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Research report

Otolith dysfunction alters exploratory movement in mice

Philip A. Blankenship[a], Lucia A. Cherep[a], Tia N. Donaldson[a], Sarah N. Brockman[b], Alexandria D. Trainer[b], Ryan M. Yoder[b], Douglas G. Wallace[a,*]

[a] Dept of Psychology, NIU, DeKalb, IL, 60115, United States
[b] Dept of Psychology, IPFW, Fort Wayne, IN, 46805, United States

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ABSTRACT

The organization of rodent exploratory behavior appears to depend on self-movement cue processing. As of yet, however, no studies have directly examined the vestibular system's contribution to the organization of exploratory movement. The current study sequentially segmented open field behavior into progressions and stops in order to characterize differences in movement organization between control and otoconia-deficient *tilted* mice under conditions with and without access to visual cues. Under completely dark conditions, *tilted* mice exhibited similar distance traveled and stop times overall, but had significantly more circuitous progressions, larger changes in heading between progressions, and less stable clustering of home bases, relative to control mice. In light conditions, control and *tilted* mice were similar on all measures except for the change in heading between progressions. This pattern of results is consistent with otoconia-deficient *tilted* mice using visual cues to compensate for impaired self-movement cue processing. This work provides the first empirical evidence that signals from the otolithic organs mediate the organization of exploratory behavior, based on a novel assessment of spatial orientation.

1. Introduction

Most species are able to navigate efficiently by relying on a strategy that takes advantage of available cues. In familiar visual environments, stationary objects (landmarks) dominantly influence navigation [1]. However, in unfamiliar environments or in darkness, self-movement cues are available to guide navigation [1–5]. Regardless of the source of sensory information, the animal's location and directional heading within the environment appears to be represented by place cells and head direction cells, respectively [6–12]. Insight into the sensory signals' influence on these representations is therefore necessary to fully understand navigation.

Numerous studies have evaluated the role of visual cues in navigation and the underlying neural representations, but only a handful of studies have tested the influence of non-visual cues. One source of non-visual cues is the vestibular system, which is necessary for place cell and head direction cell function [13–15] and contributes to navigation on various tasks [16–19]. The vestibular system comprises the semicircular canals which detect angular head acceleration in three planes, and the otolith organs which detect linear head acceleration and static head position relative to gravity. Disruption of semicircular canal function eliminates the directional tuning of head direction cells and often causes the animal to spin or circle within a limited area [20,21]. The otolith organs also contribute to head direction cell function, but this contribution appears to be relatively less than that of the canals; otoconia-deficient mice have head direction cells that are directionally tuned, but this tuning degrades over time [22]. Nevertheless, the degraded head direction signal of otoconia-deficient mice is paralleled by deficits in the performance of directional navigation tasks such as the radial arm maze, food-carrying (homing) task, and Y-maze alternation [4,23,24] for review, see [25]. However, otoconia-deficient mice were able to accurately perceive the goal location during the probe trial on a Barnes maze, suggesting place recognition was intact [23]. The available evidence thus suggests that otolith signals contribute heavily to the directional aspect of navigation in both visual and non-visual environments, but have a lesser role in the locational aspect.

Previous studies using animals with vestibular pathology have provided important insight into the unique contributions of the semicircular canals and otolith organs to navigation in a variety of tasks. In an open-field exploration task, rats with bilateral vestibu-

* Corresponding author at: Psychology Department, Northern Illinois University, DeKalb, IL, 60115-2892, United States.
E-mail address: dwallace@niu.edu (D.G. Wallace).

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lar dysfunction exhibited hyperactivity across several months [26]. This hyperactivity could affect the way an animal explores the environment, which could, in turn, affect the ability to learn to solve spatial tasks. Whether or not this hyperactivity affects learning is not well understood; however, as many studies of the vestibular contribution to navigation used tasks that involved aversive stimuli – a design factor that may influence the effects of vestibular dysfunction on navigation. For example, vestibular dysfunction is thought to be anxiogenic [27], as are aversive stimuli such as the bright overhead lights of the Barnes maze, and the food restriction used to motivate animals in the radial arm maze [28,29]. Thus, the anxiety associated with vestibular dysfunction may be compounded with that of the task itself, preventing accurate inferences about the vestibular contribution to navigational performance.

