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RESEARCH ARTICLE

Behavioral and neural responses to high-strength magnetic fields are reduced in otolith mutant mice

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Abstract

Static high magnetic fields (MFs) interact with the vestibular system of humans and rodents. In rats and mice, exposure to MFs causes perturbations such as head movements, circular locomotion, suppressed rearing, nystagmus, and conditioned taste aversion acquisition. To test the role of otoconia, two mutant mouse models were examined, *head-tilt* Nox3^{het} (*het*) and *tilted* Otop1 (*tlt*), with mutations, respectively, in Nox3, encoding the NADPH oxidase 3 enzyme, and Otop1, encoding the otopetrin 1 proton channel, which are normally expressed in the otolith organs, and are critical for otoconia formation. Consequently, both mutants show a near complete loss of otoconia in the utricle and saccule, and are nonresponsive to linear acceleration. Mice were exposed to a 14.1 Tesla MF for 30 min. After exposure, locomotor activity, conditioned taste aversion and c-Fos (in *het*) were assessed. Wild-type mice exposed to the MF showed suppressed rearing, increased latency to rear, locomotor circling, and c-Fos in brainstem nuclei related to vestibular processing (prepositus, spinal vestibular, and supragenual nuclei). Mutant *het* mice showed no response to the magnet and were similar to sham animals in all assays. Unlike *het, tlt* mutants exposed to the MF showed significant locomotor circling and suppressed rearing compared with sham controls, although they failed to acquire a taste aversion. The residual responsiveness of *tlt* versus *het* mice might reflect a greater semicircular deficit in *het* mice. These results demonstrate the necessity of the otoconia for the full effect of exposure to high MFs, but also suggest a semicircular contribution.

c-Fos; conditioned taste aversion; magnetic resonance imaging; utricle; vestibular system

INTRODUCTION

Static high magnetic fields (MFs) used by magnetic resonance imaging (MRI) machines have been shown to perturb the vestibular system of both rodents and humans. In rats and mice, exposure to static MFs of 4–14.1 Tesla (T) or greater induces head movements and circular locomotion, suppresses rearing, mediates conditioned taste aversion (CTA) acquisition (a measure of motion sickness), and causes c-Fos induction in vestibular nuclei of the brainstem (1–4). Mice have been shown to display nystagmus in MFs of 4.7 T (5). In humans, horizontal and torsional nystagmus has been documented at low fields of 1.5 T (6). Subjects and workers report vertigo, dizziness, subjective body motion, and nausea around or within MRI machines at 4 T and above (7–10).

These vestibular responses suggest that high MFs affect the inner ear. Loss of labyrinth function abolishes MF responses in both rats and humans (9, 11), implicating the inner ear as a locus for MF effects. Mutant mice that are lacking specific components of the inner ear are a convenient way to test the role of those components. In this study, we examined the responses of two mutant mouse strains, *head-tilt* Nox3^{het} (*het*) and *tilted* Opt1^{tlt} (*tlt*). Both Nox3, encoding the NADPH oxidase 3 enzyme, and Opt1, encoding the opterin1 proton channel, are normally expressed in the ototlith organs, and are critical for otoconia formation (12–14). Consequently, both *het* and *tlt* mice show a near complete loss of otoconia in the utricle and saccule, and they are nonresponsive to linear acceleration (15, 16). At the same time, they have no apparent anatomical defects in the rest of the inner ear and remain sensitive to rotational stimulation (12, 17, 18). Thus they are probes for the necessity of otoconia and the role (direct or indirect) of the otolith organs in vestibular perturbation induced by MFs, while largely preserving the rest of the vestibular apparatus.

We assessed *het* and *tlt* mice for MF-induced locomotor circling, suppression of rearing, acquisition of conditioned taste aversion, and (in *het* mice) c-Fos expression in the brainstem. A recent study by Ward et al (5). demonstrated that *het* mice do not show a nystagmus response during MF exposure, and our study is an extension of their approach and a test of the comparably deficient *tlt* mice. A preliminary report has appeared (19).

METHODS

General Methods

Animals.

Breeding pairs of *head-tilt* (*het*; B6Ei.GL- *Nox3* ^{het}/J, strain no. 002557) and *tilted* (*tlt*; B6.Cg- *Otop1* ^{tlt}/J, strain no.

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001104) mice were acquired from Jackson Laboratories. Male and female offspring were raised until at least 8 wk of age before being phenotyped and exposed to MF. Adult C57BL6/J mice were ordered from Jackson Laboratories as wild-type controls.

A swim test was used to phenotype *het* mice, *tlt* mice, and their littermates (12). Wild-type mice (+/+) swam along the surface of the water toward the side of the pool. Homozygous *het* mice (*het*/*het*) and *tlt* mice (*tlt*/*tlt*) were identified by their inability to swim while keeping their head above water; instead, they swam in circles and somersaulted toward underwater.

In addition, *tlt* mice were genotyped by PCR using primers to amplify across the Otop1 ^{tlt} C476 > A mutation, 5′-CAC TGT TTG GTC TTG GTA CC-3′ (forward) and 5′-CAG CTC ATT CCT GAC AAG-3′ (reverse). *Taq1* enzyme digest of the PCR product was used to confirm the restriction site introduced by the *tlt* allele. The PCR product was 391 bp (present in all mice), which could be completely or partially digested to 2 fragments (230 bp and 161 bp) restriction fragments in homozygous or heterozygous *tlt* mice, respectively (14).

Exposure to static high MF.

Mice were exposed for 30 min to a 14.1 T static MF in the center of a Bruker Cryo 600-MHz magnet. The magnet has a vertical bore of 89 mm in diameter and a fixed field strength of 14.1 T. The field is oriented vertically with the positive pole at the top of the magnet, and with a shim magnet extended along the bore for approximately ±15 cm from the vertical center to stabilize the MF and to give uniform field strength.

During MF exposure, mice were either restrained in tubes (*experiments 1*, *3*, and *4*) or unrestrained in cups (*experiments 2* and *5*), as previously described (1). Mice in restrained MF-exposed groups were restrained by placing them in a plastic tube made from a 50-mL conical cylinder, with the head of the mouse positioned at the cone end. A hole in the tip of the cone allowed for breathing. A plastic plug with a hole to allow for the tail of the mouse at the caudal end of the tube restrained the mouse from moving. Two restrained mice in these tubes were fitted into a plastic collar that was inserted vertically into the bore of the magnet and exposed to the 14.1 T MF.

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

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

Scoring of locomotor behavior.

After the 30-min MF or sham exposure, mice were placed in an open Plexiglas cage (37 × 47 × 20 cm) with pine-chip bedding in the same room as the magnet and sham-magnet. The locomotor behavior of the mice was recorded by video for 2–5 min, and the mice were then returned to their home cages in the animal facility room. Two minutes was chosen as the minimum time because in previous experiments almost all circling behavior ends within 2 min. Behavior was scored for *1*) number of rears (with at least one paw touching cage side), *2*) latency from the start of recording to first rear (mice that did not rear during the recording period were assigned a latency equal to recording period), or *3*) tight circling [complete and continuous turns with a diameter no longer than the body of the mouse; clockwise (CW) and counterclockwise (CC) circling were scored separately] by an individual blind to treatment condition.

Conditioned taste aversion.

Before being conditioned, mice were placed on daily water restriction, with access tapered from 3 h of water/day to 30 min of water per day over 8 days. On the first day of conditioning, mice were given 10-min access to a 0.125% saccharin solution (saccharin) in 25-mL sipper tubes as the conditioned stimulus (CS). Consumption was measured by weighing the sipper tubes before and after the 10-min session to 0.1 g. Immediately following saccharin access, the mice were taken from their home cages, carried to the magnet (~50 m), and exposed to the 14.T MF for 30 min as the unconditioned stimulus (US). Immediately after magnet or sham exposure, locomotor activity was recorded for 2 min, as described above, and mice were then returned to their home cages. The conditioning procedure was repeated for 3 consecutive days. Water was returned ad libitum after the third conditioning.

Strength of conditioning was measured by 24-h two-bottle extinction testing, beginning the day after the third MF exposure. Mice were given ad libitum 24-h access to both water and 0.125% saccharin solutions in two 25-mL sipper tubes, with the bottles placed side by side. Each day, the placement of the bottles was alternated to observe possible position bias. Consumption of both water and saccharin was measured daily by weight (0.1 g accuracy). The preference score was calculated for each mouse for each day by dividing the saccharin consumed by the total fluid consumed: saccharin/(water + saccharin). A score of 1.0 would indicate that all fluid intake was saccharin; the lower the preference score, the stronger the aversion, with aversion operationally defined as a significant decrease in preference compared with sham controls. The 24-h preference tests continued for 5–8 days.

As an additional test of capability to form taste aversions, some mice were subsequently tested with a Kool-Aid flavor as different novel CS paired with LiCl as a nonmagnetic US (*experiments 2b* and *5b*). LiCl is a commonly used toxin that robustly induces CTA in rodents, acting via chemoreceptors in the brainstem area postrema (20). Mice were placed on a water-restriction schedule as above, then given 10-min

access to a 0.05% grape Kool-Aid mixed with 0.125% saccharin. Immediately following Kool-Aid access, mice were administered LiCl injections (40 mL/kg, 0.15 M ip). Only a single Kool-Aid-LiCl conditioning trial was conducted. After LiCl injection, ad libitum water was returned to the mice. Beginning 24 h after conditioning, 24-h 2-bottle extinction tests of Kool-Aid versus water were conducted for 2 days.

Statistical analysis.

