Published in final edited form as:

Hippocampus. 2014 October ; 24(10): 1169–1177. doi:10.1002/hipo.22300.

Otoconia-Deficient Mice Show Selective Spatial Deficits

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Abstract

Damage or inactivation of the vestibular system impairs performance on various spatial memory tasks, but few studies have attempted to disambiguate the roles of the semicircular canals and otolith organs in this performance. The present study tested the otolithic contribution to spatial working and reference memory by evaluating the performance of otoconia-deficient *tilted* mice on a radial arm maze and a Barnes maze. One radial arm maze task provided both intramaze and extramaze cues, whereas the other task provided only extramaze cues. The Barnes maze task provided only extramaze cues. On the radial arm maze, *tilted* mice performed similar to control mice when intramaze cues were available, but made more working and reference memory errors than control mice when only extramaze cues were available. On the Barnes maze task, control and *tilted* mice showed similar latency, distance, and errors during acquisition training. On the subsequent probe trial, both groups spent the greatest percentage of time in the goal quadrant, indicating they were able to use extramaze cues to guide their search. Overall, these results suggest signals originating in the otolith organs contribute to spatial memory, but are not necessary for all aspects of spatial performance.

Keywords

Vestibular; Spatial Orientation; Spatial Memory; Otolith Organs; Radial Arm Maze

Introduction

Efficient navigation within an environment often involves a learned relationship among goal locations and visual cues, or landmarks. Despite this reliance on visual information, additional sensory signals can contribute to spatial performance depending on environmental conditions and task demands. One such signal originates in the vestibular system, as indicated by profound spatial deficits following vestibular dysfunction in humans and animals (Besnard et al., 2012; Brandt et al., 2005; Ossenkopp and Hargreaves, 1993; Russell et al., 2003a). Further, rats with vestibular lesions preferentially used a response strategy instead of a spatial strategy to solve a T-maze task, suggesting signals from the vestibular system contribute to the use of spatial cues for navigation (Machado et al., 2014). The vestibular contribution to spatial abilities may involve neural representations of space, such as the hippocampal place cell signal or the head direction (HD) signal, given that vestibular damage disrupts both of these representations (Russell et al., 2003b; Stackman et al., 2002;

Stackman and Taube, 1997). The vestibular system thus contributes to the performance of predominantly visual tasks and to the spatial signals thought to underlie this performance, but the nature of this vestibular contribution is not fully understood at the present time.

The vestibular system comprises the semicircular canals and otolith organs, which detect angular acceleration and linear acceleration/gravity, respectively. These signals provide complementary information regarding head movements in space, and may therefore have differential involvement in spatial representations and performance. No previous studies have directly tested the roles of the canals and otolith organs in place cell activity, but surgical blockade of the semicircular canals virtually eliminated the directional tuning of thalamic HD cells (Muir et al., 2009) and HD cells were absent in mice that lack functional horizontal canals (Taube and Valerio, 2012). In contrast, robust HD cells were recorded from otoconia-deficient *tilted* mice, but these HD cells progressively lost their directional tuning across trials (Yoder and Taube, 2009). Importantly, all of these studies revealed “bursty” cells which had firing patterns characteristic of HD cells, but that lacked significant directional tuning. It is therefore possible that these bursty cells are HD cells that lacked the sensory signals necessary to maintain their direction-specific firing. Thus, available evidence suggests that both the semicircular canals and otolith organs contribute to the HD signal, and each of these vestibular components may therefore influence performance on spatial tasks.

No previous studies have specifically tested spatial performance in animals lacking functional semicircular canals, but a recent study revealed impairments in animals with dysfunctional otolith organs. Otoconia-deficient *head tilt (het)* mice were impaired at place recognition and did not alternate above chance levels on a spatial Y-maze task, indicating a role for otolithic signals in these aspects of spatial performance (Machado et al., 2012). However, the extent to which the otolith organs contribute to other spatial abilities remains unknown. To this end, the present study tested the otolithic contribution to spatial working and reference memory on a radial arm maze discrimination task and on a Barnes maze reference memory task.

