Physiology & Behavior 97 (2009) 36–43

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Labyrinthectomy abolishes the behavioral and neural response of rats to a high-strength static magnetic field

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ARTICLE INFO

Article history:
Received 31 July 2008
Received in revised form 22 January 2009
Accepted 23 January 2009

Keywords:
Magnetic resonance imaging
Vestibular system
Vertigo
Motion sickness
Nausea

ABSTRACT

Vertigo is a commonly-reported side effect of exposure to the high magnetic fields found in magnetic resonance imaging machines. Although it has been hypothesized that high magnetic fields interact with the vestibular apparatus of the inner ear, there has been no direct evidence establishing its role in magnet-induced vertigo. Our laboratory has shown that following exposure to high magnetic fields, rats walk in circles, acquire a conditioned taste aversion (CTA), and express c-Fos in vestibular and visceral relays of the brainstem, consistent with vestibular stimulation and vertigo or motion sickness. To determine if the inner ear is required for these effects, rats were chemically labyrinthectomized with sodium arsanilate and tested for locomotor circling, CTA acquisition, and c-Fos induction after exposure within a 14.1 T magnet. Intact rats circled counterclockwise after 30-min exposure to 14.1 T, but labyrinthectomized rats showed no increase in circling after magnetic field exposure. After 3 pairings of 0.125% saccharin with 30-min exposure at 14.1 T, intact rats acquired a profound CTA that persisted for 14 days of extinction testing; labyrinthectomized rats, however, did not acquire a CTA and showed a high preference for saccharin similar to sham-exposed rats. Finally, significant c-Fos was induced in the brainstem of intact rats by 30-min exposure to 14.1 T, but magnetic field exposure did not elevate c-Fos in labyrinthectomized rats above sham-exposed levels. These results demonstrate that an intact inner ear is necessary for all the observed effects of exposure to high magnetic fields in rats.

1. Introduction

Vertigo is a commonly-reported side effect of exposure to static high magnetic fields as found in magnetic resonance imaging (MRI) machines. Surveys of technicians working around 1.5 T [1] and 4 T [2] magnets found statistically significant occurrences of vertigo, dizziness and nausea particularly associated with movement through the high fields. Users of a 7 T magnet have reported subjective sensations of movement and falling [3], and subjects in studies conducted with an 8 T magnet reported vertigo when moving in or out of the MRI machine [4–6]. These reports suggest a transient influence of high magnetic fields on the vestibular system, leading to vertigo.

As MRI machines are increasingly employed for interventional procedures [7], there may also be effects on medical personnel working within the fringe fields of large magnets. For example, while performing neurobehavioral tasks within a 1.5 T MRI machine or immediately outside 1.5, 3, and 7 T magnets, subjects showed deficits in hand-eye coordination and visual tracking [8–10]. This also suggests a magnet-induced perturbation of vestibular inputs, or their integration with visual and proprioceptive information.

Although it is hypothesized that high magnetic fields interact with the vestibular apparatus of the inner ear [11,12], there has been no direct evidence establishing a role for the inner ear in magnet-induced vertigo. In this study, we tested the role of the inner ear using chemical labyrinthectomy in an animal model of magnetic field-induced vertigo. Rodents were restrained within superconducting magnets used for nuclear magnetic resonance, or within large resistive magnets. At the behavioral level, magnetic field exposure suppressed normal rearing and induced tight locomotor circling in a counterclockwise direction for the first few minutes following exposure [13,14]. Furthermore, when the magnetic field exposure was paired with a novel taste solution (e.g. saccharin), a conditioned taste aversion (CTA) was induced [13–16] similar to that seen after pairings of taste with rotation or motion sickness. At the neural level, magnetic field exposure induced significant c-Fos immunoreactivity, a marker of neuronal activation, in specific vestibular and visceral nuclei within the rat brainstem [17].

Because these magnet-induced responses parallel responses to vestibular stimulation, rats may be experiencing vertigo similar to the self-reports of humans after magnetic field exposure. We hypothesize that the magnetic field interacts directly with the vestibular apparatus

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0031-9384/$ – see front matter © 2009 Published by Elsevier Inc.
doi:10.1016/j.physbeh.2009.01.018

of the inner ear. The behavioral effects of exposure within large magnets are proportional to the duration of exposure and intensity of the static field at the center of the magnet [13,14]. Movement through the magnets and exposure to the large gradients surrounding the center of the magnet are not sufficient to induce circling and CTA [18]. Therefore, we hypothesize that the static magnetic field per se interacts with the inner ear during exposure, which results in c-Fos induction and the observed post-exposure behaviors.

In this report we tested the role of the inner ear in these responses using chemical labyrinthectomy by intratympanic injections of the ototoxic chemical, sodium arsanilate [19–22]. Labyrinthectomized and intact rats were tested in 3 separate experiments to assess the acute effects of magnetic field exposure on locomotor activity (circling and the suppression of rearing in Experiment 1), acquisition of CTA after repeated pairing consumption of a novel saccharin solution with magnetic field exposure (Experiment 2), and finally the induction of c-Fos in vestibular and visceral relays of the brainstem after acute magnetic field exposure (Experiment 3). In all experiments, a consistent magnetic field exposure of 30 min within a 14.1 T superconducting magnet was employed, based on our previous reports that parameterized the effects of exposure intensity, duration, and position within high-strength static magnets [13,16,18,23–25]. While 14.1 T is stronger than any MRI machines currently in clinical use, experimental MRI machines are now available with magnets up to 9.4 T (for use with humans [26]) and 20 T (for use with animals [27]). In addition, we assessed the consequences of labyrinthectomy using a maximal stimulus, with the assumption that if the lesion blocked the effects of a 14.1 T magnetic field, then it would also block the effects of lower intensity fields.

2. Materials and methods

2.1. Animals

Female Sprague Dawley rats (200–225 g; Charles River Laboratories, Wilmington, MA) were housed individually in polycarbonate cages in a temperature-controlled colony room at the US National High Magnetic Field Laboratory at The Florida State University. The rats were maintained on a 12 h light/dark cycle with lights-on at 7:00 A.M. All procedures were conducted during the light cycle. The rats had *ad libitum* access to Purina Rat Chow and deionized–distilled water except where specified otherwise. Different rats were used in each experiment.