Circling and rearing behavior were analyzed with two-way ANOVA with genotype and treatment as factors or two-way ANOVA with one repeated measure when observed across multiple test days. CTA preference was analyzed with two-way ANOVA with one repeated measure across extinction days. Analyses were performed with Prism (GraphPad).

Experiment 1. Behavioral Responses to 14.1 T Exposure in *het* Mice

On 2 consecutive days, nondeprived wild-type (+/+; n = 6 female) and homozygous *het* (−/−; n = 5 female and n = 9 male) mice were restrained in 50-mL conical centrifuge tubes and exposed for 30 min within the 14.1 T magnet or sham exposed. Immediately after exposure, the mice were released into a Plexiglas cage and their locomotor activity was recorded by video for 5 min, and the behavior was scored as in *General Methods* for number of circles, latency to rear, and number of rears. Sham exposure and MF exposure were counterbalanced across the 2 days, so that every mouse received 1 MF exposure and 1 sham exposure.

Experiment 2. CTA after 14.1 T Exposure and LiCl in *het* Mice

Experiment 2a. MF-induced CTA.

Naive mice (16 wild-type and 32 $het^{-/-}$) were placed on water restriction as described above. On the first conditioning day, mice were given 10-min access to saccharin. Individual mice were then placed in plastic cups with lids and exposed for 30 min in either the 14.1 T magnet or sham exposed as described above. Thus, there were six groups: wild-type-sham (n = 8), *het*-sham (n = 14), wild-type-magnet (n = 8), *het*-magnet (n = 12).

Immediately after MF or sham exposure, mice were released into a Plexiglas cage and locomotor behavior recorded for 2 min as described above. Mice were then returned to their home cages. The conditioning procedure and behavioral recording was repeated on a total of 3 consecutive days.

After the third conditioning trial, ad libitum water was returned to the mice. Twenty-four hours later, 2-bottle, 24-h preference tests were begun between water and saccharin. The preference tests continued for a total of 8 days.

Experiment 2b. LiCl-induced CTA.

The same mice used in *experiment 2a* were again placed on water restriction. On a single conditioning day, mice were given 10-min access to Kool-Aid (0.05% grape Kool-Aid 0.125% saccharin), followed by an injection of either LiCl or NaCl (0.15 M, 40 mL/kg ip). Mice that were exposed in the MF in *experiment 2a* received LiCl injections, whereas mice that were sham exposed in *experiment 2a* received NaCl injections. After conditioning, ad libitum water was returned to the mice. Twenty-four hours later, 2-bottle, 24-h preference tests were begun between water and Kool-Aid. The preference tests continued for a total of 2 days.

Experiment 3. c-Fos Expression after 14.1 T Exposure in *het* Mice

Nondeprived female wild-type mice (n = 12) and homozygous female $het^{-/-}$ mice (n = 12) were restrained in 50-mL conical tubes and either exposed in the 14.1 T magnet or sham exposed for 30 min (n = 6 in each condition for each genotype). Immediately after magnet or sham exposure, mice were released into a Plexiglas cage and their locomotor activity video-recorded for 2 min, and the behavior scored as in *General Methods* for number of circles, latency to rear, and number of rears.

c-Fos immunohistochemistry.

One hour after magnet or sham exposure, mice were overdosed with pentobarbital sodium. When completely unresponsive, the mice were perfused transcardially, first with 100 mL of isotonic saline/0.5% sodium nitrite/1,000 U heparin and then with 400-mL phosphate-buffered 4% paraformaldehyde. The brains were removed, blocked, postfixed for 24 h, and then transferred into 30% sucrose at 4°C for 24 h to 1 wk before sectioning. Tissue from all treatment groups was processed in parallel. Coronal sections (40 μm) were cut on a freezing, sliding microtome. Alternate sections were immediately processed after cutting for c-Fos immunohistochemistry.

Free-floating tissue sections were washed twice for 15 min in 0.1 M phosphate-buffered saline (PBS) and then incubated for 30 min in 0.2% Triton X-100/1% BSA-PBS. After two washes in PBS/BSA for 15 min each, sections were incubated overnight with a rabbit anti-c-Fos antiserum (Ab-5; Oncogene Research) at a dilution of 1:20,000. After two 15-min washes in PBS/BSA, sections were then incubated for 1 h with a biotinylated goat anti-rabbit antibody (Vector Laboratories) at a dilution of 1:200. Antibody complexes were amplified using the Elite Vectastain ABC kit (Vector Laboratories) and visualized via a 5-min reaction in 0.05% 3,3-diaminobenzidine tetrahydrochloride. Sections were stored in 0.1 M PBS until mounted onto gelatin-coated glass slides, counterstained with methyl green (Vector Laboratories), and coverslipped using Permount.

Cells expressing darkly positive, nuclear c-Fos immunoreactivity were quantified using a custom software program (MindsEye 1.19 b; T. Houpt). Images were digitally captured in a 0.72 × 0.54 mm counting frame. Cell counts were restricted to three brainstem nuclei associated with vestibular responses: prepositus nucleus (Prp; bregma −6.2 to −6.0 mm, 10.3 ± 2.4 sections); medial vestibular nucleus (MeV; bregma −6.2 to −6.0 mm, 12.4 ± 2.4 sections); supragenualis nucleus (SGe; bregma −5.8 to −5.7 mm, 4.0 ± 0.8 sections); spinal vestibular nucleus (SpVe; bregma −6.96 to −6.12 mm, 22.1 ± 4.45 sections); lateral vestibular nucleus (LVe; bregma −6.24 to −5.8 mm, 11.88 ± 3.64 sections); superior vestibular nucleus (SuVe; bregma −6.0 to −5.4 mm, 15.71 ± 3.79 sections); and two nuclei associated with visceral and stress responses: nucleus of the solitary tract (NTS; bregma −7.6 to

−7.3 mm, 11.0 ± 2.3 sections) and locus coeruleus (LC; bregma −5.5 to −5.3 mm, 4.0 ± 1.1 sections). Coordinates were based on those in the atlas by Paxinos and Franklin (21). These five regions were delineated by a hand-drawn outline on the left and right sides of the brainstem. The individual mean counts per section for each region were averaged within each mouse and then averaged across mice within experimental groups.

Experiment 4. Behavioral Responses to 14.1 T Exposure in *tlt* Mice

Nondeprived female wild-type (+/+; n = 10) and female *tlt* mice (−/−; n = 10) were restrained in 50-mL conical centrifuge tubes and either exposed for 30 min within the 14.1 T MF or sham exposed (n = 5 for each genotype and treatment). Immediately after exposure, the mice were released into a Plexiglas cage, their locomotor activity was recorded by video for 3 min, and their behavior was scored as in *General Methods* for number of circles, latency to rear, and number of rears. Sham exposure and MF exposure were counterbalanced across the 2 days, so that every mouse received 1 MF exposure and 1 sham exposure.

Experiment 5. CTA after 14.1 T Exposure and LiCl in *tlt* Mice

Experiment 5a. Magnet-induced CTA.

Naive mice (12 wild-type$^{+/+}$ and 11 *tlt*$^{-/-}$) were placed on water restriction as above. On the first conditioning day, mice were given 10-min access to saccharin. Individual mice were then placed in cups and exposed for 30 min in either the 14.1 T MF, or sham exposed. Thus there were four groups: wild-type-sham (n = 6 males), homozygous-sham (n = 1 female, 3 males), wild-type-magnet (n = 6 males), and homozygous-magnet (n = 3 females, 4 males).

Immediately after MF or sham exposure, mice were released into a Plexiglas cage and locomotor behavior recorded for 2 min as described above. Number of circles, latency to rear, and number of rears were scored. Mice were then returned to their home cages. The conditioning procedure and behavioral recording were repeated on a total of 3 consecutive days.

After the third conditioning trial, ad libitum water was returned to the mice. Twenty-four hours later, 2-bottle, 24-h preference tests were begun between water and saccharin. The preference tests continued for a total of 5 days.

Experiment 5b. LiCl-induced CTA.

The same mice used in *experiment 5a* were again placed on water restriction. On a single conditioning day, mice were given 10-min access to Kool-Aid (0.05% grape Kool-Aid 0.125% saccharin), followed by an injection of either LiCl or NaCl (0.15 M, 40 mL/kg ip). Mice that were exposed in the magnet in *experiment 5a* received LiCl injections, whereas mice that were sham exposed in *experiment 5a* received NaCl injections. After conditioning, ad libitum water was returned to the mice. Twenty-four hours later, 2-bottle, 24-h preference tests were begun between water and Kool-Aid. The preference tests continued for a total of 2 days.

RESULTS

Experiment 1

Sham-exposed control mice of both genotypes did not circle at all and showed frequent rearing in the test cage. After 30-min exposure to 14.1 T MF, restrained wild-type mice showed counterclockwise locomotor circling, suppressed rearing, and increased latency to rear compared with sham-exposed controls. MF-exposed *het* mice did not circle and had rearing similar to sham-exposed mice; thus, the mutants showed no significant response to MF exposure (see Fig. 1 and Supplemental Video S1).