Materials and Methods

Subjects

All procedures involving live animals were approved by the Purdue Animal Care & Use Committee. Male control ($n = 20$) and *tilted* ($n = 20$) mice were used. These mice were descendants of an initial stock of mice (Jackson Laboratories, Bar Harbor, ME) that was bred to produce offspring that were homozygous ($-/-$) for the recessive mutation or crossed with C57BL/6J mice to produce offspring that were heterozygous ($+/-$) for the mutation. The F1 $+/-$ and $-/-$ mice were then bred to produce $+/-$ and $-/-$ offspring, with a predicted 50% frequency of each genotype. A swim test was used to determine whether mice were $+/-$ or $-/-$. Briefly, at age 12 weeks, mice were dropped from a height of ≈ 20 cm into a pool of water; $+/-$ mice immediately resurfaced and swam with their heads above water, whereas $-/-$ mice were unable to remain at the surface and required immediate rescue to prevent drowning. The swim test has previously been shown to accurately detect otoconia agenesis in $\approx 98\%$ of confirmed homozygous *tilted* mice (Ornitz et al., 1998). The remaining $\approx 2\%$ of

the homozygous mice showed intermediate swimming ability that was associated with large, malformed otoconia. In the present study, all mice categorized as $-/-$ were unable to swim, indicating complete otoconia agenesis. Mice categorized as $+/-$ and $-/-$ were then pseudo randomly selected from the entire population; in order to be included in the study, a $-/-$ mouse was required to have at least one male $+/-$ littermate which was also tested on the same task. All mice were 3–8 months of age at the beginning of testing.

6-Arm Radial Maze

Apparatus—A 6-arm radial maze was constructed from wood and painted gray, and consisted of a regular hexagonal center platform (sides = 8 cm) surrounded by six identical walled arms radiating from its sides (arm length = 60 cm, wall height = 2 cm) with a recessed food cup located near the end of each arm. The maze was positioned on a square wooden table (62 cm X 62 cm, height = 76.5 cm), under which a 100-watt upward-facing incandescent lamp provided indirect illumination. The maze and table were located near the corner of the room in a way that allowed visual detection of room asymmetry as well as various objects (sink, cabinet, etc.) that could serve as distal landmarks. This configuration was chosen because geometric information appears to contribute to spatial performance in mice (Fellini and Morellini, 2011; Fellini et al., 2006). An overhead video camera was used to record acquisition trials.

Procedure—Two versions of a discrimination task were used, with different experimentally naïve mice (control $n = 7$, *tilted* $n = 7$) used for each task. An *intramaze cue* task included salient cues (small white plastic bottles) that were placed next to the baited food cups and were visible from the center platform. An *extramaze cue* task did not have cues available at the goal locations, but instead required the animal to use distal cues to discriminate among baited and unbaited arms. Both tasks used the same procedure.

Habituation Trials: One day prior to habituation trials, animals were weighed and food was removed to reduce body weight to 85–90% of the free-feeding weight. This reduced body weight was maintained by a restricted diet throughout habituation and training trials. Water was available *ad libitum* in the home cage throughout the procedures. Habituation trials were conducted with the maze located in a different room from the one that would later be used for training. All arms were baited with 0.1 ml of 50% sweetened condensed milk. A mouse was placed on the center platform and permitted to explore the maze for one 10-min habituation trial per day, for two days.

Acquisition Trials: The food reward was placed in the cups of two non-adjacent arms, and a mouse was placed in the center of the maze where it was confined by a transparent plastic cover for 15–30 sec. A trial started when the cover was removed and ended when the mouse poked its nose into the second food cup, for a maximum of 5 min. At the end of each trial, the mouse was returned to its home cage for several minutes while the maze was cleaned with alcohol and baited for the next trial. Each mouse performed four trials per day, for 10 days, with the same two arms baited across days. The baited/unbaited arm configuration was counterbalanced across groups. The maze was cleaned thoroughly with alcohol between

trials and was rotated 180° at the end of each day in order to discourage the use of intramaze cues. An overhead video camera digitally recorded acquisition trials for backup.