The current study investigated the organization of exploratory behavior of control and otoconia-deficient mice, as they spontaneously explored within an open field environment. Rodents typically organize their exploratory behavior into a sequence of stops and progressions that are focused around one location in the environment, or home base [30,31]. The location of the home base has been characterized by long dwell times, high frequency of grooming behavior, and faster progressions toward the home base relative to departing progressions [32]. Based on this work, subsequent studies have demonstrated a role for self-movement cue processing in the organization of single exploratory trips [33]. Specifically, outward segment changes in heading during stops and translations during progressions are used to estimate direction and distance on the homeward segment. Impairments in generating self-movement cues would be predicted to disrupt behavior observed during stops, progressions, and possibly the stability of the home base, however, these disruptions may depend on the extent that visual cues can be used as a compensatory source of information to guide movement. Stops, progressions, and home base stability in control and otoconia-deficient mice were used as measures of exploratory movements under dark and light conditions.

2. Materials and methods

2.1. Animals

Female homozygous *tilted* (n = 10) and heterozygous control (n = 10) mice were obtained from the breeding colony established at Indiana University-Purdue University Fort Wayne. The colony was established from an original stock of homozygous *tilted* mice (−/−; B6.Cg-Otop1tlt/j; Jackson Laboratories, Bar Harbor, ME) that were bred to produce −/− offspring, or crossed with the background strain (+/+; C57BL/6J; Jackson Laboratories, Bar Harbor, ME) to produce +/− offspring. The resultant +/− and −/− litters of mice were bred to produce +/− and −/− offspring with a predicted 50% frequency within subsequent litters.

At 12 weeks old, the mice were classified as +/− or −/− using a swim test that has previously been shown to reliably detect otolith dysfunction in mice [34]. Swimming behavior was assessed during this test by dropping mice from a height of 20 cm into a pool of water. Mice that immediately resurfaced and engaged in swimming behavior were classified as the heterozygous control mice. Mice that failed to resurface and tumbled underwater were immediately rescued to prevent drowning and classified as homozygous *tilted* mice.

Mice were between three to eight months of age during the assessment of exploratory behavior. Prior to assessing exploratory behavior under light conditions, one control mouse had to be euthanized, resulting in nine control mice. Only female mice were used in the current study. Previous analyses of exploratory behavior have primarily used female subjects [33,35–37]. Neither sexual dimorphisms nor phase of estrous cycle has been observed to influence self-movement cue processing during food hoarding [38]; however, further work is needed to determine if these results generalize to exploratory movement.

2.2. Apparatus

The exploration arena was a white circular wooden table (112 cm diameter and 34.5 cm high). A vertical transparent plastic tab (20 cm wide × 15 cm high) was attached to the edge of the table and extended upward, where it could serve as a tactile cue and encourage home base establishment. The position of the plastic tab remained consistent for each mouse across dark and light exploration sessions; however, the position of the tab (north, east, south, or west) varied among mice. The table was located inside a wooden chamber (122.5 cm × 122.5 cm × 191 cm high) that had the walls and ceiling painted black, limiting the use of environmental cues. During dark exploration sessions, infrared emitters were used to illuminate the room. Four 25-W incandescent lights located on the ceiling of the enclosure provided illumination during light exploration sessions. Exploratory sessions were recorded at 30 frames per second by an overhead color/infrared video camera that was connected to a personal computer. All video recordings were saved for offline analysis.

2.3. Procedure

Mice were individually transported to the testing room in an opaque container. Upon entering the testing room each mouse was placed on the center of the table by the researcher and was left to explore the arena for 40 min. At the end of the session, the mouse was transported back to the colony room. The arena was thoroughly cleaned with an alcohol solution and dried prior to running the next mouse. All mice received three dark exploratory sessions prior to three light exploratory sessions.

2.4. Data analysis

Ethovision 3.0 (Noldus, NL) was used to digitize the position of the mouse during four five-minute consecutive samples throughout the 40-min dark and light exploratory sessions. Samples were taken after the first bout of grooming behavior was observed, usually within 2–5 min into the 40-min session. This behavior was chosen because previous work has demonstrated that grooming is a marker of home base establishment [30,35]. Therefore, samples were taken after the mice exhibited home base behaviors. Movement during each sample was divided into progressions (moment-to-moment speeds 3.0 cm/s or greater for at least two frames) and stops (moment-to-moment speeds less than 3.0 cm/s for at least two frames).