2.2. Chemical labyrinthectomy

Bilateral labyrinthectomy was performed by intratympanic injections (50–100 µl/ear) of sodium arsanilate (15 mg in 50 µl of 0.15 M NaCl). This procedure destroys the hair cells of the peripheral vestibular receptors. Sham-lesioned rats received intratympanic injections of saline vehicle instead of sodium arsanilate and are referred to as intact rats. Rats were given 7–17 days to recover from surgery prior to exposures.

2.3. Magnet

Exposure to the high magnetic field was conducted in a superconducting magnet with a vertical bore designed for biochemical nuclear magnetic resonance (NMR) studies. The 14 T magnet was a 600 MHz Bruker Cryo magnet with an 89 mm bore and fixed field strength of 14.1 T. It contained a shim magnet extending along the magnet bore for approximately ± 15 cm from the magnet's core, which was used to stabilize the magnetic field and give a central core field of uniform strength (we have previously mapped the magnetic field along the vertical axis [18]). The magnetic field was oriented vertically so that the positive pole was at the top of the magnet. The magnet was operated without radiofrequency pulses, so rats were exposed only to a static magnet field (B). During insertion into the magnet, however, the magnetic field would have been experienced as a time-varying field (dB/dt), relative to the moving rat. Also, the magnetic field gradient changes rapidly between the bore of the magnet and the central core, so that the rats experienced gradients of up to 54 T/m during insertion [25].

2.4. Magnet exposure and sham exposure

Prior to exposure to the magnetic field ("magnet exposure") or sham exposure, rats were placed in a Plexiglas restraint tube that had an inside diameter of 56 mm and an outside diameter of 64 mm. A cone shaped plug with a 1 cm hole at the apex was inserted in the rostral end of the restraint tube to accommodate the head of the rat and to allow fresh air for breathing. A second plug was inserted in the caudal end of the restraint tube and could be adjusted to restrain the movement of the rat. It had a 1 cm hole in the center to accommodate the rat's tail. When in the tube, the rat was almost completely immobile. Restrained animals were carried individually to the 14 T magnet where the rat was inserted head-up into the bottom of the vertical bore of the magnet. The rat was quickly raised by hand (at a speed of approximately 0.5–1 m/s) from the floor through the magnet until the rat was in the core of the magnet. In its final position, the head of the rat was located 65 cm from the opening of the bore of the magnet, such that the head and body were completely within the homogenous 14 T central field. We have previously demonstrated that maximal effects of the magnetic field are experienced when the rats are in this position; exposure to lower field strengths and higher gradients are not as effective [18]. Rats remained in the 14 T MF for 30 min. Previous measurements of temperature found that rats maintain a constant body temperature in the restraint tube for the duration of exposure both within the core of a similar magnet or when restrained outside the magnet at room temperature [15].

To control for restraint and handling, additional rats were sham-exposed. Sham-exposed rats were inserted into identical restraint tubes. Then, the sham-exposed rats were inserted vertically into an opaque polyvinylchloride pipe with dimensions and conditions similar to those of the bore of the 14 T magnet. The sham apparatus was located in the same room as the 14 T magnet, but placed outside the 5 gauss field. One MF-exposed rat and one sham-exposed rat were treated simultaneously.

2.5. Locomotor activity

Following MF or sham exposure, rats were removed from the magnet or sham-magnet, and carried to a locomotor test cage located within 4 m of the magnet (just outside the 5-gauss line). Within 30 s after exposure, the rostral plug of the restraint tube was removed; and the rat was allowed to emerge into an open polycarbonate cage (37 cm wide by 47 cm long by 20 cm high). The floor of the cage was covered with chip bedding. The locomotor behavior of each rat was recorded on videotape for 2 min after release into the cage. Then, the rat was returned to its home cage and carried back to the animal facility. An observer blind to the rats' treatment scored the videotapes later. Instances of tight circling behavior and rearing behavior (both forepaws on the side of the cage) were quantified. Circles were counted if the rat moved continuously around a full circle with a diameter less than the length of the rats' body. Partial circles or circles interrupted by stationary pauses were not counted, because full circles provided an unambiguous measure that has been consistent across scorers, and because all rats walking in a constrained area must make some partial turns regardless of treatment.

2.6. Statistical analysis

All data were analyzed using Statistica software (Statsoft, Tulsa, OK) as described below. When the ANOVA revealed a significant difference, Newman–Keuls multiple comparison test was performed to determine significant differences between specific groups.

2.7. Experiment 1: Locomotor activity

In order to access the role of the inner ear in the locomotor effects of the high magnetic field, rats underwent bilateral labyrinthectomy (LBX, $n=8$) or sham-lesioning (intact, $n=8$) and then were observed after a single sham exposure and then retested on the following day after a single exposure to 14 T. On the first test day, rats were individually restrained outside the magnet for 30 min (sham exposure). Following restraint, the rats were released into the open-field testing chamber. Locomotor behavior was recorded on videotape for 2 min. The rats were then returned to their home cages. On the following day, the rats were restrained and exposed individually within the 14 T magnetic field for 30 min (magnet exposure). After magnet exposure, the rats were released into the open-field testing chamber and locomotor behavior was recorded on videotape for 2 min.

The videotapes were later scored for circling and rearing behavior. A 2-way repeated measures ANOVA was calculated for the total number of circles and number of rears following sham and magnet exposure. The two main factors were group (LBX or intact) and exposure (the repeated measure).

2.8. Experiment 2: Conditioned taste aversion

In order to access the role of the inner ear in the acquisition of magnet-induced CTA, a total of 22 rats underwent either bilateral labyrinthectomy (LBX, $n=10$) or sham lesioning (intact, $n=12$) and then were observed following 3 pairings of saccharin and exposure to the high magnetic field.