Scoring and Data Analysis: Performance during acquisition trials was scored based on reference memory (RM), working memory-correct (WM-C) and working memory-incorrect (WM-I) errors, as well as latency. A choice was counted when all four paws crossed the threshold of an arm, which usually resulted in traversal of the entire arm to the food cup. However, a correct choice was only counted if the mouse approached the food cup; entry into a baited arm without approach to the food cup was counted as an error (described below). Three types of errors could be committed: RM errors occurred with the first entry into an unbaited arm; an RM error could also occur if the mouse entered a baited arm but did not approach the food cup. This partial entry was classified as an RM error because the mouse had made a choice (arm entry) that did not meet the criteria to be classified as a correct choice. WM-C errors occurred when a mouse re-entered an arm that previously contained a reward. WM-I errors occurred when a mouse reentered an arm that never contained a reward. Latency to complete each trial was the elapsed time between the start and end of a trial. Percentage of correct arm choices (correct choices divided by overall choices, multiplied by 100), frequency of each type of error, and latency were averaged within each trial block (day). Mean time per arm visit was calculated as the trial latency divided by the number of choices within a trial.

Statistical analyses were performed with StatView (SAS, Cary, NC) and SPSS (IBM, Armonk, NY). Group performance was compared with a separate 2×10 mixed ANOVA (Group X Trial Block) for each performance measure. The Greenhouse-Geisser correction was used when sphericity violations were indicated by Mauchly's test. Significant main effects and interaction effects were further evaluated with a Student-Newman-Keuls (SNK) test.

Barnes maze

Apparatus—The Barnes maze consisted of a circular wooden table (69 cm diam.), painted white, with 16 holes (4.5 cm diam.) located along the edge, as described previously (O'Leary et al., 2011). A black wooden escape box (length = 18.5 cm, width = 12.5 cm, depth = 8 cm) could be mounted under one of four holes, with several stairs leading into the box. A black wooden subfloor prevented entry to all non-escape holes. Two downward-facing 150-watt incandescent light bulbs mounted overhead served as an aversive stimulus, with all other room lights extinguished. The maze was located near the corner of the room (in the same position that was used for the radial maze), and various objects around the room (cabinet, sink, desk, etc.) were illuminated and could potentially serve as landmarks. An overhead video camera was used to digitally record data for offline analysis with Ethovision (Noldus, Leesburg, VA).

Procedure—Experimentally naïve control (n = 6) and *tilted* (n = 6) mice were used. The maze task procedure was based on the one described by O'Leary et al., 2011, but did not include a reversal test. Briefly, mice were tested in squads of four, with two control and two *tilted* mice per squad. Within each squad, each mouse was assigned to a different escape

hole, and target hole assignment was counterbalanced across groups. The maze was cleaned with alcohol between trials to remove odor cues, and was rotated 90° at the end of each day.

Habituation Trials: One 5-min habituation trial was conducted to familiarize mice with the maze and escape box. With the overhead light turned on, a transparent cylinder with an opaque roof confined each mouse to the maze next to the assigned escape hole, and the mouse was permitted to enter the escape hole and explore the immediate area. If mice did not freely enter the escape hole within 5 min, the experimenter encouraged the mouse to enter the escape box by nudging the mouse toward the escape hole. If the mouse did not enter the escape box, the mouse was placed in the escape box for 30 sec before it was returned to its home cage.

Acquisition Trials: Four acquisition trials were conducted per day, across four days. Mice were confined to the center of the maze for 15–30 sec by a transparent cylinder with an opaque roof, after which the cylinder was removed to allow the mouse to search for the escape hole. A trial started when the cylinder was removed and ended when the mouse placed all four paws in the escape hole, for a maximum of 5 min; mice that did not find the escape hole within 5 min were guided to the escape hole by the experimenter. Mice remained in the escape box for 30 sec before being returned to the home cage, where they remained for a 10–15 min intertrial interval period.

Probe Trial: A single 5-min probe trial was conducted one day following the acquisition trials, with no escape box available.