Multiple measures were used to characterize organization of exploratory behavior. First, the total distance traveled and total time spent stopping was calculated for each sample. Both of these measures provide general measures of locomotor function. Next, several measures were developed to describe behavior during progressions. Peak speed and distance traveled were calculated for all progressions and averaged for each sample. Average peak speed and distance traveled provided additional measures of general locomotor function. The path circuity of each progression was calculated by dividing the Euclidean distance by the actual traveled distance for each progression. Path circuity values range from 1.0 to 0.0 with lower values representing more circuitous paths through the environment. Previous work with rats has demonstrated that progressions are typically non-circuitous paths through the envi-

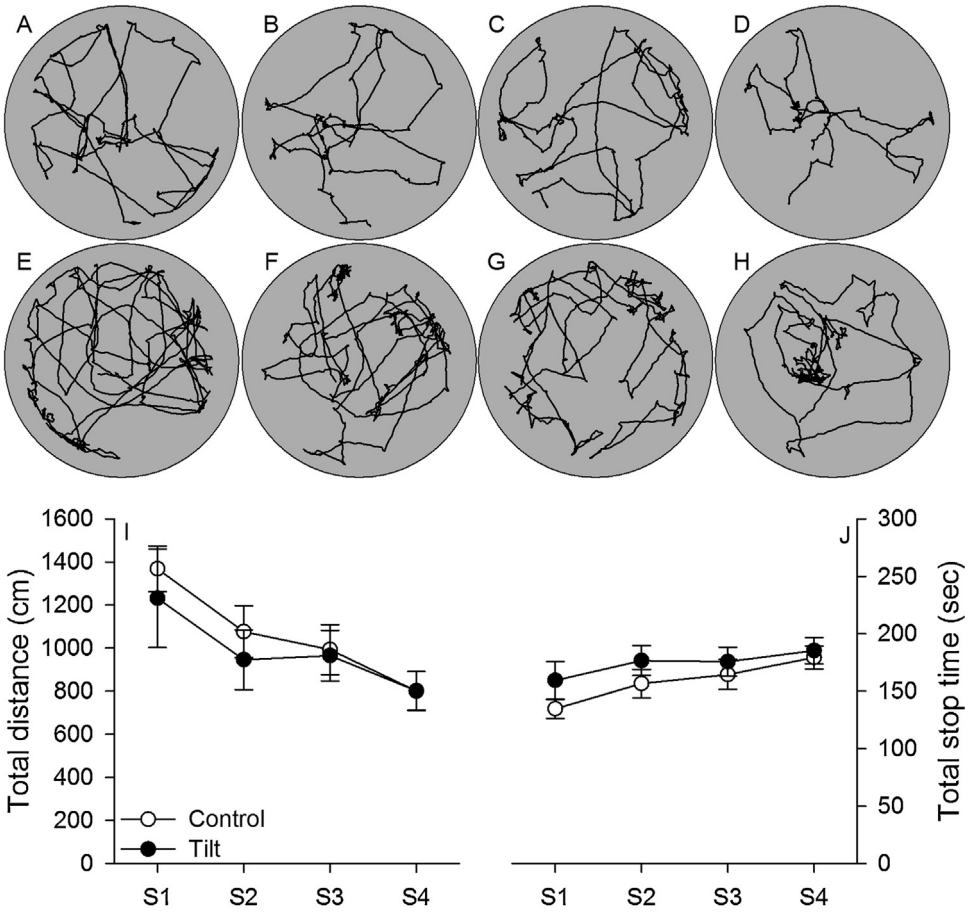

Fig. 1. Circular panels plot paths (black line) followed by a representative control (A, B, C, D) and *tilted* (E, F, G, H) mice during each sample under dark conditions. Each group's average total distance traveled (panel I) and stop time (panel J) are plotted for the four five-minute samples under dark conditions.

ronment independent of access to visual cues [33]. These measures were averaged across all progressions within a sample.

Several measures were developed to characterize stopping behavior. First, the average stop duration was calculated for each sample. Previous work has demonstrated that reduction in stop durations contributes to the increase in hyperactivity associated with damage to the hippocampal formation [39]. Second, progressions are typically non-circuitous trajectories through an environment with most of the path's change in heading occurring during periods of relatively slow speed [33]. The change in the path heading that occurs during a stop between two progressions can be quantified by calculating the angle subtended by the following points: preceding progression peak speed location, average stop location, and subsequent progression peak speed location. As a result, each mouse will have a set of changes in headings that can be averaged for each sample. Finally, distribution of stopping behavior in the environment has been associated with home base establishment [30,40–42]. Cartesian coordinates (x, y) associated with each stop were converted to polar coordinates (theta, r), and circular statistics [43] were used to characterize the concentration and stability of stops across samples. The duration of each stop was converted into an individual observation at a specific heading (i.e., one second was equal to one observation). First order circular statistics (parameter of concentration and average heading) were calculated for all stops from each sample from an individual mouse. The resulting parameter of concentrations from each mouse was used as a measure of stop density across samples. Second order circular statistics were applied to the average heading from each sample, with the resulting parameter of concentration used as a measure of stop stability across samples.