Twelve to 14 days after surgery, rats were conditioned using a procedure similar to earlier experiments [13–15,18,24]. Rats were placed on a water-restriction schedule. The initial session was 3 h, and the session times were diminished each day across 8 days so that the day before conditioning the rats received their water in a single 10-min session.

On conditioning days, the conditioned stimulus was 0.125% (wt/vol) sodium saccharin in deionized–distilled water. Rats were given a single pairing of saccharin and exposure to the magnetic field ($n=6$ LBX, $n=6$ intact) or sham exposure ($n=4$ LBX, $n=6$ intact) on 3 consecutive days. On each conditioning day, all rats were given access to saccharin for 10 min and were required to consume at least 2 ml (five rats failed to drink 2 ml during this 10-min presentation of saccharin, and their bottles were returned to the cage for an additional 5 min during which time they met the criterion).

Immediately after saccharin access, rats were individually restrained and either inserted into the 14 T magnet for 30 min (magnet exposure) or sham-exposed for 30 min. Rats were then released from restraint into the open-field testing chamber and locomotor behavior was recorded on videotape for 2 min. Rats were then returned to their home cages without access to water. After the last pairing, rats were given *ad libitum* access to water.

Two-bottle, 24-h preference tests were begun on the day after the last conditioning trial and continued for 14 days to test for CTA acquisition and rate of extinction. Each day two bottles were placed on the cages, one containing the saccharin solution and the other containing distilled water. Fluid consumption was measured every 24-h and a preference score was calculated as a ratio of saccharin intake to total fluid intake. The preference tests were continued for 14 post-conditioning days. Because saccharin access during the preference tests was not paired with any exposure, the preference tests constituted extinction trials. A CTA was considered significant if the preference score of the magnet-exposed group was significantly less than the preference score of the sham group. A CTA was considered extinguished when the average saccharin preference of magnet-exposed rats was not significantly different from the sham-exposed rats. Thus, preferences across the extinction trials showed the persistence and rate of extinction of the CTA.

Total number of circles and rears observed after sham or magnet exposure on the first day of conditioning were analyzed by 2-way ANOVA. The two main factors were group (intact or LBX) and exposure (sham or magnet). Significant differences in conditioning day intake were determined using 2-way repeated measures ANOVAs that compared intake by group across the 3 conditioning days. Likewise, significant differences in preference scores were determined using 2-way repeated measures ANOVAs that compared the preference scores of sham-and magnet-exposed rats across 14 days of 2-bottle preference tests following 3 pairings. The two main factors were group (sham-exposed intact, magnet-exposed intact, sham-exposed LBX, or magnet-exposed LBX) and test day (the repeated measure).

2.9. Experiment 3: c-Fos induction

In order to access the role of the inner ear in the neural effects of the magnetic field exposure, the brainstem of labyrinthectomized rats and intact rats was processed for c-Fos expression induced by sham or magnet exposure. Rats underwent either bilateral labyrinthectomy (LBX, $n=12$) or sham surgery (intact, $n=10$) as described above.

Rats were placed in the restraint tube and either inserted upward into the 14 T magnet and exposed to the magnetic field for 30 min ($n=6$ LBX rats and $n=6$ intact rats), or sham-exposed for 30 min ($n=6$ LBX rats, $n=4$ intact rats). Following magnet or sham-exposure, rats were released into the open-field chamber and locomotor behavior was recorded on videotape for 2 min. Rats were then returned to their home cage and left undisturbed for 60 min.

One hour after the end of magnet or sham exposure, rats were overdosed with sodium pentobarbital. Rats were examined at 1 h because c-Fos protein is maximally expressed 1–3 h after stimulation in many neural tissues [28]; we have previously reported c-Fos induction in the rat brainstem 1 h after magnet exposure [17]. Once completely unresponsive, rats were transcardially perfused, first with 100 ml heparinized isotonic saline containing 0.5% $NaNO_2$, then with 400 ml 4% paraformaldehyde in 0.1 M sodium phosphate buffer. Brains were removed, blocked and post-fixed for 2 h and transferred into 30% sucrose for cryoprotection.

2.10. Tissue processing

Coronal sections were cut at 40 μm on a freezing, sliding microtome. Seventy sections were cut through the medulla from the caudal subpostremal end of the nucleus of the solitary tract (NTS; bregma −13.8 mm) to the rostral extent of the medial vestibular nucleus (MeV; bregma −11.0 mm). In addition, 30 sections were cut through the pons from the caudal supragenualis nucleus (SG; bregma −10.52 mm), through the locus coeruleus (LC) to the rostral tip of the lateral parabrachial nucleus (latPBN; bregma −9.3 mm). Coordinates are from the atlas of Paxinos and Watson [29].

Alternative tissue sections were processed for c-Fos immunohistochemistry. Free floating sections were washed twice in 0.1 M sodium phosphate-buffered saline (PBS), then permeabilized in 0.2% Triton, 1% bovine serum albumin (BSA) in PBS for 30 min. After two washes in PBS–BSA, sections were incubated overnight with a rabbit anti-c-Fos polyclonal antiserum raised against human c-Fos residues 4–17 (Oncogene Sciences Ab-5, 1:20,000 dilution). After incubation for 1 h with a biotinylated anti-rabbit goat antibody bound secondary antibody was amplified with a Vector Elite ABC kit. Antibody complexes were visualized by a 5 min reaction with diaminobenzidine.