Scoring and Data Analysis: Performance measures for acquisition trials included the latency, distance, and the number of errors that occurred before entering the escape hole, as reported previously (O’Leary et al., 2011). An error was counted when a mouse poked its head into a hole that did not lead to the escape box. Multiple nose pokes into a single hole counted as a single error unless these multiple pokes were separated by a poke into a different hole. A failure occurred if the mouse did not enter the escape hole by the end of the 5 min trial. For the probe trial, the maze was divided into four quadrants: with the “correct” quadrant including the escape hole used during acquisition trials. The percentage of time spent in each quadrant was used as a measure of spatial reference memory for the goal location, as described previously (Germain et al., 2013; O’Leary and Brown, 2012; O’Leary et al., 2011).

Search Strategy Analysis: Video records from acquisition trials were evaluated offline and the search strategy was categorized as described previously (O’Leary and Brown, 2012). Briefly, search paths could be based on spatial, serial, and random strategies. A spatial strategy was used when an animal moved from the center toward the goal location and did not explore a hole more than two holes away from the correct hole in either direction; this range corresponds to a quadrant of the maze. A serial strategy occurred when an animal moved along the edge of the maze and passed three or more adjacent holes before approaching the target hole. A random strategy, also referred to as a “mixed” strategy, occurred when an animal walked along the edge of the maze and then crossed the center, or displayed a path that could not be classified as a single search strategy (spatial or serial).

Statistical analyses were performed with StatView and SPSS. Group performance was compared during acquisition with a 2×4 mixed ANOVA (Group X Trial Block) for each performance measure. The Greenhouse-Geisser correction was used when Mauchly's test indicated a sphericity violation. Significant main effects and interaction effects were further evaluated with an SNK *post hoc* test. For the probe trial, t-tests were used to compare the percentage of time between quadrants.

Results

Radial Arm Maze – Intramaze Cues

Control and *tilted* mice performed similarly on the radial arm maze when intramaze cues were available, suggesting *tilted* mice were able to learn to navigate to a visible goal location. Group percentage of correct arm choices in the maze task across trial blocks are depicted in Fig. 1A. Control and *tilted* mice showed similar percentages of correct arm choices (Group, $F(1,12) = .618$, $p = .45$) and the percentage of correct choices changed across trials (Trial Block, $F(9,108) = 22.046$, $p < .01$). The percentage of correct arm choices was increased in Trial Blocks 2–10, relative to Trial Block 1 (SNK, all p 's $< .05$). Importantly, control and *tilted* mice showed similar rates of performance improvement across trials (Group X Trial Block, $F(9,144) = .992$, $p = .45$).

Like the percentage of correct arm choices, latency to complete the intramaze cue task did not differ between control and *tilted* mice (Fig. 1B). Control and *tilted* mice spent similar amounts of time solving the task (Group, $F(1, 12) = .498$, $p = .53$). Latency to solve the task decreased across trials (Trial Block, corrected $F(2.917, 35.010) = 25.001$, $p < .01$), and was significantly shorter in Trial Blocks 2–10, relative to Trial Block 1, (SNK, all p 's $< .01$). The rate of latency decrease did not differ between groups, (Group X Trial Block, corrected $F(2.917, 35.010) = .270$, $p = .84$). Overall, these results suggest that *tilted* mice were not impaired at locomotion in general, and were unimpaired at using a taxon strategy to solve the radial arm maze task.

Radial Arm Maze – Extramaze Cues

Subjectively, during the first several days of training, *tilted* mice appeared to spend a greater amount of time on the central platform before choosing to enter each arm, relative to control mice. In contrast, *tilted* mice did not enter fewer arms during any trial blocks. The mean percentage of correct arm choices per trial is depicted in Figure 2A. Control and *tilted* mice showed different percentages of correct arm choices, with control mice having a greater percentage of correct choices than *tilted* mice (Group, $F(1,12) = 64.82$, $p < .01$). Overall performance did improve across trials (Trial Block, $F(9,108) = 14.24$, $p < .01$), with Trial Blocks 6–10 having greater percentages of correct arm choices than Trial Block 1 (SNK, all p 's $< .05$). The rate of performance improvement differed between control and *tilted* mice (Group X Trial Block, $F(9,108) = 5.06$, $p < .01$), with control mice having greater percentages than *tilted* mice on days 3–4 and 6–10 (SNK, all p 's $< .05$). Thus, both control and *tilted* mice improved across trials, but control mice improved more rapidly than *tilted* mice.