Repeated measures ANOVAs were used to evaluate main effects of group, sample, and group by sample interactions with alpha set at 0.05. Partial eta squared values (η^2_p) were reported for each main effect and interaction as a measure of effect size. The Greenhouse-Geisser correction was used in analyses where Mauchly's test indicated violations of the assumption of sphericity, and Fisher's Least Significant Difference (LSD) post-hoc test was used to evaluate significant main effects and interactions. T-tests were used to assess group differences on parameter of concentration calculated from average heading across the four samples. Degrees of freedom were adjusted when Levene's test indicated violations of the assumption of homogeneity of variance.

3. Results

3.1. Dark exploration

Mice exhibited exploratory behavior across all four five-minute samples (see Fig. 1a–d and e–h) under dark conditions. The ANOVA conducted on total distance traveled during each sample revealed a significant main effect of sample [$F(3,54) = 9.510$, $p < 0.01$, $\eta^2_p = 0.346$]; however, neither the main effect of group [$F(1,18) = 0.248$, $p = 0.624$, $\eta^2_p = 0.014$] nor Group × Sample interaction [$F(3,54) = 0.275$, $p = 0.843$, $\eta^2_p = 0.015$] were significant (Fig. 1i). A post hoc trend analysis revealed a significant linear decrease across samples [$F(1,18) = 15.651$, $p = 0.001$, $\eta^2_p = 0.465$]. The ANOVA conducted on total stop time during each sam-

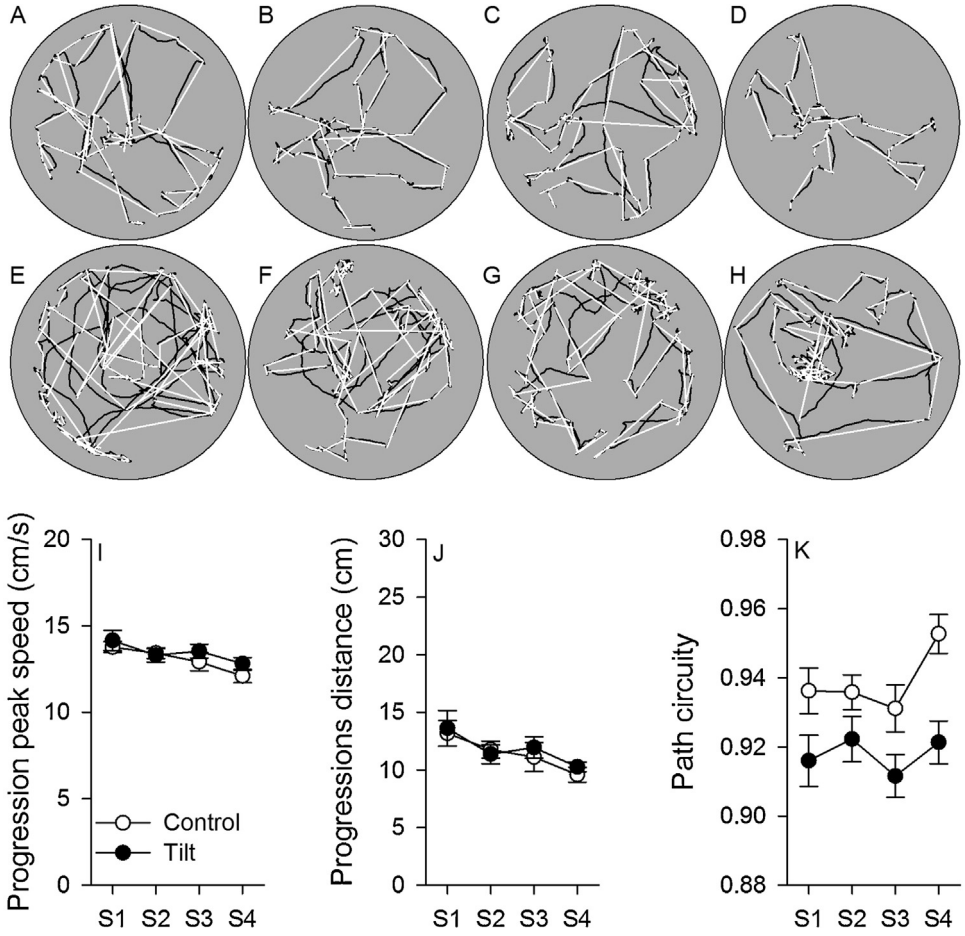

Fig. 2. Circular panels plot the path (black line) and progression Euclidean distances (grey lines) for the same representative control (A, B, C, D) and *tilted* (E, F, G, H) mice under dark conditions. Each group's average progression peak speed (I), distance (J), and path circuity (K) are plotted for the four five-minute samples under dark conditions.

