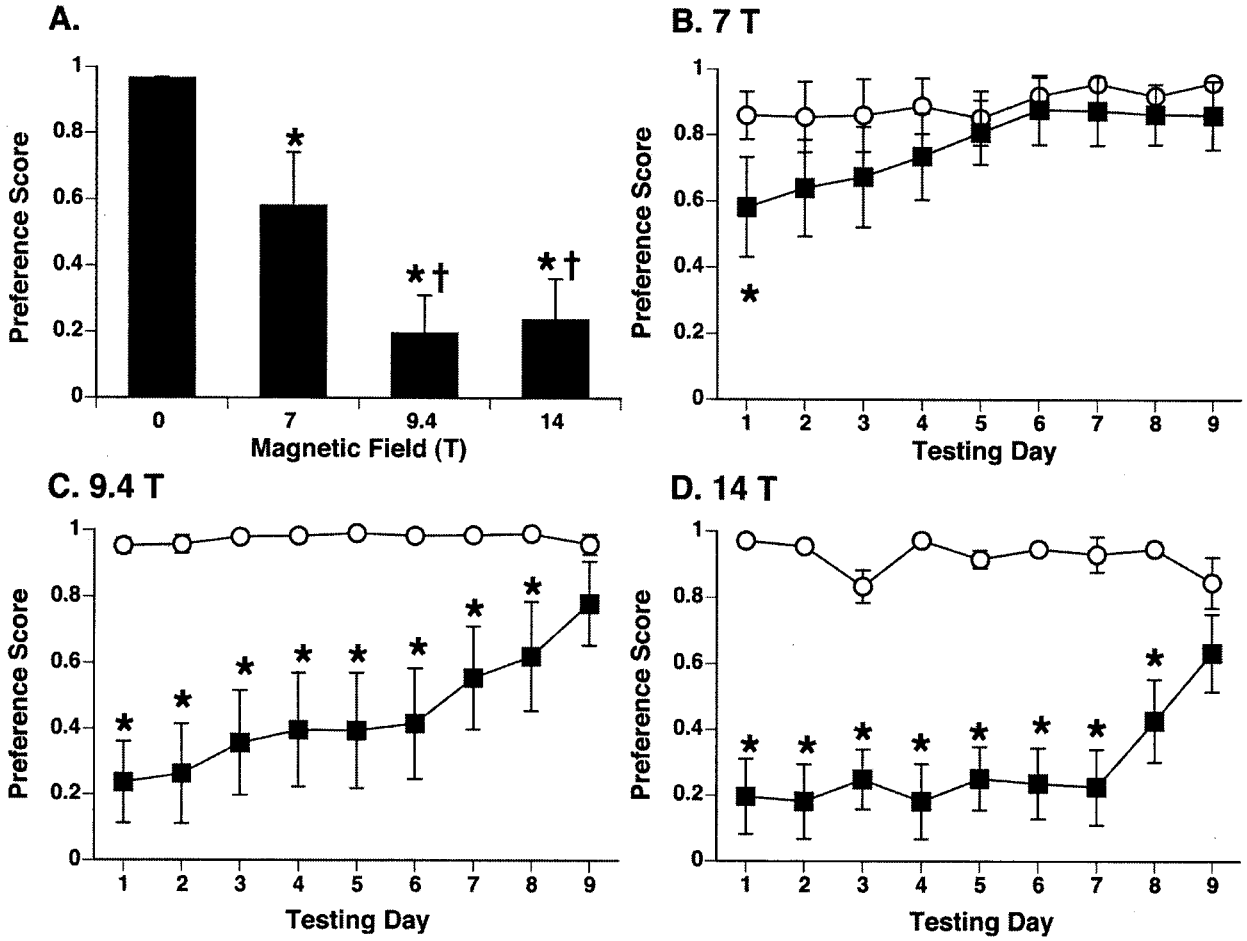

Figure 5. CTAs induced by three pairings of G+S intake with restraint within 7, 9.4, or 14 T magnetic fields for 0–30 min. Significant CTAs were observed in all magnet-exposed rats on the first 24 hr two bottle preference test (*A*). Over subsequent 24 hr two bottle test days, the CTA of 7 T-exposed rats extinguished on the second day (*B*), whereas the CTAs of 9.4 T- and 14 T-exposed rats persisted for 8 d of preference testing (*C*, *D*). $^{*}p < 0.05$ versus sham-exposed rats; $^{\dagger}p < 0.05$ versus 7 T-exposed rats. Data for 9.4 T exposure are replotted from Nolte et al. (1998).