Evaluation of specific error types reveals increased error frequency for *tilted* mice, relative to controls. Control and *tilted* mice committed different frequencies of RM errors (Fig. 2B), with *tilted* mice making more RM errors than control mice (Group, $F(1,12) = 38.86$, $p < .01$). The frequency of RM errors changed across Trial Blocks (Trial Block, $F(9,108) = 8.80$, $p < .01$), with fewer errors occurring during Trial Blocks 6–10, relative to Trial Block 1 (SNK, all p 's $< .01$). The rate at which RM errors decreased was different between groups (Group X Trial Block, $F(9,108) = 7.02$, $p < .01$), with *tilted* mice committing more RM errors than control mice in Trial Blocks 4 & 6–10 (SNK, all p 's $< .05$). Thus, *tilted* mice made more RM errors overall and showed a slower rate of RM error decrease than control mice.

Overall, *tilted* mice made more spatial WM errors than control mice. The occurrence of WM-C errors (Fig. 2C) was greater for *tilted* mice than control mice (Group, $F(1,12) = 11.09$, $p < .01$). However, the number of WM-C errors did not change across trials (Trial Block, corrected $F(4.03,48.31) = 1.07$, $p = .38$), but groups showed different rates of WM-C errors across trial blocks (Group X Trial Block, corrected $F(4.03, 48.31) = 2.68$, $p = .04$). Interestingly, control mice made more WM-C errors than *tilted* mice in Trial Block 1, but made fewer WM-C errors in Trial Blocks 4 and 7–9 (SNK, all p 's $< .05$). This difference appears to be driven by the increased number of WM-C errors for control mice, which was significantly decreased in all subsequent trial blocks (SNK, all p 's $< .01$ vs. Trial Block 1). Control and *tilted* mice also differed on the numbers of WM-I errors (Fig. 2D), with *tilted* mice making more errors than control mice (Group, $F(1,12) = 5.73$, $p = .03$). The overall number of WM-I errors changed across trials (Trial Block, $F(9,108) = 2.01$, $p = .04$), with fewer errors in Trial Block 10, relative to Trial Block 1 (SNK, $p < .05$). The rate at which WM-I errors changed across trial blocks was different between groups (Group X Trial Block, $F(9,108) = 4.78$, $p < .01$), with a greater number of WM-I errors for *tilted* mice in Trial Blocks 4 and 6–10, (SNK, all p 's $< .05$). Thus, both types of WM errors occurred more frequently for *tilted* mice than for control mice.

Latency to complete the radial maze task differed between control and *tilted* mice (Fig. 2E). Tilted mice showed greater latency to solve the radial maze than control mice (Group, $F(1,12) = 5.36$, $p = .039$). The overall latency decreased across trials (Trial Block, corrected $F(3.86,46.36) = 18.13$, $p < .01$), with significantly shorter latency in Trial Blocks 4–10 (SNK, all p 's $< .01$ vs. Trial Block 1). The rate at which latency decreased across trial blocks did not differ between groups (Group X Trial Block, corrected $F(3.86, 46.36) = 1.03$, $p = .40$). To determine whether the increased latency for *tilted* mice resulted from slower movements or from a greater number of arm visits, time per arm was calculated as the latency to task completion divided by the number of arms visited, as a function of trial block (Fig. 2F). Control and *tilted* mice did not differ on time per arm (Group, $F(1,12) = 1.27$, $p = .28$), although time per arm was different across days (Trial Block, $F(9,108) = 2.79$, $p < .01$), with reduced time per arm in Trial Blocks 6–10 versus Trial Block 2 (SNK, all p 's $< .05$). The rate at which time per arm changed across trial blocks did not differ significantly between groups (Group X Trial Block, $F(9,108) = 1.27$, $p = .26$). Thus, the greater overall latency required for task completion in *tilted* mice can be explained by the increased number of arms visited.