ple revealed a significant main effect of sample [$F(3,54) = 5.729$, $p < 0.01$, $\eta^2_p = 0.241$]; however, neither the main effect of group [$F(1,18) = 1.236$, $p = 0.281$, $\eta^2_p = 0.064$] nor Group × Sample interaction [$F(3,54) = 0.466$, $p = 0.707$, $\eta^2_p = 0.025$] were significant (Fig. 1j). A post hoc trend analysis revealed a significant linear increase across samples [$F(1,18) = 17.760$, $p = 0.001$, $\eta^2_p = 0.497$]. Distance traveled decreased while total stop time increased across samples; however, groups did not differ on either measure.

Mice appeared to organize their exploration under dark conditions into a sequence of progressions and stops (see Fig. 2a–d and e–h). Kinematic (peak speed) and topographic (travel distance, path circuity) characteristics of progressions were examined in both groups across samples. The ANOVA conducted on progression peak speed during each sample revealed a significant main effect of sample [$F(3,54) = 6.074$, $p < 0.01$, $\eta^2_p = 0.252$]; however, neither the main effect of group [$F(1,18) = 0.978$, $p = 0.336$, $\eta^2_p = 0.052$] nor Group × Sample interaction [$F(3,54) = 0.543$, $p = 0.655$, $\eta^2_p = 0.029$] were significant (Fig. 2i). A post hoc trend analysis revealed a significant linear decrease in peak speed [$F(1,18) = 10.980$, $p < 0.01$, $\eta^2_p = 0.379$] across samples. The ANOVA conducted on progression travel distance during each sample revealed a significant main effect of sample [$F(3,54) = 5.942$, $p = 0.001$, $\eta^2_p = 0.248$]; however, neither the main effect of group [$F(1,18) = 0.162$, $p = 0.692$, $\eta^2_p = 0.009$] nor Group × Sample interaction [$F(3,54) = 0.217$, $p = 0.885$, $\eta^2_p = 0.012$] were significant (Fig. 2j). A post hoc trend analysis revealed a significant linear decrease in distance traveled [$F(1,18) = 8.880$, $p < 0.01$, $\eta^2_p = 0.330$] across samples. The ANOVA conducted on progression path circuity values across samples revealed a significant main effect of group [$F(1,18) = 14.291$, $p < 0.01$, $\eta^2_p = 0.443$], with control mice showing greater path circuity scores (more direct progressions) than *tilted* mice; however, neither sample [$F(3,54) = 2.641$, $p = 0.059$, $\eta^2_p = 0.128$] nor Group × Sample interaction [$F(3,54) = 0.850$, $p = 0.473$, $\eta^2_p = 0.045$] were significant (Fig. 2k). Thus, no group differences were observed in progression peak speeds or travel distances, although both measures decreased across samples. In contrast, circuity of progressions were observed to differ between groups, with the *tilted* group exhibiting more circuitous progressions relative to the control group.

Stops varied in duration and functioned as transitions between progressions (see Fig. 3a–d and e–h). Average stop duration and change in heading were examined for group differences across the four samples. Lack of sphericity in average stop duration resulted in the application of a Greenhouse-Geisser correction ($\varepsilon = 0.729$). The ANOVA conducted on average stop duration did not reveal significant main effects of group [$F(1,18) = 2.248$, $p = 0.151$, $\eta^2_p = 0.111$], sample [$F(2.187, 39.359) = 2.408$, $p = 0.099$, $\eta^2_p = 0.118$], or Groups × Sample [$F(2.187, 39.359) = 0.181$, $p = 0.909$, $\eta^2_p = 0.010$] interaction (Fig. 3i). The ANOVA conducted on average change in heading across samples revealed a significant effect of group [$F(1,18) = 17.872$, $p < 0.01$, $\eta^2_p = 0.498$], with *tilted* mice showing a greater change in heading than control mice (Fig. 3j). The main effect of sample was also significant [$F(3,54) = 7.542$, $p < 0.01$, $\eta^2_p = 0.295$]; however, there was not a significant Group by Sample interaction [$F(3,54) = 0.557$, $p = 0.646$, $\eta^2_p = 0.030$]. Post hoc trend analysis revealed a significant linear