For circling, two-way ANOVA with factors "Genotype" and repeated "Treatment" revealed significant effects of Genotype [F (1, 18) = 424.0, $P < 0.0001$], Treatment [F (1, 18) = 427.8, $P < 0.0001$], and a significant interaction [F (1, 18) = 427.8, $P < 0.0001$]. For rearing, there was a significant effect of Genotype

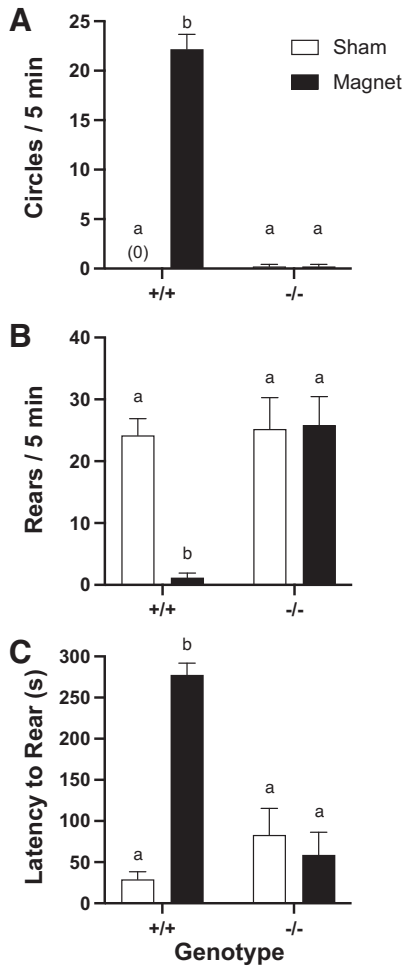

Figure 1. Locomotor behavior after restrained 14.1 T magnet exposure or sham exposure in wild-type (n = 6) and *het* mutant mice (n = 14). Compared with their own sham exposure, wild-type mice showed significantly increased locomotor circling (*A*; mean circles/2 min ± SE), decreased rearing (*B*; mean rears/2 min ± SE), and increased latency to rear (*C*; mean seconds to first rear ± SE). Locomotor behavior of *het* mice after magnet exposure was not different from their own sham exposure: *het* mice only rarely circled, and showed high levels of rearing with low latency in both conditions. Groups with different letters were significantly different from each other, $P < 0.05$ (Sidak's post hoc test).

[F (1, 18) = 7.723, $P < 0.05$], with no effect of treatment or an interaction effect. For latency to rear, there were significant effects of Treatment [F (1, 18) = 14.78, $P < 0.01$], Genotype [F (1, 18) = 4.98, $P < 0.05$], and a significant interaction [F (1, 18) = 21.88, $P < 0.001$].

Experiment 2

Acute behavioral responses after 14.1 T exposure.

Consistent with an earlier report of unrestrained exposure in cups (1), none of the mice circled. After 30-min exposure to 14.1 T MF, wild-type animals showed reduced rearing and increased latency to rear compared with sham-exposed controls. MF-exposed *het* mice showed no significant reduction in rearing or increase in latency to rear on *day 1* and actually showed an increase in rearing and a decrease in latency to rear on *days 2* and *3* compared with sham-exposed controls (see Fig. 2).

For rearing, two-way ANOVA with factors "Group" and repeated "Days" revealed significant effects of Group [F (3, 38) = 15.13, $P < 0.0001$), Day [F (1.663,63.19] = 8.204, $P < 0.01$), and a significant interaction [F (6, 76) = 3.705, $P < 0.01$]. For latency to rear, there were significant effects of Group [F (3, 38) = 16.70, $P < 0.0001$], Day [F (1.950, 74.09) = 7.004, $P < 0.01$], and a significant interaction [F (6, 76) = 5.925, $P < 0.0001$].

MF-induced CTA.

Sham-exposed wild-type mice showed a high preference for saccharin over water on the first day. MF-exposed wild-type mice showed a significantly lower preference for saccharin on the first 2-bottle test day; their CTA extinguished over 4–5 days. All MF-exposed mutant *het* groups showed a high preference for saccharin on all days that was not significantly different from their sham controls or wild-type sham-exposed mice. Thus, repeated pairing of 14.1 T MF exposure with saccharin induced a CTA in wild-type mice but not in *het* mutants (see Fig. 3, *A* and *B*).

For *day 1* preference, two-way ANOVA with factors "Genotype" and "Treatment" revealed significant effects of Genotype [F (1, 38) = 45.07, $P < 0.0001$], Treatment [F (1, 22) = 57.21, $P < 0.0001$], and a significant interaction [F (1, 38) = 44.16, $P < 0.0001$]. For CTA extinction, two-way ANOVA with factors "Group" and repeated "Day" revealed significant effects of Group [F (3, 38) = 10.32, $P < 0.0001$), Day [F (2.147, 81.58] = 27.20, $P < 0.0001$), and a significant interaction [F (21, 266) = 12.68, $P < 0.0001$].

LiCl-induced CTA.

To demonstrate that the *het* mutant mice could acquire a CTA when a nonmagnetic US was used, mice were given access to Kool-Aid paired with LiCl. Mice given LiCl showed a low preference for Kool-Aid compared with saline controls regardless of genotype (see Fig. 3*C*).

For preference after LiCl treatment, two-way ANOVA with factors "Genotype" and "Treatment" revealed a significant effect of Treatment [F (1, 29) = 92.17, $P < 0.0001$].

Experiment 3

Acute behavioral responses.

Sham-exposed control mice of both wild-type and *het* genotypes did not circle at all and showed frequent rearing in the test cage. After 30-min exposure to 14.1 T MF, consistent with the behavior seen in *experiment 1*, restrained wild-type mice circled counterclockwise, showed a suppressed number of rears, and an increased latency to rear compared with sham-exposed controls. MF-exposed *het* mice did not circle and had similar rearing behavior to wild-type sham controls, again demonstrating mutants show no significant behavioral response to MF exposure (see Fig. 4).

For circling, two-way ANOVA with factors "Genotype" and "Treatment" revealed significant effects of Genotype, Treatment, and a significant Interaction of equivalent values: [F (1, 20) = 15.44, $P < 0.001$]. For rearing, there was a significant effect of Treatment [F (1, 20) = 18.56, $P < 0.001$] and a significant interaction [F (1, 20 = 15.41, $P < 0.001$] but no effect of Genotype. For latency to rear, there were significant effects of Treatment [F (1, 20) = 52.76, $P < 0.0001$], Genotype [F (1, 20) = 72.17, $P < 0.0001$], and a significant interaction [F (1, 20) = 55.99, $P < 0.001$].

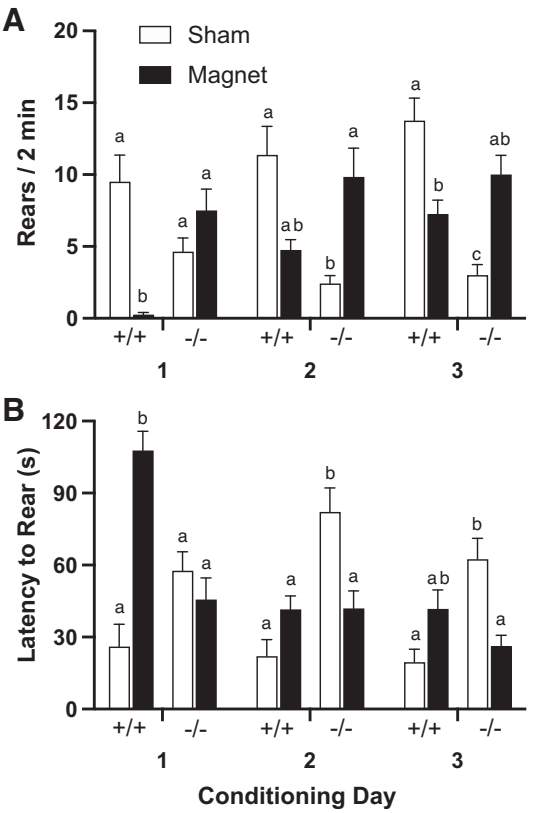

Figure 2. Locomotor behavior after free moving 14.1 T magnet exposure or sham in wild-type and *het* mutant mice across 3 days of saccharin-magnet conditioning. *A*: rearing (mean rears/2 min ± SE). Compared with sham-exposed wild-type mice (n = 8), rearing was significantly reduced in magnet-exposed wild-type mice (n = 8) on *day 1*, but they showed increased rearing after magnet exposure on *days 2* and *3*. In contrast, magnet-exposed *het* mice (n = 12) showed more rearing than sham-exposed *het* mice (n = 14) on all 3 days. Rearing in magnet-exposed mutants was not significantly different from wild-type sham on any day. *B*: latency to rear (mean seconds to first rear ± SE). Wild-type magnet-exposed mice showed a significant increase in latency on *day 1* compared with sham-exposed wild-type mice, but this effect was lost on *days 2* and *3*. Latency to rear was lower, but not significantly, in magnet-exposed *het* compared with sham-exposed *het*. Groups with different letters were significantly different from each other, $P < 0.05$ (Tukey's HSD). HSD, honestly significant difference.