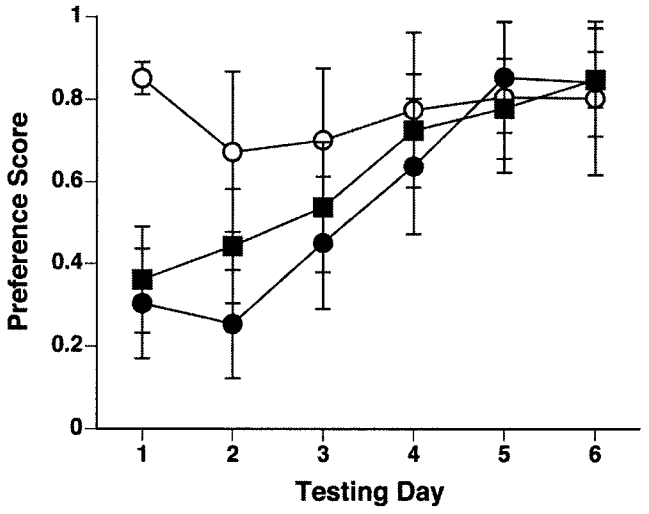

Figure 6. CTAs induced by a single pairing of G+S with 14 T restraint in head-up or head-down orientations. After 10 min of access to G+S, rats were restrained in either a head-up orientation (*black squares*) or head-down orientation (*black circles*) for 30 min within the 14 T magnetic field. Although rats circled in opposite directions on release from restraint (with head-up rats circling counterclockwise and head-down rats circling clockwise), there was little difference in the CTA acquisition of the two groups. Control rats were sham-exposed while head-down for 30 min (*white circles*).

tight-circling locomotor activity and suppressed rearing, and both magnets induced CTA. The direction of circling (but not the magnitude of CTA) was dependent on the rat's orientation within the field. By quantifying both locomotor activity and CTA expression, and including data from a previous report (Nolte et al., 1998), a graded response to field strength was found with rank ordering of 14 > 9.4 > 7 T. Furthermore, the threshold of the magnetic field exposure sufficient for CTA acquisition after one pairing with G+S was found to be close to one pairing of 30 min at 7 or 9.4 T, and between one pairing of 0 and 1 min at 14 T. Thus the behavioral effects on rats of magnetic fields are graded and specific to field intensities and durations of exposure.

We have now observed circling and CTA acquisition on different days on three magnets from two different manufacturers. The replication of the qualitative effects of magnetic field exposure is significant: the behavioral effects appear to be caused by the presence of a high-strength magnetic field and not by an artifact of specific equipment or a specific test session. Similarly, the observation of graded effects is significant, because the three magnets have variable field strengths but are otherwise similar in terms of materials, circuitry, coolants, interior temperature, and noise, for example.

Locomotor effects

The magnetic fields induced circling and suppressed rearing proportional to field strength. These locomotor effects are compara-

ble with the circling seen after unilateral hemilabyrinthectomy (Kaufman et al., 1999) and the suppression of rearing seen after horizontal rotation (Ossenkopp et al., 1994). The tight circling seen after magnetic field exposure may also be an indirect result of activation of other motor systems. Unilateral lesions within the striatum, ascending dopaminergic pathways, cerebellum, or vestibular nuclei can induce spontaneous circling or susceptibility to circling after drug or vestibular stimulation (Shima, 1984). Because of the complicated reciprocal connections of the vestibular and motor systems, it is impossible to localize the site of magnetic field action from the intact rat's behavioral response alone.

Rats did not always circle on release from a magnetic field, although the proportion of rats that circled increased with the strength of the magnetic field. The variation in the initiation of circling and the number of times an individual rat circles may reflect variation in the susceptibility of individual rats or variation in experimental parameters.

Although the magnitude of circling behavior is variable, the direction of circling has been completely consistent across all experiments to date. Furthermore, the direction was determined by the rats' orientation within the field. Not all rats circled on release, but if a rat did circle, it always circled counterclockwise if restrained head-up or clockwise if restrained head-down. Because the field strength of superconducting NMR magnets is fixed and their polarity is difficult to reorient, we reoriented the rat by inversion within the vertical bore of the magnet. Therefore, we cannot determine whether the direction of circling is attributable to the relative polarity of the magnetic field or the head-up–head-down orientation of the rat. Future experiments in large-bore, resistive magnets in which the rat's body and the polarity of the magnet can be arbitrarily oriented will help define the parameters of this effect.