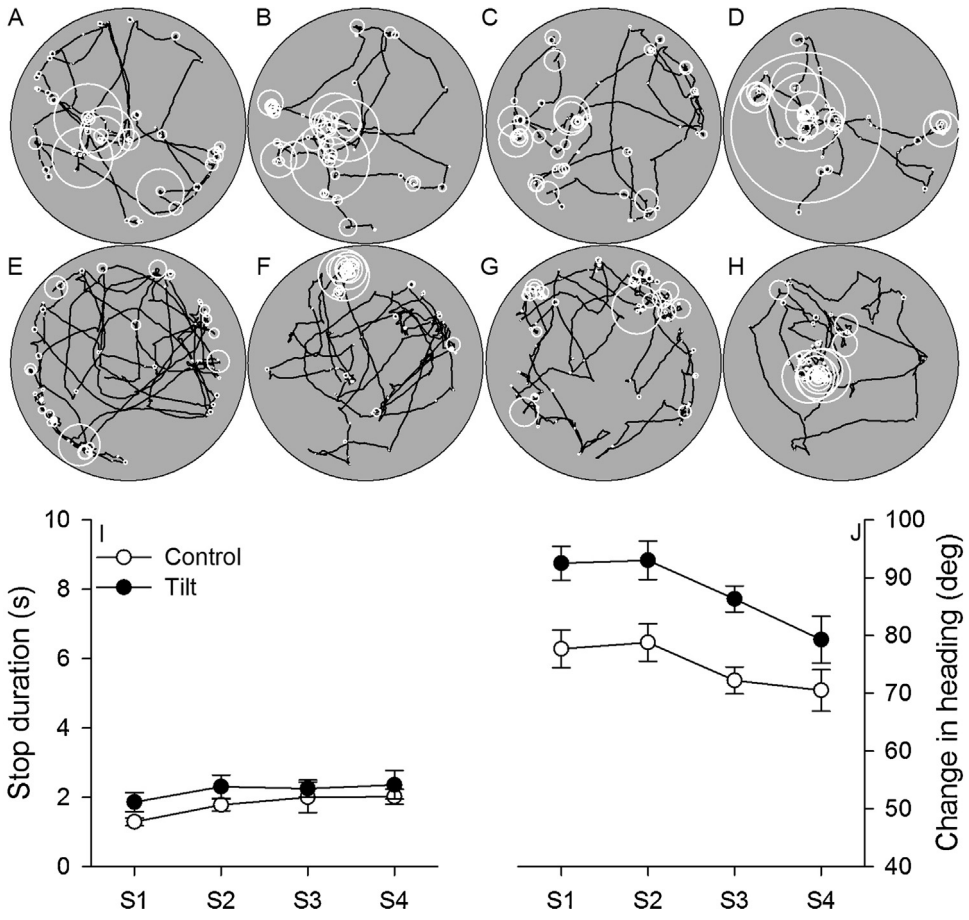

Fig. 3. Circular panels plot the path (black line) and stop location (grey circles) with diameter representing relative stop duration for the same representative control (A, B, C, D) and *tilted* (E, F, G, H) mice under dark conditions. Each group's average stop duration (I) and between progression change-in-heading (J) are plotted for the four five-minute samples under dark conditions.

decrease [$F(1,18) = 18.014$, $p < 0.01$, $\eta^2_p = 0.500$] in changes in heading across samples. Both groups thus exhibited similar stop durations that remained consistent across samples. *Tilted* mice exhibited significantly larger change in heading after stops, relative to control mice, and both groups' change in heading decreased across samples.

Under dark conditions, mice tended to cluster their stops around a specific location within a sample and a similar pattern of clustering was observed across samples (see Fig. 4a–d (control) and e–h (*tilted*)). These stops did not appear to be organized around the tactile cue (plastic tab), but were randomly distributed across the arena. The parameter of concentration (i.e., descriptive circular statistic) was used to quantify changes in the density of clustering for each mouse across samples [39]. The ANOVA conducted on parameter of concentration revealed a significant effect of sample [$F(3,54) = 2.962$, $p < 0.05$, $\eta^2_p = 0.141$]; however, neither the main effect of group [$F(1,18) = 0.629$, $p = 0.438$, $\eta^2_p = 0.034$] nor the Group x Sample interaction [$F(3,54) = 0.173$, $p = 0.914$, $\eta^2_p = 0.009$] were significant (Fig. 4i). Post hoc trend analysis revealed a significant linear increase [$F(1,18) = 6.955$, $p < 0.05$, $\eta^2_p = 0.279$] across samples. Groups exhibited similar stop clustering within samples, but stop clustering density increased across samples. The directional heading of stop clusters for each mouse was then averaged across the four samples, and the parameter of concentration of this distribution was calculated to determine whether the stop clusters remained stable (occurred in the same location) across samples. Levene's test for equality of variances was significant and prompted adjusting the degrees of freedom. The *t*-Test conducted on the parameter of concentration revealed a significant group difference [$T(10.988) = 2.475$, $p = 0.031$, $d = 1.1069$], with significantly more stable clusters in the control mice, relative to the *tilted* mice (Fig. 4j).