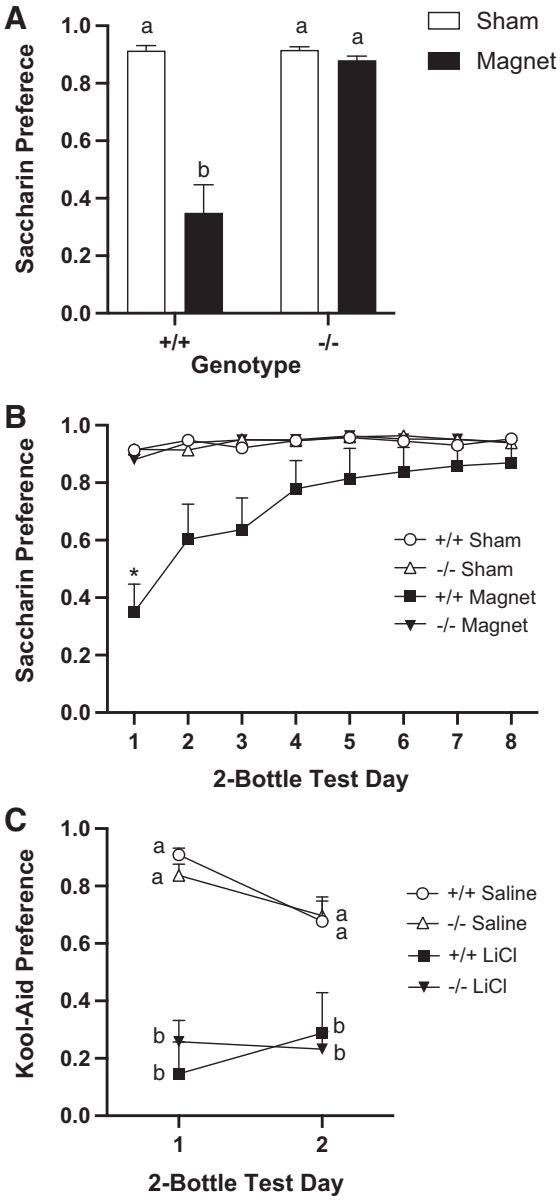

Figure 3. CTA after magnet exposure or LiCl injection in wild-type and *het* mutant mice (n = 8–14/group). *A*: CTA initial magnitude (mean preference ± SE) measured on the first day of 2-bottle testing after three pairings of saccharin with magnet exposure or sham exposure. Wild-type mice receiving magnet exposure paired with saccharin showed a significantly reduced preference compared with sham-exposed mice. Sham- and magnet-exposed *het* mice showed a high preference and hence no CTA. *B*: CTA extinction. Wild-type mice receiving magnet exposure paired with saccharin showed gradual extinction of their CTA; all other mice continued to show a high preference for saccharin. *C*: LiCl-induced CTA in 2 consecutive 24-h 2-bottle preference tests. After pairing saccharin with LiCl injections (black symbols), both wild-type and *het* mice showed significant aversions to saccharin compared with saline-injected mice (white symbols). *Significantly different from sham or saline controls; groups with different letters were significantly different from each other, $P < 0.05$ (Tukey's HSD). HSD, honestly significant difference.

Quantification of c-Fos immunoreactivity.

One hour after MF or sham exposure mice were euthanized and processed for c-Fos immunoreactivity. Significantly greater c-Fos expression was measured in the Prp and SG of MF-exposed wild-type mice compared with sham controls; *het* mice showed no significant increase in c-Fos expression after MF exposure, resembling their sham controls in all tested regions (see Figs. 5 and 6).

For NTS, two-way ANOVA with factors "Genotype" and "Treatment" revealed a significant effect of Genotype [F (1, 20) = 7.323, $P < 0.01$] and no effect of Treatment or an interaction. For Prp, there were significant effects of Genotype [F (1, 20) = 15.72, $P < 0.001$], Treatment [F (1, 20) = 11.90, $P <$

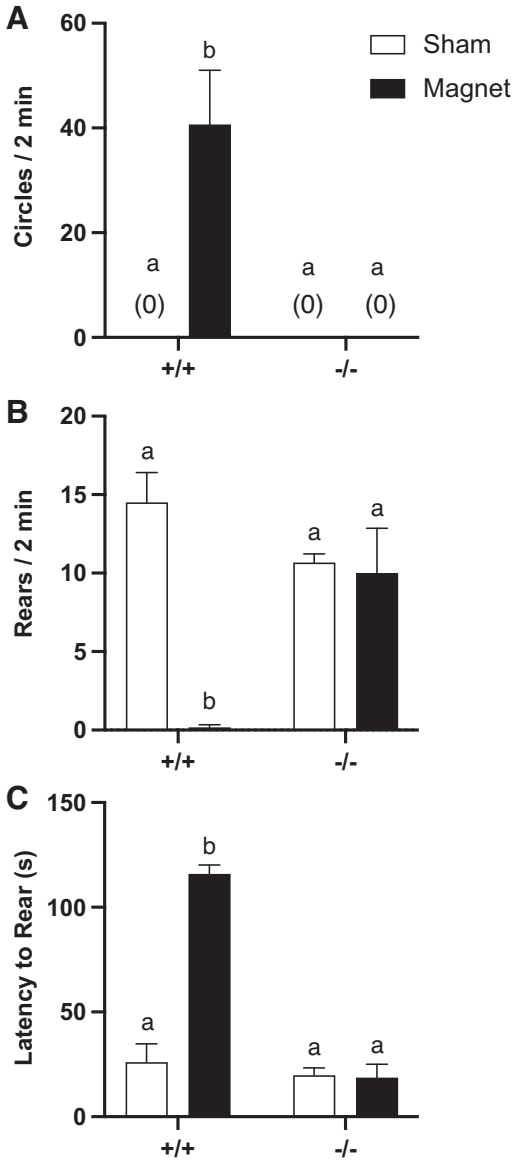

Figure 4. Locomotor after restrained 14.1 T magnet exposure or sham exposure in wild-type and *het* mutant mice (n= 6/group) before c-Fos immunohistochemistry. *A*: circling behavior (mean circles/2 min ± SE). Only magnet-exposed wild-type mice showed significant locomotor circling compared with the sham group; magnet-exposed *het* mice did not circle at all. *B*: rearing (mean rears/2 min ± SE). Rearing was reduced to zero in magnet-exposed wild-type mice; *het* mutants were indistinguishable from sham-exposed mice. *C*: latency to rear (mean sec to first rear ± SE). Magnet-exposed wild-type mice showed a significant increase in latency compared with shams; there was no difference between *het* groups. Groups with different letters were significantly different from each other, $P < 0.05$ (Tukey's HSD). HSD, honestly significant difference.

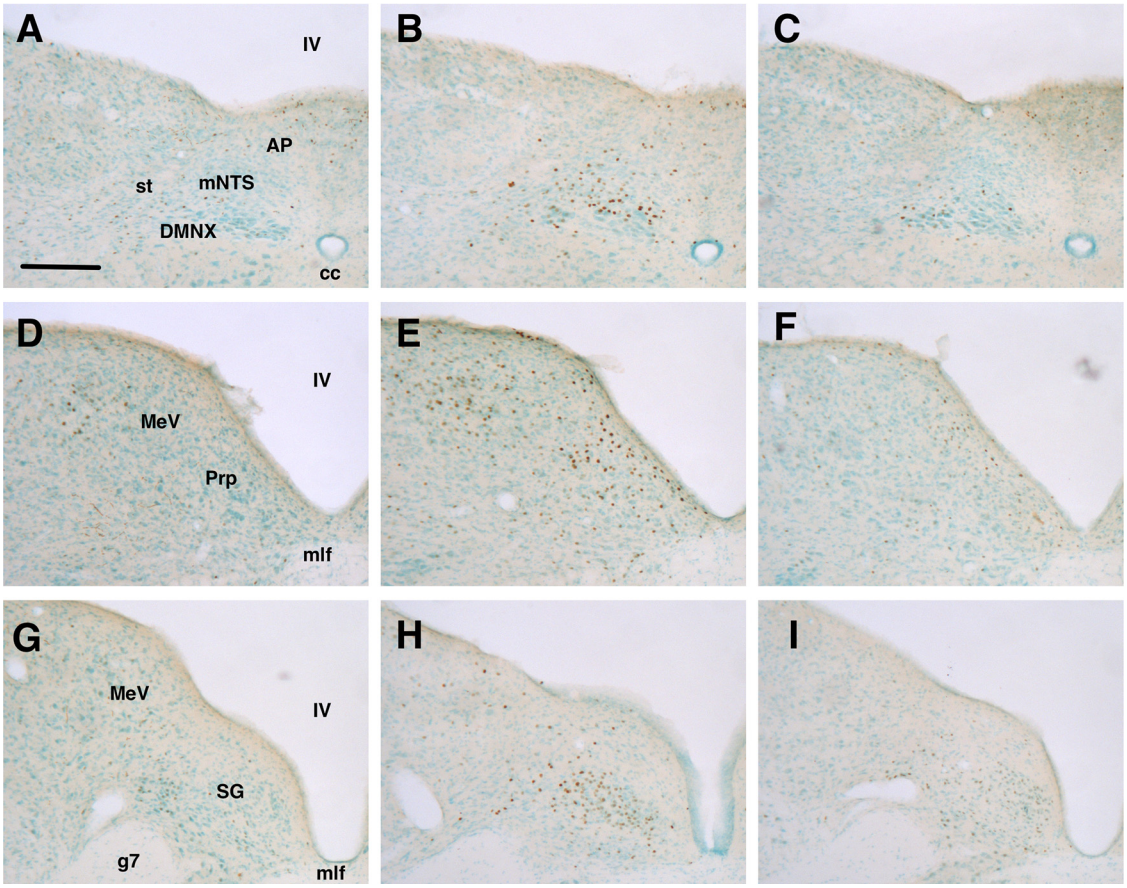

Figure 5. Photomicrographs of c-Fos immunohistochemistry in the brainstem of wild-type (*A*, *B*, *D*, *E*, *G*, and *H*) and *het* mutant mice (*C*, *F*, and *I*) after sham exposure (*A*, *D*, and *G*) or 14.1 T magnetic field exposure (*B*, *C*, *E*, *F*, *H*, and *I*). Wild-type, but not *het* mutant mice, showed increased c-Fos-positive nuclei in the NTS, Prp, and SG compared with sham-exposed mice. Scale bar: 200 μm. AP, area postrema; cc, central canal; DMNX, dorsal motor nucleus of the vagus; g7, genu of the seventh nerve; IV, fourth ventricle; MeV, medial vestibular nucleus; mlf, medial longitudinal fascile; NTS, nucleus of the solitary tract; Prp, prepositus nucleus; SG, supragenualis nucleus; st, solitary tract.