2.11. c-Fos data analysis

Images of brain regions (720 × 540 μm, 1.1 pixels/μm) were digitized with a MTI CCD72S grayscale camera mounted on an Olympus AX70 microscope. Cells expressing positive c-Fos immunohistochemistry were counted automatically by a Macintosh image analysis program (MindsEye, T. Houpt). Cells were counted in several visceral nuclei,

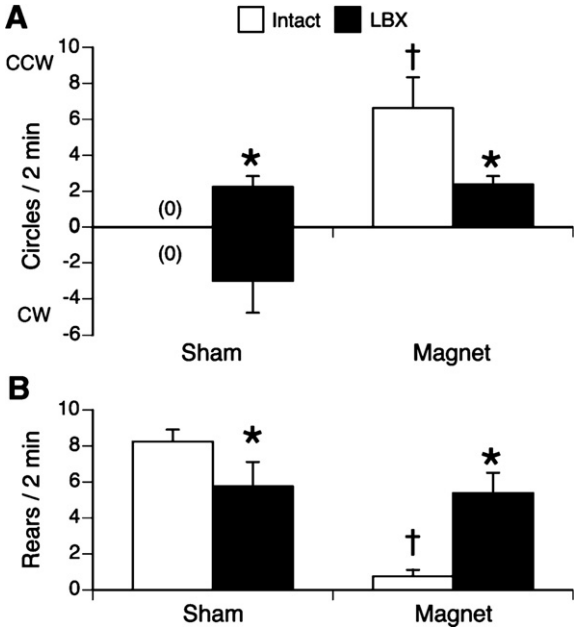

Fig. 1. Open-field locomotor behavior was scored for tight circling (A) and rearing (B) following 30 min of sham exposure (test day 1) and 30 min exposure to 14 T magnetic field (test day 2). A) Intact rats (white bars) circled more after magnet exposure than sham exposure. Labyrinthectomized rats (LBX, black bars) circled more than sham-exposed intact rats but less than magnet-exposed intact rats; magnet exposure did not increase circling in LBX rats. CW=clockwise, CCW=counterclockwise. B) Exposure to magnetic field suppressed rearing in intact rats but not LBX rats. * $p<0.05$ vs. intact rats after same treatment; † $p<0.05$ vs. sham-exposed intact rat.

including NTS (mean of 11 sections per rat), LC (3 sections), and latPBN (8 sections). Stained cells were also quantified in the MeV (20 sections per rat), nucleus prepositus (Prp; 20 sections), nucleus supragenualis (SG; 9 sections) and superior vestibular nucleus (SuV; 9 sections), all of which express c-Fos after vestibular stimulation [30]. Bilateral cell counts were averaged across sections of each nuclei within each rat; the mean counts were then averaged across rats in each experimental group. Two-way ANOVAs were used to determine differences in number of c-Fos-positive cells within each nuclei, with surgical group (intact or LBX) and exposure (sham- or magnet-exposed) as the factors.

3. Results

3.1. Experiment 1: Locomotor behavior

Two-way ANOVA revealed a significant interaction of surgical group and exposure for both circling [total number of clockwise and counterclockwise circles; $F(1,14)=18.26$; $p<0.001$] and rearing [$F(1,14)=47.48$; $p<0.001$] (see Fig. 1). Following sham exposure, intact rats reared but did not walk in circles in either direction. After magnet exposure, however, rearing was suppressed and most of the intact rats walked (7 of 8) in counterclockwise circles.

Conversely, all LBX rats showed both rearing and circling after both sham exposure and magnet exposure. Following sham exposure, LBX rats circled both clockwise and counterclockwise; after magnet exposure, LBX rats circled only in the counterclockwise direction.

In this experiment, sham and magnet exposures were not counterbalanced within groups so that all intact and LBX rats experienced restraint during sham exposure first, and subsequently experienced a second restraint during magnet exposure. An undetected interaction might have occurred, therefore, between repeated restraint on the response to the magnetic field. In other words, repeated restraint might have either enhanced the response of intact rats or diminished the response of LBX rats after magnet exposure. Nonetheless, LBX rats failed to show the same response as intact rats to magnet exposure, and we have no reason to believe that LBX rats are any more sensitive to repeated restraint than intact rats.

3.2. Experiment 2: Conditioned taste aversion

On the first conditioning day, there was a significant effect of surgical group (but not exposure) on total number of circles observed [$F(1,18)=15.88$, $p<0.001$], such that LBX rats circled more in either direction than intact rats, regardless of sham or magnet exposure. Four of 6 intact rats showed counterclockwise circling after magnet exposure, but the increase in number of circles was not significant (Fig. 2A). There was a significant interaction of group and exposure on