Barnes Maze

Overall, control and *tilted* mice performed similarly on the Barnes maze RM task. A search strategy analysis indicates that both groups used a serial search strategy most often during the first two days. Control mice trended toward favoring a spatial strategy but did not significantly favor any single strategy by the last day; *tilted* mice did not show this trend (all p 's > .05; Fig. 3A,B). Regardless of the search strategy used, control and *tilted* mice performed similarly during acquisition training. For the latency measure, both groups performed similarly (Group, $F(1,10) = .307$, $p = .59$), with decreased latency after day 1 (Trial Block, corrected $F(2.06, 20.56) = 8.70$, $p < .01$; SNK, all p 's < .05 vs. Trial Block 1; Fig 3C). Latency decreased at similar rates for both control and *tilted* mice (Group X Trial Block, corrected $F(2.06, 20.56) = 2.00$, $p = .19$). Like the latency measure, the error rates also indicate similar performance between control and *tilted* mice (Group, $F(1, 10) = .018$, $p = .90$; Fig 3D). Overall frequency of errors decreased after day 1, (Trial Block, corrected $F(1.74, 17.43) = 5.42$, $p = .02$; SNK, all p 's < .05 vs. Trial Block 1), and the rate of error decrease did not differ between groups (Group X Trial Block, corrected $F(1.74, 17.43) = .083$, $p = .90$). The distance measure showed similar results, with no difference between groups (Group, $F(1, 10) = .038$, $p = .85$), but a significant decrease in distance after day 1 (Trial Block, $F(3, 30) = 8.54$, $p < .01$; SNK, all p 's < .01 vs. Trial Block 1; Fig 3E). The distance traveled decreased at similar rates for both groups (Group X Trial Block, $F(3, 30) = .816$, $p = .50$). Thus, control and *tilted* performed similarly on the Barnes maze task.

Even though neither group showed a significant preference for a spatial search strategy on the last day of acquisition training, both control and *tilted* mice spent the greatest percentage of time in the goal quadrant during the probe trial (all p 's < .01), and this percentage was similar between groups, $t(10) = .134$, $p = .90$ (Fig. 3F). Additionally, both groups spent similar percentages of time in the quadrant to the right of the goal, $t(10) = .008$, $p = .99$, to the left of the goal, $t(10) = .791$, $p = .45$, and opposite the goal, $t(10) = .876$, $p = .40$. Thus, despite a lack of preference for a spatial search strategy during acquisition training, both control and *tilted* mice were able to learn the location of the goal relative to distal spatial cues.

Discussion

We evaluated the spatial ability of otoconia-deficient *tilted* mice on three maze tasks, providing evidence for an otolithic contribution to spatial memory performance on select tasks. *Tilted* mice were able to use the combination of proximal and distal cues to solve a radial arm maze discrimination task but were impaired when only distal cues were available. Control mice preferentially used a spatial search strategy by the end of acquisition training, whereas *tilted* mice did not show this preference. However, *tilted* mice were unimpaired at using distal spatial cues during the probe trial, indicating otolithic involvement in select aspects of spatial performance.

The *tilted* mice's impaired spatial performance on the radial arm maze and intact performance on the Barnes maze provides insight into the otolithic contribution to spatial memory. Several previous studies found that rats with complete lesions of the vestibular apparatus were impaired on a radial arm maze discrimination task, committing more WM

and RM errors than controls (Besnard et al., 2012; Ossenkopp and Hargreaves, 1993). The present results indicate that similar results can occur with dysfunction limited to the otolith organs, as *tilted* mice also showed increased WM and RM errors across trials. By day 3, both types of WM errors virtually disappeared for control mice, and nearly all subsequent errors were of the RM type. In contrast, neither type of WM errors decreased significantly for *tilted* mice, suggesting they either failed to learn the task or failed to reliably discriminate among arms. A general memory deficit does not appear to have impaired *tilted* mice's performance, as they were able to learn to use intramaze cues to solve an otherwise identical task. We therefore infer that the performance deficits of *tilted* mice resulted from an impaired ability to discriminate among arms. Despite the spatial deficits on the radial arm maze, *tilted* mice performed relatively well on the Barnes maze reference memory task. Importantly, however, control mice failed to use a spatial search strategy more often than a serial strategy by the fourth day of training. This result is similar to that of a previous study, where C57Bl/6J mice showed a similar pattern of strategies on a small Barnes maze similar to the present one (O'Leary and Brown, 2012). This phase of the Barnes maze task was therefore not a good test of spatial memory. However, during the probe trial on day 5, *tilted* mice spent most of the time searching within the goal quadrant, indicating they were able to use distal spatial cues. Thus, signals from the otolith organs appear to be necessary for the use of spatial cues on the radial arm maze task, but not for the present Barnes maze task.