0.01], but no Interaction. For SG, there was only a significant effect of Genotype [F (1, 20) = 9.381, $P < 0.01$]. For MeV, there was only a significant effect of Genotype [F (1, 20) = 27.92, $P < 0.0001$]. For LC, there was a significant effect of Genotype [F (1, 20) = 16.76, $P < 0.001$] and an Interaction [F (1, 20) = 4.719, $P < 0.05$]. For SpVe, there was a significant effect of treatment [F (1, 19) = 9.67, $P < 0.01$]. No significant differences between groups were found for the LVe and SuVe.

Experiment 4

No circling was observed in either wild-type or *tlt* mutant mice after restrained sham exposure: both genotypes showed numerous tight circles after MF exposure. Both genotypes also showed an increased latency to rear after MF exposure compared with sham exposure. Wild-type mice showed significantly fewer rears after MF exposure compared with sham exposure; the reduction of rearing in mutant *tlt* mice after MF exposure was not significant (see Fig. 7 and Supplemental Video S1).

Two-way ANOVA with factors "Genotype" and "Treatment" revealed only a significant effect of Treatment on all behaviors: circling [F (1, 8) = 24.96, $P < 0.01$], rearing [F (1, 8) = 12.55, $P < 0.01$], and latency to rear [F (1, 8) = 42.53, $P < 0.001$].

Experiment 5

Acute behavioral responses after 14.1 T exposure.

None of the mice circled after MF exposure in cups. After 30-min exposure to 14.1 T MF, unrestrained wild-type mice showed reduced rearing and increased latency to rear compared with sham-exposed controls on the first 2 days. Although MF-exposed *tlt* mice showed a mean decrease in rearing and an increase in latency to rear on all days compared with their sham-exposed controls, these effects were not significant (see Fig. 8).

For rearing, two-way ANOVA with factors "Group" and repeated "Day" revealed a significant effect of Group [F (3, 20) = 109.0, $P < 0.0001$] and an Interaction [F (6, 40) = 2.569, $P < 0.05$]. For latency to rear, there were significant effects of Group [F (3, 20) = 8.958, $P < 0.001$], and Day [F (1.591, 31.82) = 4.737, $P < 0.05$].

MF-induced CTA.

Sham-exposed wild-type mice showed a high preference for saccharin over water on the first day. MF-exposed wild-type mice showed a significantly lower preference for saccharin on the first 2-bottle test day, and their CTA extinguished over 4–5 days. MF-exposed *tlt* mice showed a high

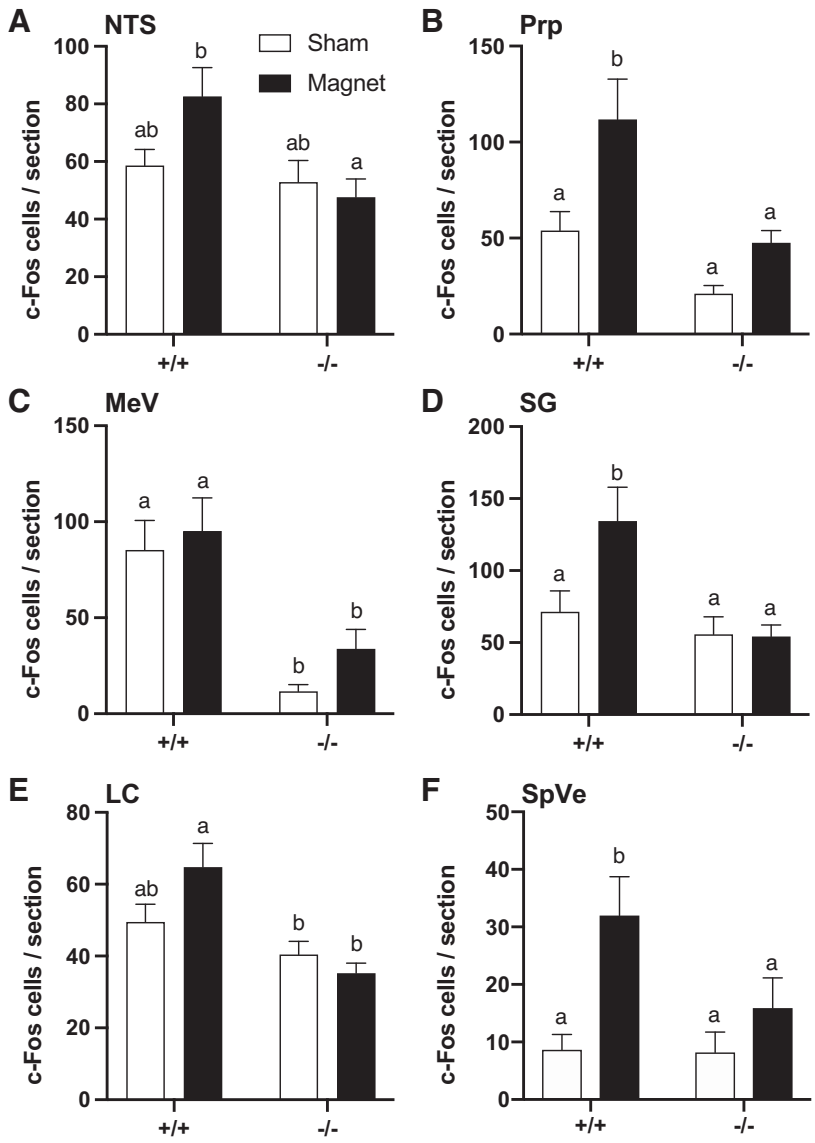

Figure 6. Quantification of c-Fos expression (mean number of c-Fos expressing cells/section) after restrained 14.1 T magnet exposure or sham exposure in wild-type and *het* mutant mice (n = 6/group, same mice as Fig. 4). Magnet exposure significantly increased c-Fos expressing cells compared with sham controls in the NTS (*A*), Prp (*D*), SG (*E*), and SpVe (*F*) of wild-type mice but not *het* mice. In the MeV (*C*), *het* mutants had significantly lower c-Fos expression irrespective of exposure condition. In the locus coeruleus (LC), there was little or no difference between all groups (*B*). Groups with different letters were significantly different from each other, $P < 0.05$ (Tukey's HSD). HSD, honestly significant difference; MeV, medial vestibular nucleus; NTS, nucleus of the solitary tract; Prp, prepositus nucleus; SG, supragenualis nucleus; SpVe, spinal vestibular nucleus.

preference for saccharin on all days that was not significantly different from their sham controls or wild-type sham-exposed mice. Thus, repeated pairing of 14.1 T MF exposure with saccharin induced a CTA in wild-type mice but not in *tlt* mutants (see Fig. 9, *A* and *B*).

For *day 1* preference, two-way ANOVA with factors "Genotype" and "Treatment" revealed only a significant effect of Treatment [F (1, 19) = 11.27, $P < 0.01$]. For CTA extinction, two-way ANOVA with factors "Group" and repeated "Day" revealed significant effects of Group [F (3, 19) = 3.498, $P < 0.05$], Day [F (2.279, 43.30) = 8.645, $P < 0.001$], and a significant interaction [F (12, 76) = 3.292, $P < 0.001$].

LiCl-induced CTA.

To demonstrate that the *tlt* mutant mice could acquire a CTA when a nonmagnetic US was used, mice were given access to Kool-Aid paired with LiCl. Mice given LiCl showed a low preference for saccharin regardless of genotype compared with saline controls (see Fig. 9*C*).

For preference after LiCl treatment, two-way ANOVA with factors "Genotype" and "Treatment" revealed a significant effects of both Genotype [F (1, 20) = 5.421, $P < 0.05$] and Treatment [F (1, 20) = 245.7, $P < 0.0001$].

DISCUSSION

The role of otoconia in MF-induced perturbation was assessed using two mutant strains of mice (*het* and *tlt*) lacking proteins critical for otoconia genesis. As previously reported, wild-type mice exposed to a static 14.1 T MF showed locomotor circling behavior; a significant reduction in rearing; acquired a CTA after pairing MF exposure with saccharin; and significant c-Fos expression in brainstem vestibular and visceral nuclei (1–4). Mutant *het* mice lacking otoconia showed no perturbation of locomotor behavior after MF exposure, however, with normal rearing and no circling, comparable to sham-exposed mice. Correlated with the lack of behavioral responses, *het* mice also showed decreased induction of c-Fos in visceral and vestibular brain