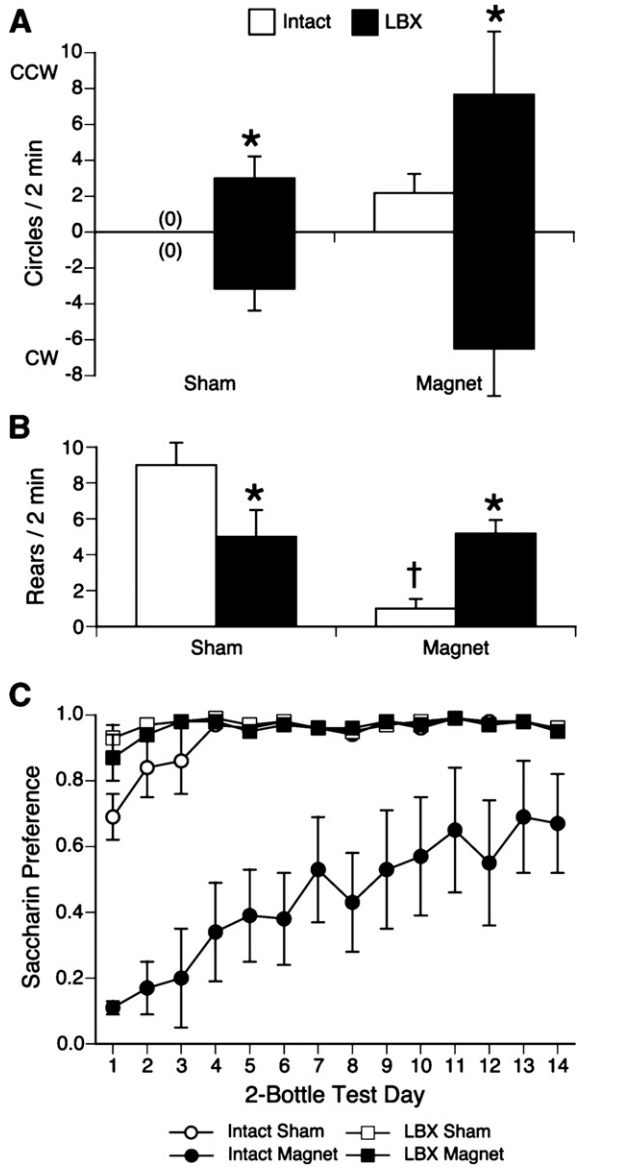

Fig. 2. Locomotor activity (A, B) and CTA acquisition (C) after the pairing of saccharin and 14 T magnetic field exposure. After the first exposure, there was no difference between sham-exposed and magnet-exposed labyrinthectomized rats (LBX, black bars) in either circling (A) or rearing (B). Compared to sham exposure, magnet exposure suppressed rearing in intact rats (white bars); the increase in circling after magnet exposure in intact rats was not significant. * $p<0.05$ vs. intact rats after same treatment, † $p<0.05$ vs. sham-exposed intact rats. (C) In 2-bottle preference tests following 3 pairings of saccharin and exposure, sham-exposed rats (white symbols) showed a high preference for saccharin. Magnet-exposed intact rats (black circles) acquired a strong CTA, as shown by a significantly lower preference for saccharin that persisted for the first 12 days of testing. The saccharin preference of magnet-exposed LBX rats (black squares) was not different from sham-exposed intact rats or sham-exposed LBX rats on any day, demonstrating that LBX rats did not acquire a magnet-induced CTA.

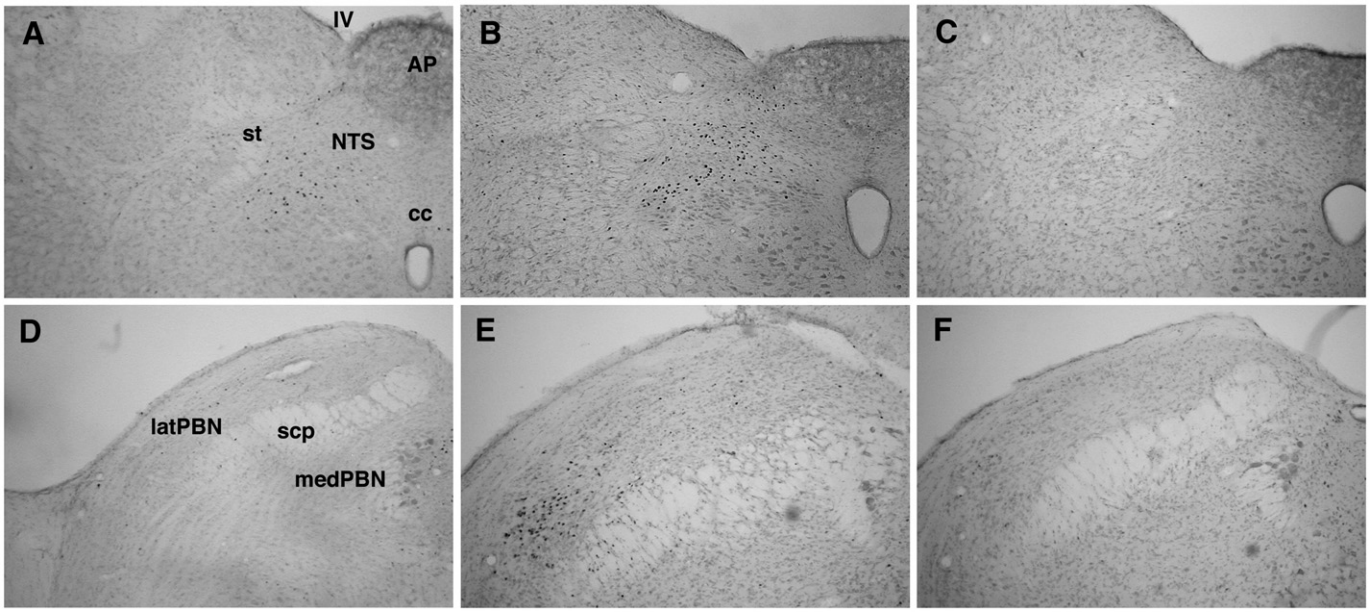

Fig. 3. Examples of c-Fos induction in visceral nuclei of the brainstem after 30-min sham exposure in an intact rat (A, D), or after 30-min exposure to 14.1 T magnetic field in an intact rat (B, E) or LBX rat (C, F). AP=area postrema, cc=central canal, NTS=nucleus of the solitary tract, latPBN=lateral parabrachial nucleus, medPBN=medial parabrachial nucleus st=solitary tract, IV=fourth ventricle.

rearing [$F(1,18) = 16.5$, $p<0.001$]. Rearing by intact rats was significantly reduced after magnet exposure compared to both sham-exposed intact rats and magnet-exposed LBX rats (Fig. 2B).

There was no significant difference in saccharin intake on the first day of conditioning, among the four groups (mean intake 7.2 ± 0.5 g). On the second day of conditioning, the mean intake of the magnet-exposed intact rats was 6.2 ± 1.0 g, and the intake of the other 3 groups was 10.6 ± 0.9 g. Across the 3 days of conditioning, however, there was a significant interaction of group and day [$F(6,36) = 2.57$, $p<0.05$], such that by the third day of conditioning the saccharin intake of intact rats undergoing magnet exposure (5.9 ± 0.2 g) was significantly lower than the saccharin intake of the other three groups (12.2 ± 1.0 g).

During 2-bottle preference tests after 3 pairings of saccharin and sham- or MF-exposure, 2-way ANOVA revealed a significant interaction of group and extinction day [$F(39,234) = 2.22$, $p<0.0005$]. The preference scores of magnet-exposed intact rats were significantly lower than both magnet-exposed LBX rats and sham-exposed rats for the first 12 days of extinction (Fig. 2C). Magnet-exposed LBX rats were not different from sham-exposed rats on any test day. Thus, intact rats formed a significant CTA after MF exposure, while LBX rats showed no CTA at all.