The *tilted* mice's relatively accurate use of spatial cues during the Barnes maze probe trial is not entirely surprising for several reasons. First, rats with complete vestibular lesions were unimpaired on an open field homing task when distal visual cues were available, suggesting spatial memory can occur without signals from either the canals or otolith organs (Wallace et al., 2002). In a different experiment, vestibular rats accurately solved a relatively simple navigation task in an open field when a landmark was available (Stackman and Herbert, 2002). Both of these tasks, along with the present Barnes maze task, required navigation to a single goal defined by its position relative to landmarks. In contrast, the present radial arm maze task required animals to learn to navigate among multiple goals. It is therefore possible that task demands produced the differential results between maze tasks. However, impairments have been reported for vestibular rats performing single-goal navigation tasks on the radial arm maze (Russell et al., 2003a), suggesting the number of goals is not the only factor underlying spatial deficits in vestibular animals. A second explanation for the differential performance between mazes is the different types of stimuli used to motivate the animals. The radial maze used food restriction to encourage animals to search for food, whereas the Barnes maze required satiated animals to escape the bright light and open space. Some theorists have suggested that individual variation in sensitivity to hunger can influence performance on appetitive tasks, whereas performance on aversive tasks such as the Barnes maze are not influenced by these factors (O'Leary and Brown, 2012). Overall performance was indeed different between the radial maze and Barnes maze tasks, but whether or not this was caused by the nature of the stimuli cannot be inferred from the present results. Additional studies are therefore necessary to determine how task complexity, maze size, and stimulus characteristics influence spatial performance in mice with vestibular dysfunction.

An additional possibility is that congenital otolith dysfunction disrupted visual functions necessary for task performance. The semicircular canal-mediated vestibulo-ocular reflex

(VOR), which enables gaze stability during head movement, is known to be attenuated in *het* mice (Harrod and Baker, 2003). However, if the observed spatial deficits were caused by attenuated VOR or other visual deficits, we would have expected *tilted* mice to be impaired at using distal cues during the probe trial of the Barnes maze. Thus, the spatial deficits of *tilted* mice did not appear to result from visual deficits.

Tilted mice's spatial deficits are similar to those in animals with hippocampal dysfunction, suggesting the otolithic contribution to spatial performance may involve hippocampal circuits. The hippocampus is necessary for efficient performance on spontaneous alternation tasks and for recognition tasks that include a spatial component such as object-in-place recognition, but not for non-spatial recognition tasks such as novel object recognition (Barker and Warburton, 2011; Douglas and Isaacson, 1964; Roberts et al., 1962). Similarly, otoconia-deficient *het* mice failed to spontaneously alternate above chance level and were impaired at place recognition, but showed intact non-spatial object recognition (Machado et al., 2012). The hippocampus is also necessary for the use of extramaze cues but not for the use of intramaze cues to solve the radial arm maze (Olton et al., 1978; Packard et al., 1989; White and Gaskin, 2006; White et al., 2013). Similarly, *tilted* mice in the present radial arm maze task were unable to use extramaze cues to locate the goals, but were not impaired at using intramaze cues. It is therefore possible that at least a portion of *tilted* mice's spatial deficits resulted from impaired function of the hippocampus or related brain regions.