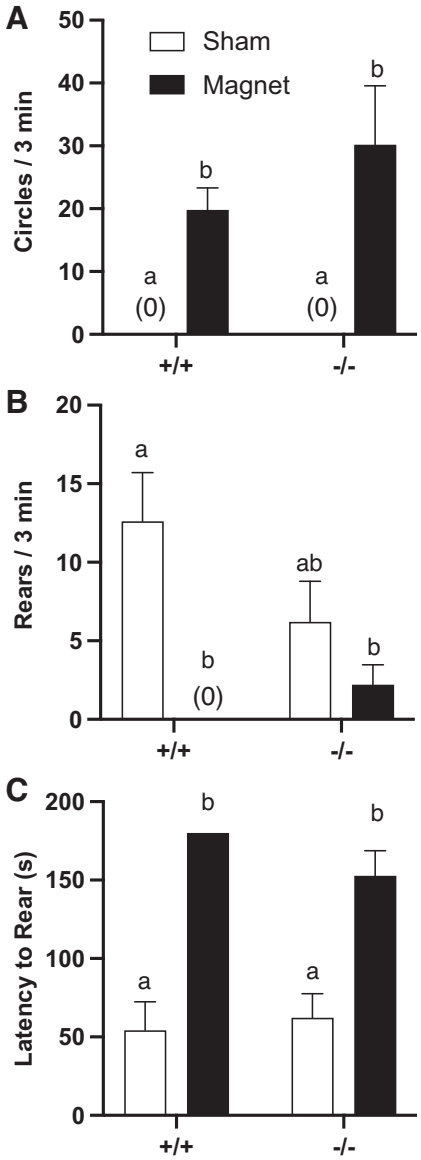

Figure 7. Locomotor behavior after restrained 14.1 exposure or sham exposure in wild-type and *tlt* mutant mice (n = 5/group). *A*: circling behavior (mean circles/2 min ± SE). No mice circled in the sham condition for either genotype. Magnet exposure induced circling in both genotypes. *B*: rearing (mean rears/2 min ± SE). Rearing was significantly reduced in magnet-exposed wild-type mice compared with sham exposure, and *tlt* mutants showed a nonsignificant reduction in rearing after magnet exposure. *C*: latency to rear (mean seconds to first rear ± SE). Wild-type and mutant magnet-exposed mice showed significant increase in latency compared with their own sham exposures. Groups with different letters were significantly different from each other, $P < 0.05$ (Tukey's HSD). HSD, honestly significant difference.

regions after MF exposure. These results in *het* mice are consistent with a recent report that MF-induced nystagmus is also absent in *het* mice (5). Mutant *tlt* mice, which also largely lack otoconia, also failed to acquire MF-induced CTA, although they did show MF responses in rearing and circling similar to wild-type mice. The failure of *het* and *tlt* mice to acquire MF-induced CTA was not due to general learning deficits, as both strains readily acquired LiCl-induced CTA.

These results are consistent with the inner ear, and specifically the otolith organs, as the site of MF effects, manifesting as vestibular perturbation, in rodents and humans. MF exposure, similar to vestibular stimulation, induces circling locomotion (2), suppressed rearing (2), suppressed intake (23), c-Fos in brainstem vestibular nuclei (4), and nystagmus in rodents (5). An aversive quality to MF exposure is implied by high MF avoidance (24) and CTA acquisition (19). Vestibular perturbation is also observed in humans, with nystagmus and subjective experience of bodily rotation in the bore of high-strength MRI machines (9). The essential role of the inner ear has been shown by the lack of response to MF of labyrinthectomized rats (11) and humans (9).

The c-Fos observed after MF exposure in key vestibular nuclei further supports action on the vestibular sensory organs. Stimulation of the otoliths and semicircular canals is known to result in significant c-Fos expression in the vestibular nuclei (25–29). The lack of c-Fos in *het* mice is consistent with a loss of inner ear stimulation, comparable to a previous report that *het* mice showed significantly less c-Fos in forebrain nuclei after vestibular stimulation (30).

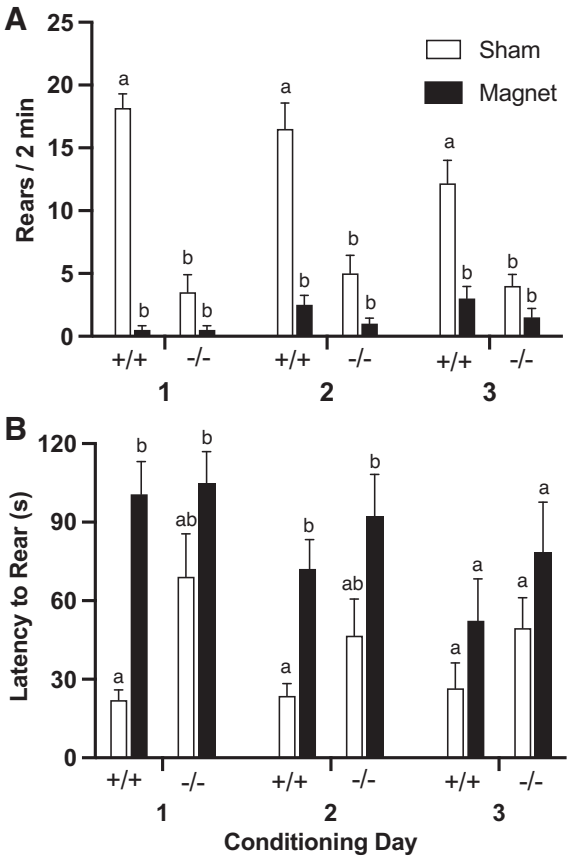

Figure 8. Locomotor behavior after free moving 14.1 exposure or sham exposure in wild-type and *tlt* mutant mice (n = 6/group). Compared with sham exposure, wild-type magnet-exposed mice showed significantly reduced rearing on all 3 days (*A*; mean rears/2 min ± SE), and a significantly increased latency to rear (*B*; mean seconds to first rear ± SE) on *days 1* and *2*. Magnet-exposed *tlt* mice were not significantly different from their own sham on all days for rearing and latency to rear, but *tlt* sham mice had significantly less rearing compared with wild-type sham. Groups with different letters were significantly different from each other, $P < 0.05$ (Tukey's HSD). HSD, honestly significant difference.

The *het* and *tlt* mutants are models of otolith organ dysfunction resulting from mutations in genes that are critical for the formation of otoconia: NOX3 for *het* and OTOP1 in the *tlt*. The *het* mice appear otherwise anatomically normal with intact ossicles, cochlea, and semicircular canals (17) along with hair cells and synapses (18). The *tlt* mice are the same, except for the occasional presence of abnormally large otoconia in the saccule (12, 31). Functionally, both mutants are unable to swim, lack linear vestibular-evoked potentials, and lack gravity-dependent vestibular-ocular reflexes (VORs) (13, 15, 16). Therefore, deficits in MF responsiveness are likely due to the lack of otoconia or otolith organ functioning, either directly or indirectly; i.e., changes in MF responsiveness may also be due to deficits in canal function accompanying the otolith deficit.

However, *tlt* mice still show circling and suppressed rearing after MF exposure, possibly because they have a graded responsiveness to the MF. The threshold for MF responsiveness may be increased in *tlt* mice; for example, although CTA is not induced by 14.1 T exposure, the high MF still induces circling and suppresses rearing. It is possible that at lower field strengths, all MF responses might be absent in *tlt*.

Alternatively, the *tlt* mice may be more responsive if they have residual otoconia or have greater semicircular canal function than the *het* mice. The presence of residual otoconia in the *tlt* is unlikely: the *tlt* mice in this study were all unable to swim, whereas the rare mouse possessing large otoconia in the saccule shows an intermediate swimming ability (12).

Mutant *tlt* mice have better semicircular canal integration compared with *het* mutants: *tlt* mice show a more robust rotational VOR (32) and enhanced optokinetic compensation (31) compared with *het* mice, which show deficits in rotationally stimulated VOR. The rotational deficit in *het* mice has been suggested to be due to their mutation compromising not only otolith organs but also canal afferent output or central canal processing, as evidenced by VOR deficits in het mice (33).

In other words, *tlt* mice may be a more specific otoconia-knockout model, whereas the *het* mice have both a profound otolith deficit and also some semicircular canal integration deficits. Taken together, these results show that the presence of otoconia is necessary for full responses to static high MF, but the greater deficit in *het* mice implicates a role for semicircular integration as well.

The proximal mechanism by which MF interacts with the inner ear is still unknown. The lack of responsiveness in the otoconia mutant mice suggests several logical possibilities:

1) The MF may apply a force to the otoconia themselves. The MF could exert torque on the otoconia themselves, causing bending of utricular hair cells and opening of their mechanosensitive ion channels. This is unlikely,