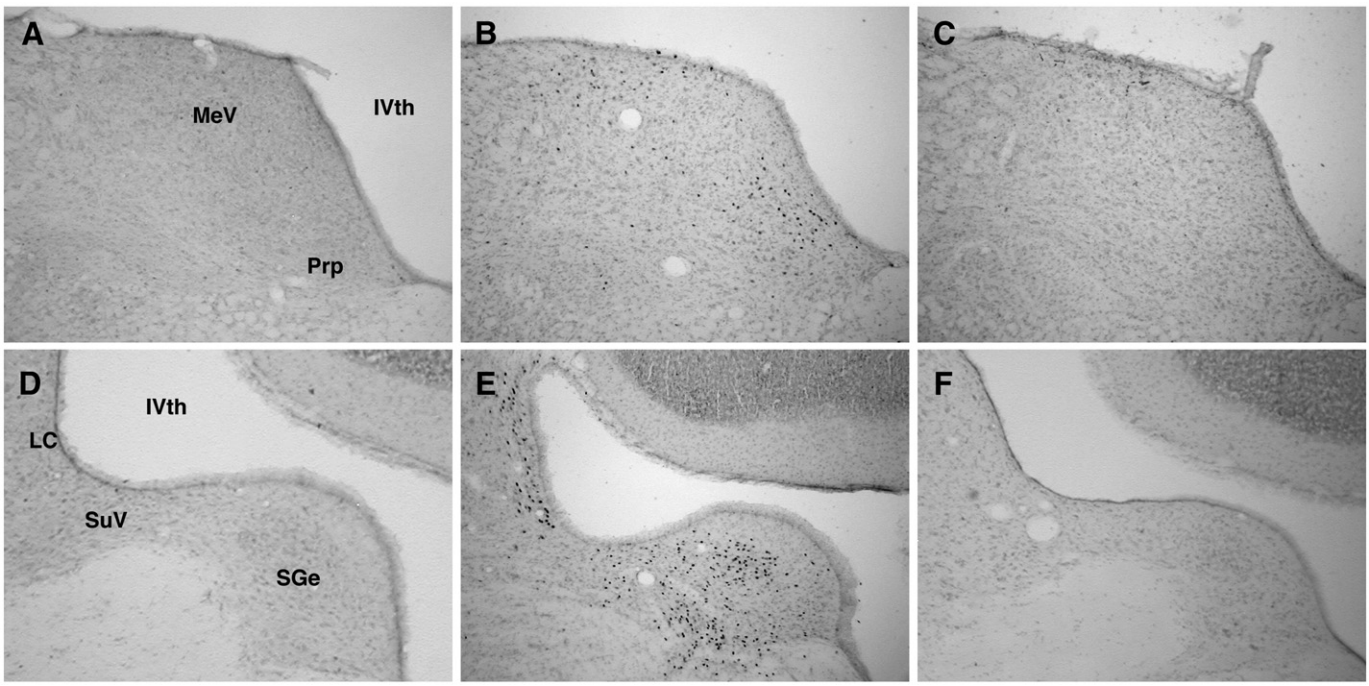

Fig. 4. Examples of c-Fos induction in vestibular nuclei of the brainstem after 30-min sham exposure in an intact rat (A, D), or after 30-min exposure to 14.1 T magnetic field in an intact rat (B, E) or LBX rat (C, F). LC=locus coerelus, MeV=medial vestibular nucleus, SuV=superior vestibular nucleus, SGe=supragenualis nucleus.

3.3. Experiment 3: c-Fos induction

As in the previous experiments, exposure to the 14 T magnetic field induced counterclockwise circling (9.3 ± 3.3 circles over 2 min) and suppressed rearing (1.2 ± 0.8 rears over 2 min) in intact rats. After both sham- and magnet exposure, LBX rats showed high levels of rearing (magnet: 5.3 ± 1.3, sham: 5.3 ± 1.5) and circling in both the clockwise (magnet: 4.3 ± 1.5, sham: 6.2 ± 2.3) and counterclockwise directions (magnet: 13.0 ± 4.4, sham: 14.7 ± 3.1).

3.4. c-Fos expression in visceral relays

Two-way ANOVA revealed a significant interaction of surgery (intact or labyrinthectomy) and exposure (sham or magnet exposure) on c-Fos expression in the NTS [$F(1,18) = 14.8$; $p<0.005$], PBN [$F(1,18) = 11.8$; $p<0.005$], and LC [$F(1,18) = 28.0$; $p<0.001$]. Compared to either group of sham-exposed rats, there were significantly more c-Fos-positive cells following magnet exposure in intact rats in the NTS, PBN and LC (Figs. 3 and 5A). In the NTS, abundant c-Fos-positive cells were observed in the medial divisions of the intermediate, subpostremal, and caudal NTS while little or no c-FLI was observed in the lateral divisions or rostral (gustatory) NTS. In the PBN, c-FLI was concentrated in the external lateral, central lateral and ventrolateral regions. In the LC, C-FLI was densely spread throughout the nuclei.

In contrast, both magnet-exposed LBX rats and sham-exposed LBX rats had significantly fewer c-Fos positive cells than magnet-exposed intact rats in the NTS, PBN, and LC.

3.5. c-Fos expression in vestibular relays

Two-way ANOVA revealed a significant interaction of surgery and exposure on c-Fos expression in the MeV [$F(1,18) = 33.8$; $p<0.001$], Prp [$F(1,18) = 135.2$; $p<0.001$], SuV [$F(1,18) = 23.7$; $p<0.001$], and SGe [$F(1,18) = 26.2$; $p<0.001$]. Compared to either group of sham-exposed rats, after magnetic field exposure there were more c-Fos-positive cells in intact rats in the MeV, Prp, SuV and Sge (Figs. 4 and 5B). In particular, c-Fos positive cells were densely induced by magnetic field exposure in the Prp and SG, while within the MeV and SuV, the pattern of c-Fos-positive cells was more scattered and sparse.

However, magnetic field exposure induced significantly fewer c-Fos-positive cells in LBX rats compared to intact rats in the MeV, Prp, SuV and SGe. Furthermore, magnet-exposed LBX rats showed significantly fewer c-Fos-positive cells than sham-exposed LBX rats in the SuV.