The hippocampus and other limbic structures contain spatial representations and contribute to performance on many spatial tasks including the radial arm maze (Olton and Papas, 1979; Taube et al., 1992; Vann and Aggleton, 2003). One such spatial representation is the place cell signal, which is present in hippocampus and associated regions, and provides a neural representation of location within the environment (Cho and Sharp, 2001; Moser et al., 2008; O'Keefe and Dostrovsky, 1971; Sharp and Green, 1994; Taube, 1995). The place cell signal is heavily influenced by signals from the vestibular system, suggesting that either the semicircular canals or otolith organs, or both, provide a necessary component of this representation (Russell et al., 2003b; Sharp et al., 1995; Stackman et al., 2002). However, the available evidence suggests that otolith signals may not be necessary for place cell activity. Relatively normal place cells were recorded from rats while they navigated a three-dimensional track in outer space, where the otolithic representation of gravity would have been absent (Knierim et al., 2000). A recent study also identified relatively normal place cells in head-fixed mice while they performed a virtual navigation task, suggesting the place cell signal can persist in the absence of translation (Chen et al., 2013). Nevertheless, a smaller percentage of complex spike cells functioned as place cells during the virtual task, suggesting that translation signals contribute to the activity of some place cells. Another limbic spatial representation is the HD signal, which provides a neural representation of directional heading within the environment and depends on vestibular input (Stackman et al., 2002; Stackman and Taube, 1997; Taube et al., 1990). The semicircular canals provide a crucial component of the HD signal, as thalamic HD cells were absent in chinchillas following surgical blockade of the semicircular canals, and in mice lacking functional horizontal semicircular canals (Muir et al., 2009; Taube and Valerio, 2012). In contrast, HD cells were identified in the thalamus of *tilted* mice, but these cells lost their directional tuning across trials (Yoder and Taube, 2009). Other brain regions, such as the parietal

cortex, also receive signals originating in the vestibular system and contribute to spatial performance, but we currently have no evidence to indicate whether parietal cortical functions depend specifically on signals from the otolith organs (for review, see Yoder and Taube, 2014). Thus, at the present time, the only spatial representation that is known to specifically require signals from the otolith organs is the HD signal. The degraded HD signal of *tilted* mice may therefore contribute to their spatial deficits.

Summary and Conclusion

Signals from the otolith organs were necessary for spatial working and reference memory on a radial arm maze discrimination task when only extramaze cues were available, but not when intramaze cues were present to signal the goal locations. However, otolith signals were not necessary for spatial reference memory on a Barnes maze task when only extramaze cues were available, suggesting signals from the otolith organs contribute to select aspects of spatial memory. Based on available evidence, we infer that this otolithic contribution involves the HD signal, but additional studies are required to determine whether other brain signals are also involved.

Acknowledgments

Grant sponsor: NIH **Grant number:** DC012630

Grant sponsor: Indiana University-Purdue University Fort Wayne **Grant number:** Faculty Summer Grant

The authors thank Doug Wallace for helpful comments on an earlier version of the manuscript.

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Figure 1.

Cued navigation performance was similar for control and *tilted* mice. **A**, The percentage of correct arm choices increased across trial blocks at similar rates between groups. **B**, Latency to complete the task decreased across trial blocks at similar rates between groups. Mean $\pm$ SEM.

Figure 2.

Tilted mice were impaired at place learning on the radial arm maze. **A**, Percentage of correct arm choices increased more rapidly across trial blocks for control mice than for *tilted* mice. **B–D**, Reference Memory (RM), Working Memory – Correct (WM-C), and Working Memory – Incorrect (WM-I) errors decreased across trial blocks for control mice but not for *tilted* mice. **E**, Latency to complete the task decreased across trial blocks for both groups. **F**, Time per arm did not differ between control and *tilted* mice. Mean $\pm$ SEM.

Figure 3.

Control and *tilted* mice performed similarly on the Barnes maze. **A,B**, Both groups of mice used a serial search strategy most often during the first two days of training, but did not significantly favor a particular strategy during the last two days. **C–E**, Control and *tilted* mice showed similar latency, errors, and distance during acquisition training. **F**, Both groups spent the greatest percentage of time in the goal quadrant during the probe trial on Day 5. Mean $\pm$ SEM.