3.2. Light exploration

Mice exhibited exploratory behavior across all four five-minute samples (see Fig. 5a–d (control) and e–h (*tilted*)) under light conditions. The ANOVA conducted on total distance traveled during each sample did not reveal a significant effect of group [$F(1,17) = 0.219$, $p = 0.219$, $\eta^2_p = 0.013$], sample [$F(3,51) = 2.665$, $p = 0.058$, $\eta^2_p = 0.136$], or Group × Sample interaction [$F(3,51) = 1.857$, $p = 0.148$, $\eta^2_p = 0.098$] (Fig. 5i). Control and *tilted* mice therefore showed similar travel distances, and these distances did not change across samples. The ANOVA conducted on total stop time during each sample revealed a significant main effect of sample [$F(3,51) = 4.570$, $p < 0.01$, $\eta^2_p = 0.212$]; however, neither the main effect of group [$F(1,17) = 0.134$, $p = 0.718$, $\eta^2_p = 0.008$] nor Group × Sample interaction [$F(3,51) = 2.147$, $p = 0.106$, $\eta^2_p = 0.112$] were significant (Fig. 5j). A post hoc trend analysis revealed a significant linear increase across samples [$F(1,17) = 6.568$, $p < 0.05$, $\eta^2_p = 0.279$]. Only total stop time was observed to significantly increase across samples and groups did not differ on either measure.

Mice appear to organize their exploration under light conditions into a sequence to progressions and stops (see Fig. 6a–d (control) and e–h (*tilted*)). Kinematic (peak speed) and topographic (travel distance, path circuity) characteristics of progressions were exam-

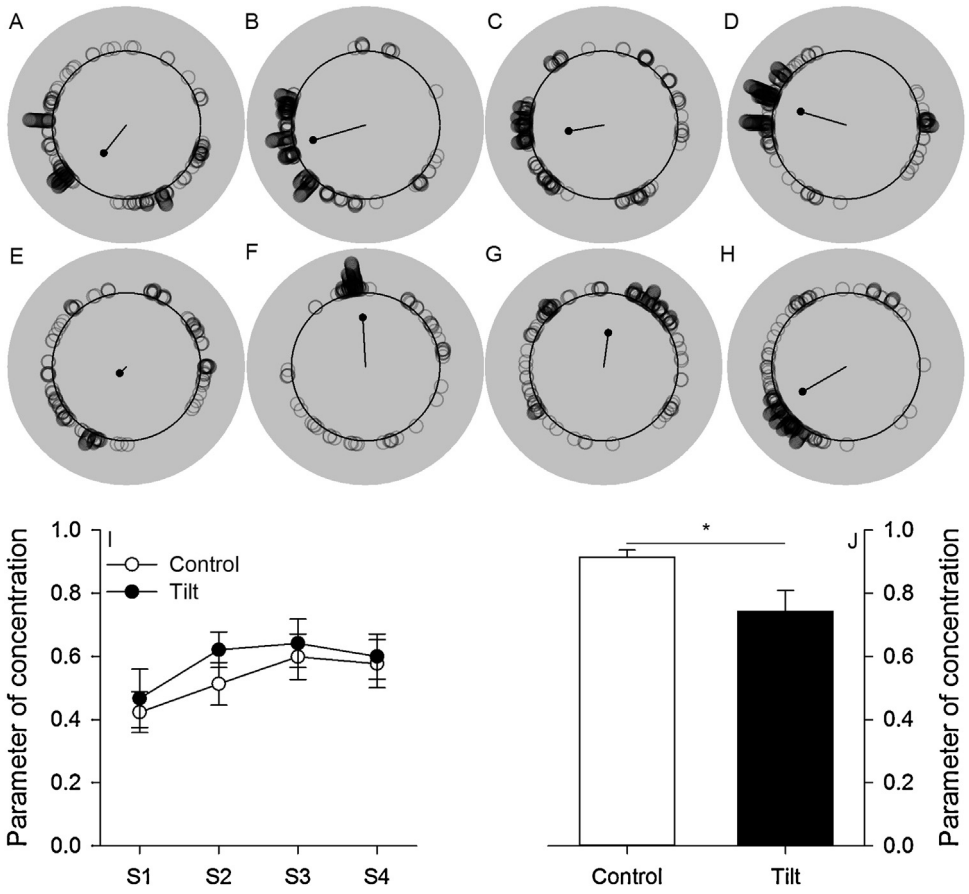

Fig. 4. Circular panels plot stop frequencies relative to polar heading direction for the same representative control (A, B, C, D) and *tilted* (E, F, G, H) mice under dark conditions. Average heading (theta) and parameter of concentration (r) are provided for each sample and represented by the polar line graph originating at the center of each plot. Group average parameter of concentration is plotted for each sample under dark condition (I). Average parameter of concentration (calculated from each mouse's four average heading directions) is plotted for both groups (J). (* < 0.05).