The consistent effects of the magnetic field on the direction of circling strongly suggest unilateral or asymmetrical stimulation of the inner ear, vestibular nuclei, or motor pathways. Furthermore, the correlation of circling direction with the rat's orientation within the field suggests an inherent asymmetry in the interaction of the magnetic field with the vestibulomotor system of the rat. Although the mechanism and site of action is obscure, the engagement of the vestibulomotor system by magnetic fields suggests candidate neural sites involved in the rat's detection of the magnetic field. In fact, c-Fos immunohistochemistry in the rat brainstem 1 hr after 9.4 T magnetic field exposure has revealed activation of vestibular and visceral nuclei (Snyder et al., 2000). We cannot, however, rule out effects of the magnetic field on the basal ganglia–midbrain dopaminergic system (Shima, 1984) or prefrontal cortex (Nakamura-Palacios et al., 1999) that can also lead to circling behavior and indirectly to vestibular activation. The use of females (Choleris et al., 2000) or other rodent strains (Cransac et al., 1997; Richter et al., 1999) that are more sensitive to vestibular stimulation may be useful.

Taste aversion

CTA learning has been used to reveal the detection and responsiveness of animals to many pharmacological, vestibular, or radiation treatments. It has a number of advantages for the exploration of the effects of magnetic fields. The rate of CTA acquisition, the magnitude of the CTA, and rate of extinction can be used as measures of the strength of the detected US.

Because CTA learning tolerates a long delay (Smith and Roll, 1967) between presentation of the conditioned stimulus (the taste) and the unconditioned treatment (magnet exposure) and is largely insensitive to environmental context (Garcia and Koelling, 1966), the magnetic field exposure could be separated in time and space from the taste stimulus. Because the expression of a CTA can be measured for days after acquisition, the graded effects of the magnetic fields could be measured by two bottle preference tests in extinction trials independent of any short-term effects of the magnetic field on the rat. Finally, because of the rat's sensitivity to taste–toxin associations, a CTA can often reveal the rat's ability to detect an unconditioned stimulus even in the absence of any other observable behavioral effect. Thus, rats were shown to acquire a CTA against G+S after only a 1 min exposure to a 14 T magnetic field.

As with locomotor effects, the magnitude of the CTA was related to the intensity of the magnetic field. Pairing the G+S solution with one 30 min exposure to the 7 T magnet was not sufficient to induce a significant CTA; in the previous study, one 30 min exposure to a 9.4 T magnet was sufficient to induce a weak but significant CTA (Nolte et al., 1998). Thus we have identified a minimal intensity of the magnetic field sufficient for CTA induction under our conditions. However, different behavioral effects observed under different conditions may reveal different thresholds of sensitivity to magnetic fields. Thus, the 7 T magnet was not without effect: circling was occasionally observed after 7 T exposure, and a CTA could be acquired with three pairings of G+S with 30 min of exposure to the 7 T magnet.

It is important to emphasize that rats will learn a CTA not only to toxic, aversive, or nauseating stimuli but will also avoid tastes paired with many treatments that are not physically toxic (e.g., rotation) or obviously aversive [e.g., orexigenic drugs (Stephan et al., 1999) or self-administered drugs of abuse (Parker, 1995)]. Furthermore, although rats will easily form taste aversions to treatments that induce nausea in humans, many treatments that do not induce nausea in humans will induce CTA in rats. Therefore, we cannot conclude that magnetic fields are aversive, toxic, or malaise inducing just because rats acquire a CTA after magnetic field exposure. Nonetheless, because both magnetic fields and vestibular stimulation by rotation or labyrinthectomy can induce circling behavior and CTAs (Braun and McIntosh, 1973; Green and Rachlin, 1973; Hutchison, 1973; Arwas et al., 1989; Fox et al., 1990), and because magnetic fields and rotation induce similar c-Fos patterns in the rat brainstem (Kaufman et al., 1991, 1992, 1993; Kaufman, 1996; Marshburn et al., 1997), we hypothesize that the rats may be experiencing a vestibular disturbance during magnetic field exposure comparable with the self-reports of vertigo and nausea in humans working with high-strength magnetic fields.

The inner ear may be the site of magnetic field transduction in the rat. Schenck (1992) has proposed a model in which small movements of the semicircular canals in a magnetic field would induce a magnetohydrodynamic force on the conductive endolymph, causing apparent rotation. In the absence of consistent visual or proprioceptive information, the conflicting vestibular input could cause motion sickness. We have adopted Schenck's model of vestibular disturbance as a working hypothesis for the detection mechanism in rats. This model has the virtue of relying on well established forms of sensory transduction in the vestibular system and does not require any mechanisms specific for magnetic field detection, such as biomagnetite crystals.

Although we hypothesize that the labyrinth of the inner ear is the site of magnetic field detection, other sensory pathways may be necessary or contribute to visceral stimulation mediating CTA acquisition [e.g., vagal afferents (Coil et al., 1978) or the area postrema (Ritter et al., 1980)]. The elucidation of the sensory inputs and central processing mediating the effects of magnetic

field exposure on rats may help predict human susceptibility and responses in future generations of high magnetic field instruments such as MRI machines.

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