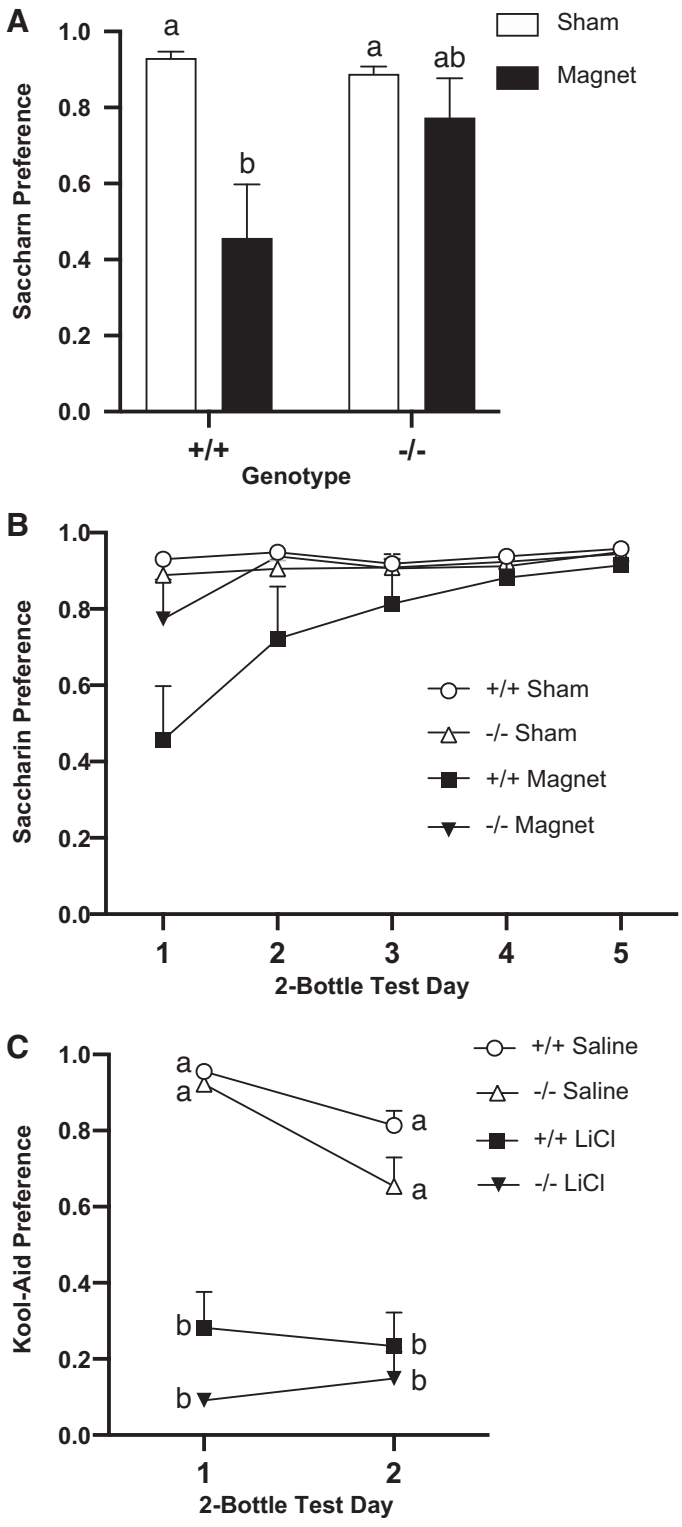

Figure 9. CTA after free moving 14.1 exposure or LiCl injection in wild-type and *tlt* mutant mice (n = 5–6/group). *A*: CTA initial magnitude (mean preference ± SE) measured on the first day of 2-bottle testing after 3 pairings of saccharin with magnet exposure or sham exposure. Wild-type mice receiving magnet exposure paired with saccharin showed a significantly reduced preference compared with sham-exposed mice. Sham- and magnet-exposed *tlt* mice showed a high preference and hence no CTA. *B*: CTA extinction. Wild-type mice receiving magnet exposure paired with saccharin showed gradual extinction of their CTA; all other mice continued to show a high preference for saccharin. *C*: LiCl-induced CTA in 2 consecutive 24-h 2-bottle preference tests. After saccharin was paired with LiCl injections (black symbols), both wild-type and *tlt* mice showed significant aversions to saccharin compared with saline-injected mice (white symbols). Groups with different letters were significantly different from each other, $P < 0.05$ Tukey's HSD. HSD, honestly significant difference.

however, due to the low magnetic susceptibility of calcium carbonate, the primary constituent of otoconia (34, 35).

2) MF transduction may require the semicircular canals, and otoconia mutants might also have compromised semicircular function. The semicircular canals appear structurally normal in both mutants, but *het* mice have been shown to be deficient in their rotational VOR, with lower gain compared with wild-type controls in response to transient horizontal and sinusoidal rotations (5, 33). It is also possible that *het* and *tlt* mice may have different frequency responsiveness; i.e., if the *tlt* was more responsive to low frequencies, it might be more sensitive to magnetic field effects. The greater deficit in MF responsiveness in the *het* mice is therefore consistent with a contribution of the semicircular canals.
3) MF transduction may be dependent on an incidental property of the otolith organs required for semicircular stimulation, for example, the flow of K^+ ions into utricular hair cells underlying a Lorentz force. In a static field, if charged particles are moving in a current perpendicular to the field direction, a Lorentz force is exerted orthogonal to both the field and current. A flow of K^+ ions from the direction of the caudal/superior side of the vestibule to the ventral/inferior hair cells of the utricle has been proposed as the substrate of moving charges; a Lorentz force here would cause a magnetohydrodynamic pressure on the cupula of the horizontal canal, leading to aberrant rotational signaling (5, 9, 36). Based on prior modeling (36, 37), the Lorentz forces would act on the greater surface area of the cupula and not the on the utricular maculae, because the greatest current density is proposed to be above the maculae, and not adjacent to it.

Consistent with the geometry required for a Lorentz force, the effects of the high MF are dependent on head position. In rodents, MF effects are greatest when the rostral-caudal axis is parallel to the MF (2, 22, 38). In humans, nystagmus is greatest with the superior-inferior axis parallel to the MF (9). This head position, in both rodent and human, would bring the plane of the horizontal canal parallel to the MF and a putative K^+ cross-current perpendicular to the MF.

However, direct experimental evidence regarding the K^+ current path and affected structures is still lacking. Although there is undoubtedly a substantial K^+ influx into the utricular hair cells, even at rest, calculations of the Lorentz force (9) assume the current flows in a dorsal-to-ventral direction into the utricular macula, rather than horizontally from the source of K^+ from dark cells adjacent to the macula.

Furthermore, it is not clear how an otoconia deficit in *het* and *tlt* mice results in a deficit of current flow into the hair cells of the utricle. Both *het* and *tlt* mice appear to have intact utricular hair and supporting cells. These strains also show robust resting discharge activity in macular primary afferents (39) and abundant ribbon synapses in type II hair cells in the utricle (18). The presence of resting discharge implies that the mutants still have some influx of K^+ at rest (40). Possibly the lack of otoconia results in fewer K^+ channel openings and thus less overall K^+ current in the mutants, resulting in reduced Lorentz force and decreased MF responsiveness.

Perspectives and Significance

Our study demonstrates the otoconia are necessary for the full behavioral effects of MF exposure, with a potential contributory role of the semicircular canals. However, the extent of semicircular canal contribution remains to be determined. Other otolith mutants such as inner ear defect (*ied*) and murgulhador (*mlh*) mice could determine if a graded MF response can be correlated with the extent of VOR deficiency. Observing mice with deficits primarily in the semicircular canals but with intact otoliths; for example, *ZPLD1* mutants may also tease apart the relative contributions of these structures (41).

DATA AVAILABILITY

Data will be made available upon reasonable request.

SUPPLEMENTAL DATA

Supplemental Video S1: https://doi.org/10.5281/zenodo.7884035.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.C.S. and T.A.H. conceived and designed research; J.M.C., A.H., B.K., J.C.S., and T.A.H. performed experiments; J.M.C., A.H., J.C.S., and T.A.H. analyzed data; J.M.C., J.C.S., and T.A.H. interpreted results of experiments; J.M.C. and T.A.H. prepared figures; J.M.C. and T.A.H. drafted manuscript; J.M.C. and T.A.H. edited and revised manuscript; J.M.C. and T.A.H. approved final version of manuscript.

REFERENCES

1. **Lockwood DR**, **Kwon B**, **Smith JC**, **Houpt TA.** Behavioral effects of static high magnetic fields on unrestrained and restrained mice. *Physiol Behav* 78: 635–640, 2003. doi:10.1016/S0031-9384(03)00040-4.
2. **Houpt TA**, **Pittman DW**, **Barranco JM**, **Brooks EH**, **Smith JC.** Behavioral effects of high-strength static magnetic fields on rats. *J Neurosci* 23: 1498–1505, 2003. doi:10.1523/JNEUROSCI.23-04-01498.2003.
3. **Houpt TA**, **Cassell J**, **Carella L**, **Neth B**, **Smith JC.** Head tilt in rats during exposure to a high magnetic field. *Physiol Behav* 105: 388–393, 2012. doi:10.1016/j.physbeh.2011.08.030.
4. **Snyder DJ**, **Jahng JW**, **Smith JC**, **Houpt TA.** c-Fos induction in visceral and vestibular nuclei of the rat brain stem by a 9.4 T magnetic field. *Neuroreport* 11: 2681–2685, 2000. doi:10.1097/00001756-200008210-00015.