4. Discussion

These experiments demonstrate that the vestibular apparatus of the inner ear is a critical site for the behavioral and neural effects of high-strength magnetic fields in rats. As in previous studies, exposure to a high magnetic field induced locomotor circling, suppressed rearing, induced CTA, and induced c-Fos expression in intact rats [13,15,17]. In labyrinthectomized rats, however, magnetic field exposure failed to suppress rearing, did not increase circling, failed to induce CTA after 3 pairings with saccharin, and did not induce c-Fos above control levels in visceral and vestibular relays of the brainstem. Overall, magnet-exposed labyrinthectomized rats were indistinguishable from sham-exposed labyrinthectomized rats. These results provide direct evidence for an interaction of high magnetic fields with the inner ear as the mechanism for vertigo in humans.

In this and previous studies, exposure of intact rats within the bore of high strength magnets suppressed rearing and induced tight locomotor circling. The direction of circling is dependent on the orientation of rats in the field. Rats exposed with their heads towards the positive pole of the magnetic field circle counter clockwise, while rats exposed with their heads towards the negative pole circle clockwise [13,14]. These responses parallel the effects of vestibular perturbations: whole-body rotation suppresses rearing [31] and causes circular swimming [32], while unilateral treatments induce locomotor circling [33]. The locomotor effects of vestibular stimulation require an intact inner ear, e.g., labyrinthectomized rats are not affected by whole-body rotation [31]. Similarly, labyrinthectomized rats reared after both sham exposure and magnetic field exposure, and they did not show any increase in counterclockwise circling after

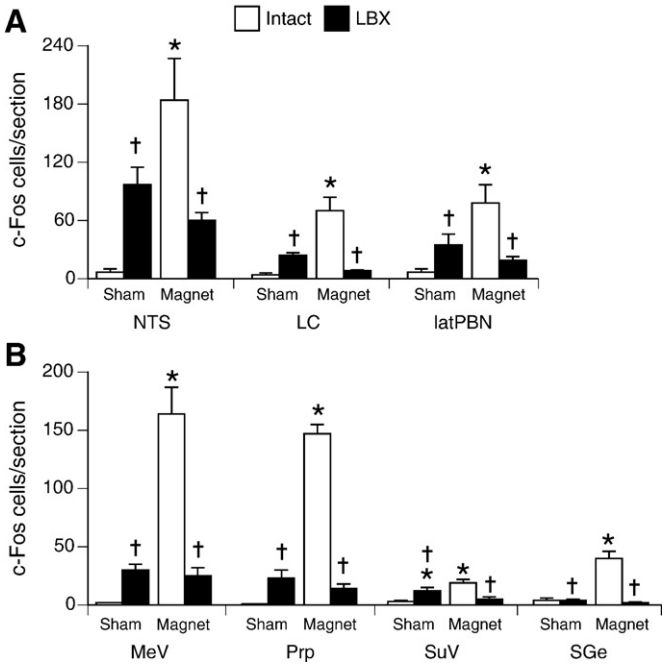

Fig. 5. Quantification of c-Fos positive cells in intact rats (white bars) or labyrinthectomized (LBX; black bars) after sham exposure or magnet exposure in vestibular relays (A) and visceral relays (B) of the rat brainstem. Labyrinthectomy significantly reduced the neuronal response to 14.1 T MF exposure in all areas such that it was not different from sham-exposed levels. * $p<0.05$ vs. sham-exposed intact group; † $p<0.05$ vs. intact magnet-exposed group. Abbreviations as in Figs. 2 and 4.

magnetic field exposure. Thus the locomotor effects of magnetic field exposure require an intact inner ear.

This interpretation is complicated, however, by the fact that labyrinthectomized rats spontaneously exhibit tight, bidirectional circling even after sham exposure. Spontaneous circling is frequently observed in bilateral labyrinthectomized animals and mutant rodents with vestibular deficiencies (e.g. [34–37]). While unilateral labyrinthectomized animals tend to circle towards the side of the lesion, bilateral labyrinthectomized animals circle in either direction, perhaps due to central asymmetries or an inability to regulate directional turns [37]. Labyrinthectomized animals may also show greater locomotor activity and less rearing [20,38], although in the present study labyrinthectomized rats reared the same amount as intact rats.

Given the baseline of spontaneous circling of LBX rats, it is difficult to compare the circling response of intact and LBX rats. The more informative comparison, therefore, is the circling between sham-and magnet-exposed groups, rather than between the intact and LBX rats. In both experiments 1 and 2, intact rats only showed circling behavior after magnet exposure (Figs. 1A and 5A), consistent with our earlier reports. If magnetic field exposure induced circling independent of the vestibular system, we might expect an additive effect of magnetic exposure and spontaneous circling in LBX rats. However, there was no statistically significant difference in circling between magnet-exposed and sham-exposed LBX rats in either experiment (The apparent, but non-significant, increase of circling in magnet-exposed LBX rats compared to magnet-exposed intact rats after magnet exposure in experiment 2 is probably due to variability in spontaneous circling by the LBX rats.). In other words, magnetic field exposure had no effect on circling of LBX rats in either experiment. We do not think this reflects a ceiling on circling in LBX rats, as we have occasionally observed individual intact rats circling 50+ times within the 2-min test period.

Another potential confound is that in experiment 1 all rats were first sham exposed and then exposed to the magnetic field, so it is possible that prior experience of restraint (i.e. habituation to stress) affected the locomotor response. However, the rats in experiment 2 were in balanced independent groups, and they showed the same pattern of responding without prior restraint, so that habituation does not account for the lack of locomotor response by the LBX rats.

Consistent with the induction of motion sickness by magnetic field exposure, intact rats acquired a strong and persistent CTA after 3 pairings of saccharin and restraint within the magnet. Labyrinthectomized rats did not acquire a CTA, and their preference for saccharin was indistinguishable from the high saccharin preference of sham-exposed rats. The blockade of CTA by labyrinthectomy is likely to be specifically caused by blockade of vestibular input, which can serve as the unconditioned stimulus in CTA acquisition. For example, vestibular stimulation by rotation can induce CTA in rats and humans [39–43] and labyrinthectomy blocks rotation-induced conditioned taste aversion [21]. Ablation of the external vestibular apparatus also eliminates the emetic response to motion in squirrel monkeys [44] and humans with unilateral or bilateral labyrinthectomy experience less nausea than subjects with an intact vestibular apparatus when exposed to a moving visual field [45]. Some gustatory nerves traverse the middle ear, so it is possible that an inner ear lesion might alter taste discrimination and thus compromise CTA learning. Labyrinthectomized rats, however, were still capable of detecting saccharin as evidenced by their high preference for saccharin over water. Also, labyrinthectomy does not cause a general deficit in CTA learning, because labyrinthectomized rats are still capable of acquiring LiCl-induced CTA [21].

As with previous findings, restraint within the high magnetic field induced significant c-Fos expression in visceral and vestibular relays of the brain stem of intact rats [17]. In contrast, exposure to the magnetic field did not induce significant c-Fos expression in visceral or vestibular relays of labyrinthectomized rats. These c-Fos results parallel the pattern of c-Fos induction in LBX and intact rats following vestibular stimulation. For example, whole-body rotation induces c-Fos patterns in the rat brainstem in a pattern similar to that seen after magnetic field exposure, and labyrinthectomy blocks rotation induced c-Fos [30,46,47].

Thus, our behavioral and c-Fos results after magnetic field exposure and labyrinthectomy are consistent with neural activation of vestibular pathways via the inner ear. The exact site within the inner ear affected by the magnetic field, and the mechanism of sensory transduction remain undetermined. The intratympanic injections of sodium arsanilate used in these studies destroys the hair cells of both the external vestibular organs and the auditory apparatus [48,49]. Therefore, it is a logical possibility that the removal of auditory input also diminished the response to the magnetic field. It is hard to conceptualize, however, in what way the magnetic field might interact with the cochlea to induce the responses we observe in rats. For example, it is unlikely that auditory stimulation alone would make rats walk in circles. Likewise, sound is not an effective unconditioned stimulus in CTA learning. A strong auditory stimulus can induce c-Fos in the rat brainstem, and the induction of c-Fos by sound is attenuated by cochlear lesion [50]. Auditory stimuli induce very different patterns of c-Fos compared to vestibular stimuli, however, with expression predominately in auditory relays rather than vestibular relays [51].

Within the vestibular apparatus, it has been proposed that high magnetic field might exert a magnetohydrodynamic force on the endolymph within the semicircular canals, or an ansiotropic torque on regular macromolecules such as the otoconia within the otolith organs [12]. Chemical labyrinthectomy destroys both the semicircular canals and otilithic organs, and thus abolishes the input of both rotational acceleration and linear acceleration sensors to the central vestibular network. Therefore, we cannot distinguish the contribution of the semicircular canals or otolith organs to the effects of magnetic field exposure. Future experiments will require either specific surgical lesions or the use of mutant animals with deficits in specific components of the external vestibular apparatus.

Although blockade by labyrinthectomy supports a direct effect on the inner ear, the magnetic field could also be affecting the vestibular system via inputs other than the inner ear. The central vestibular system receives convergent sensory input from the visual and proprioceptive systems and the inner ear; motion sickness arises when conflicting information is provided by the different senses. By removing a critical comparative input, labyrinthectomy also attenuates vestibular perturbation derived from the other sensory systems, e.g. responses to optokinetic stimuli [45]. Furthermore, visual excitation ("magnetophosphenes") is another commonly reported side effect of exposure to high magnetic fields [52]. Different lesions, such as optic enucleation, will be required to rule out a contribution by pathways other than the inner ear.

The results of the present study do not distinguish between effects induced by exposure to the static magnetic field at the center of the magnet and effects induced by rapid movement through the high gradients surrounding the magnet. When inserted into the magnet, the moving rats experienced the static field as a time-varying magnet field (dB/dt), and they passed through the spatially-varying gradient along the vertical axis of the bore (dB/dz). This is similar to the experience of human subjects moving into MRI machines, although humans are usually moved slower and, because of the larger dimensions of human MRI machines, the spatial gradients are less extreme. The vestibular perturbations around large magnets have been ascribed to head movements within the field gradients, and not as the result of static field exposure within the homogeneous core per se [1,2,10]. The spatially-varying field gradient would induce a force pulling magnetic materials towards the core, and head movement would create a time-varying field that could induce electrical currents in biological conductors. Thus the inner ear could be mechanically or electrically stimulated by movement in the gradient field of the magnet. A static field can also exert a mechanical effect, however. If a structure with magnetic susceptibility is not aligned parallel to the

magnetic field, then it will experience torque to rotate into alignment. Given the 3-dimensional structure of the vestibular apparatus, it may be possible that one or more components are experiencing torque within the static field.

We have collected evidence for effects of both the field gradient and the static field. In preliminary studies comparing the effect of rapid vs. slow insertion into the 14 T magnet (e.g. 0.1 m/s vs. 1 m/s), we have found that more rapid insertion results in greater post-exposure circling. The suppression of rearing and the acquisition of CTA is not affected by insertion speed, however. Furthermore, exposure to the static core field of the magnet appears to contribute to the behavioral effects. Insertion and removal alone does not induce circling, rearing or CTA [13]. Also, the direction of circling after magnetic field exposure depends on the rat's orientation within the static field, and not by insertion [13,14]. The most effective position for inducing behavioral effects is the center of the magnet, and exposure to the field gradient on either side of the homogeneous core has significantly less effect [18]. Therefore, we hypothesize that both passage through the field gradient and exposure to the static field contribute to the behavioral and neural effects of high magnetic fields, and the vestibular apparatus of the inner ear is required for both effects.

Acknowledgements

Supported by National Institute on Deafness and Other Communication Disorders Grants RO1DC4607 (TAH, JCS), F31DC6521 (AMC), and T32DC0044 (AMC). We thank Drs. Timothy Cross and Zhehong Gan of the United States National Magnetic Field Laboratory for providing access to the magnet.

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