5. **Ward BK**, **Lee YH**, **Roberts DC**, **Naylor E**, **Migliaccio AA**, **Della Santina CC.** Mouse magnetic-field nystagmus in strong static magnetic fields is dependent on the presence of Nox3. *Otol Neurotol* 39: e1150–e1159, 2018. doi:10.1097/MAO.0000000000002024.
6. **Marcelli V**, **Esposito F**, **Aragri A**, **Furia T**, **Riccardi P**, **Tosetti M**, **Biagi L**, **Marciano E**, **Di Salle F.** Spatio-temporal pattern of vestibular information processing after brief caloric stimulation. *Eur J Radiol* 70: 312–316, 2009. doi:10.1016/j.ejrad.2008.01.042.
7. **Schenck JF**, **Dumoulin CL**, **Redington RW**, **Kressel HY**, **Elliott RT**, **McDougall IL.** Human exposure to 4.0-Tesla magnetic fields in a whole-body scanner. *Med Phys* 19: 1089–1098, 1992. doi:10.1118/1.596827.
8. **Schaap K**, **Christopher-de Vries Y**, **Mason CK**, **de Vocht F**, **Portengen L**, **Kromhout H.** Occupational exposure of healthcare and research staff to static magnetic stray fields from 1.5-7 Tesla MRI scanners is associated with reporting of transient symptoms. *Occup Environ Med* 71: 423–429, 2014. doi:10.1136/oemed-2013-101890.
9. **Roberts DC**, **Marcelli V**, **Gillen JS**, **Carey JP**, **Della Santina CC**, **Zee DS.** MRI magnetic field stimulates rotational sensors of the brain. *Curr Biol* 21: 1635–1640, 2011. doi:10.1016/j.cub.2011.08.029.
10. **de Vocht F**, **van-Wendel-de-Joode B**, **Engels H**, **Kromhout H.** Neurobehavioral effects among subjects exposed to high static and gradient magnetic fields from a 1.5 Tesla magnetic resonance imaging system–a case-crossover pilot study. *Magn Reson Med* 50: 670–674, 2003. doi:10.1002/mrm.10604.
11. **Cason AM**, **Kwon B**, **Smith JC**, **Houpt TA.** Labyrinthectomy abolishes the behavioral and neural response of rats to a high-strength static magnetic field. *Physiol Behav* 97: 36–43, 2009. doi:10.1016/j.physbeh.2009.01.018.
12. **Ornitz DM**, **Bohne BA**, **Thalmann I**, **Harding GW**, **Thalmann R.** Otoconial agenesis in tilted mutant mice. *Hear Res* 122: 60–70, 1998. doi:10.1016/S0378-5955(98)00080-X.
13. **Paffenholz R**, **Bergstrom RA**, **Pasutto F**, **Wabnitz P**, **Munroe RJ**, **Jagla W**, **Heinzmann U**, **Marquardt A**, **Bareiss A**, **Laufs J**, **Russ A**, **Stumm G**, **Schimenti JC**, **Bergstrom DE.** Vestibular defects in head-tilt mice result from mutations in Nox3, encoding an NADPH oxidase. *Genes Dev* 18: 486–491, 2004. doi:10.1101/gad.1172504.
14. **Hurle B**, **Ignatova E**, **Massironi SM**, **Mashimo T**, **Rios X**, **Thalmann I**, **Thalmann R**, **Ornitz DM.** Non-syndromic vestibular disorder with otoconial agenesis in tilted/mergulhador mice caused by mutations in otopetrin 1. *Hum Mol Genet* 12: 777–789, 2003. doi:10.1093/hmg/ddg087.
15. **Jones SM**, **Erway LC**, **Bergstrom RA**, **Schimenti JC**, **Jones TA.** Vestibular responses to linear acceleration are absent in otoconia-deficient C57BL/6JEi-het mice. *Hear Res* 135: 56–60, 1999. doi:10.1016/s0378-5955(99)00090-8.
16. **Jones SM**, **Erway LC**, **Johnson KR**, **Yu H**, **Jones TA.** Gravity receptor function in mice with graded otoconial deficiencies. *Hear Res* 191: 34–40, 2004 [Erratum in *Hear Res* 194: 143, 2004]. doi:10.1016/j.heares.2004.01.008.
17. **Bergstrom RA**, **You Y**, **Erway LC**, **Lyon MF**, **Schimenti JC.** Deletion mapping of the head tilt (het) gene in mice: a vestibular mutation causing specific absence of otoliths. *Genetics* 150: 815–822, 1998. doi:10.1093/genetics/150.2.815.
18. **Hoffman LF**, **Ross MD**, **Varelas J**, **Jones SM**, **Jones TA.** Afferent synapses are present in utricular hair cells from otoconia-deficient mice. *Hear Res* 222: 35–42, 2006. doi:10.1016/j.heares.2006.05.013.
19. **Houpt TA**, **Smith JC.** Conditioned taste aversion induced by exposure to high magnetic fields. In: *Conditioned Taste Aversion: Behavioral and Neural Processes*, edited by Reilly S, Schachtman R. Oxford: Oxford University Press, 2009, p. 422–444.
20. **Spencer CM**, **Eckel LA**, **Nardos R**, **Houpt TA.** Area postrema lesions attenuate LiCl-induced c-Fos expression correlated with conditioned taste aversion learning. *Physiol Behav* 105: 151–160, 2012. doi:10.1016/j.physbeh.2011.08.022.
21. **Paxinos G**, **Franklin KBJ.** *The Mouse Brain in Stereotaxic Coordinates* (3rd ed.). New York: Academic Press, 2007.
22. **Houpt T**, **Pittman D**, **Riccardi C**, **Cassell J**, **Lockwood D**, **Barranco J**, **Kwon B**, **Smith J.** Behavioral effects on rats of high strength magnetic fields generated by a resistive electromagnet. *Physiol Behav* 86: 379–389, 2005. doi:10.1016/j.physbeh.2005.08.008.
23. **Houpt TA**, **Cassell JA**, **Riccardi C**, **Kwon B**, **Smith JC.** Suppression of drinking by exposure to a high-strength static magnetic field. *Physiol Behav* 90: 59–65, 2007. doi:10.1016/j.physbeh.2006.08.027.
24. **Houpt TA**, **Cassell JA**, **Riccardi C**, **DenBleyker MD**, **Hood A**, **Smith JC.** Rats avoid high magnetic fields: dependence on an intact vestibular system. *Physiol Behav* 92: 741–747, 2007. doi:10.1016/j.physbeh.2007.05.062.
25. **Holstein GR**, **Friedrich VL Jr**, **Martinelli GP**, **Ogorodnikov D**, **Yakushin SB**, **Cohen B.** Fos expression in neurons of the rat vestibulo-autonomic pathway activated by sinusoidal galvanic vestibular stimulation. *Front Neurol* 3: 4, 2012. doi:10.3389/fneur.2012.00004.
26. **Baizer JS**, **Corwin WL**, **Baker JF.** Otolith stimulation induces c-Fos expression in vestibular and precerebellar nuclei in cats and squirrel monkeys. *Brain Res* 1351: 64–73, 2010. doi:10.1016/j.brainres.2010.05.087.
27. **Kaufman G**, **Anderson J**, **Beitz A.** Fos-defined activity in rat brainstem following centripetal acceleration. *J Neurosci* 12: 4489–4500, 1992. doi:10.1523/JNEUROSCI.12-11-04489.1992.
28. **Gustave Dit Duflo S**, **Gestreau C**, **Lacour M.** Fos expression in the rat brain after exposure to gravito-inertial force changes. *Brain Res* 861: 333–344, 2000. doi:10.1016/s0006-8993(00)02044-8.
29. **Pompeiano O**, **d'Ascanio P**, **Centini C**, **Pompeiano M**, **Balaban E.** Gene expression in rat vestibular and reticular structures during and after space flight. *Neuroscience* 114: 135–155, 2002. doi:10.1016/s0306-4522(02)00202-6.
30. **Fuller PM**, **Jones TA**, **Jones SM**, **Fuller CA.** Evidence for macular gravity receptor modulation of hypothalamic, limbic and autonomic nuclei. *Neuroscience* 129: 461–471, 2004. doi:10.1016/j.neuroscience.2004.05.059.
31. **Andreescu CE**, **De Ruiter MM**, **De Zeeuw CI**, **De Jeu MTG.** Otolith deprivation induces optokinetic compensation. *J Neurophysiol* 94: 3487–3496, 2005. doi:10.1152/jn.00147.2005.
32. **Khan SI**, **Della Santina CC**, **Migliaccio AA.** Angular vestibuloocular reflex responses in Otop1 mice. I. Otolith sensor input is essential for gravity context-specific adaptation. *J Neurophysiol* 121: 2291–2299, 2019. doi:10.1152/jn.00811.2018.
33. **Harrod CG**, **Baker JF.** The vestibulo ocular reflex (VOR) in otoconia deficient head tilt (het) mutant mice versus wild type C57BL/6 mice. *Brain Res* 972: 75–83, 2003. doi:10.1016/S0006-8993(03)02505-8.
34. **Schmidt V**, **Hirt AM**, **Hametner K**, **Günther D.** Magnetic anisotropy of carbonate minerals at room temperature and 77 K. *Am Min* 92: 1673–1684, 2007. doi:10.2138/am.2007.2569.
35. **Lide DR** (editor)**.** *CRC Handbook of Chemistry and Physics* (87th ed.). Boca Raton, FL: CRC Press, 2006.
36. **Antunes A**, **Glover PM**, **Li Y**, **Mian OS**, **Day BL.** Magnetic field effects on the vestibular system: calculation of the pressure on the cupula due to ionic current-induced Lorentz force. *Phys Med Biol* 57: 4477–4487, 2012. doi:10.1088/0031-9155/57/14/4477.
37. **Ward BK**, **Roberts DC**, **Otero-Millan J**, **Zee DS.** A decade of magnetic vestibular stimulation: from serendipity to physics to the clinic. *J Neurophysiol* 121: 2013–2019, 2019. doi:10.1152/jn.00873.2018.
38. **Houpt TA**, **Kwon B**, **Houpt CE**, **Neth B**, **Smith JC.** Orientation within a high magnetic field determines swimming direction and laterality of c-Fos induction in mice. *Am J Physiol Regul Integr Comp Physiol* 305: R793–R803, 2013. doi:10.1152/ajpregu.00549.2012.
39. **Jones TA**, **Jones SM**, **Hoffman LF.** Resting discharge patterns of macular primary afferents in otoconia-deficient mice. *J Assoc Res Otolaryngol* 9: 490–505, 2008. doi:10.1007/s10162-008-0132-0.
40. **Rüsch A**, **Eatock RA.** A delayed rectifier conductance in type I hair cells of the mouse utricle. *J Neurophysiol* 76: 995–1004, 1996. doi:10.1152/jn.1996.76.2.995.
41. **Vijayakumar S**, **Jones SM**, **Jones TA**, **Tian C**, **Johnson KR.** Spontaneous mutations of the Zpld1 gene in mice cause semicircular canal dysfunction but do not impair gravity receptor or hearing functions. *Sci Rep* 9: 12430, 2019. doi:10.1038/s41598-019-48835-5.