ined in both groups across samples. The ANOVA conducted on progression peak speed during each sample failed to reveal a significant main effect of group [$F(1,17) = 0.559$, $p = 0.465$, $\eta^2_p = 0.032$] nor the main effect of sample [$F(3,51) = 1.409$, $p = 0.251$, $\eta^2_p = 0.077$] (Fig. 6i). However, there was a significant Group × Sample interaction [$F(3,51) = 3.108$, $p < 0.05$, $\eta^2_p = 0.155$]. Interestingly, groups did not show significantly different peak speeds within any of the samples [Fisher's LSD, all p's > 0.05]. The ANOVA conducted on progression travel distance during each samples did not reveal a significant main effect of group [$F(1,17) = 0.957$, $p = 0.342$, $\eta^2_p = 0.053$], sample [$F(3,51) = 0.696$, $p = 0.559$, $\eta^2_p = 0.039$], nor Group × Sample interaction [$F(3,51) = 1.823$, $p = 0.155$, $\eta^2_p = 0.097$] (Fig. 6j). The ANOVA conducted on progression path circuity values across samples did not reveal a significant main effect of group [$F(1,17) = 4.219$, $p = 0.056$, $\eta^2_p = 0.199$], sample [$F(3,51) = 0.987$, $p = 0.406$, $\eta^2_p = 0.055$], nor Group × Sample interaction [$F(3,51) = 2.182$, $p = 0.102$, $\eta^2_p = 0.114$] (Fig. 6k). Under light conditions, *tilted* mice exhibited somewhat faster peak speeds than control mice during the fourth sample, although this difference did not reach statistical significance. Progression distance traveled and path circuity did not differ between groups nor among samples.

Stops varied in duration and functioned as transitions between progressions (see Fig. 7a–d (control) and e–h (*tilted*)). Average stop duration and change in heading were examined for group differences across the four samples. Lack of sphericity homogeneity of covariance in average stop duration resulted in the application of Greenhouse-Geisser correction ($\varepsilon = 0.506$). The ANOVA conducted on average stop duration did not reveal significant main effects of group [$F(1,17) = 2.354$, $p = 0.143$, $\eta^2_p = 0.122$], sample [$F(1.519, 25.826) = 2.373$, $p = 0.124$, $\eta^2_p = 0.123$], or Group × Sample interaction [$F(1.519, 25.826) = 0.705$, $p = 0.466$, $\eta^2_p = 0.040$] (Fig. 7i). The ANOVA conducted on average change in heading across samples revealed a significant effect of group [$F(1,17) = 6.276$, $p < 0.05$, $\eta^2_p = 0.270$], with *tilted* mice having greater change in heading than control mice; however, neither sample [$F(3,51) = 0.203$, $p = 0.894$, $\eta^2_p = 0.012$] nor Group by Sample interaction [$F(3,51) = 0.700$, $p = 0.556$, $\eta^2_p = 0.040$] were significant (Fig. 7j). Under light conditions, *tilted* mice exhibited significantly larger change in heading during stops relative to control mice; however, both groups exhibited similar stop durations that did not vary across samples.

Similar to dark conditions, mice tended to cluster their stops around a specific location within a sample and a similar pattern of clustering was observed across samples (see Fig. 8a–d (control) and e–h (*tilted*)), and these stops did not appear to be organized around the tactile cue (plastic tab). The parameter of concentration (i.e., descriptive circular statistic) was used to quantify density of clustering for each mouse across samples. The ANOVA conducted on parameter of concentration did not reveal a significant effect of group [$F(1,17) = 0.002$, $p = 0.961$, $\eta^2_p < 0.001$], sample [$F(3,51) = 2.754$, $p = 0.052$, $\eta^2_p = 0.139$], or Group × Sample interaction [$F(3,51) = 1.026$, $p = 0.389$, $\eta^2_p = 0.057$] (Fig. 8i). Groups thus exhibited similar stop clustering within samples, and stop clustering density did not significantly change across samples. The directional heading of stop clusters for each mouse was then

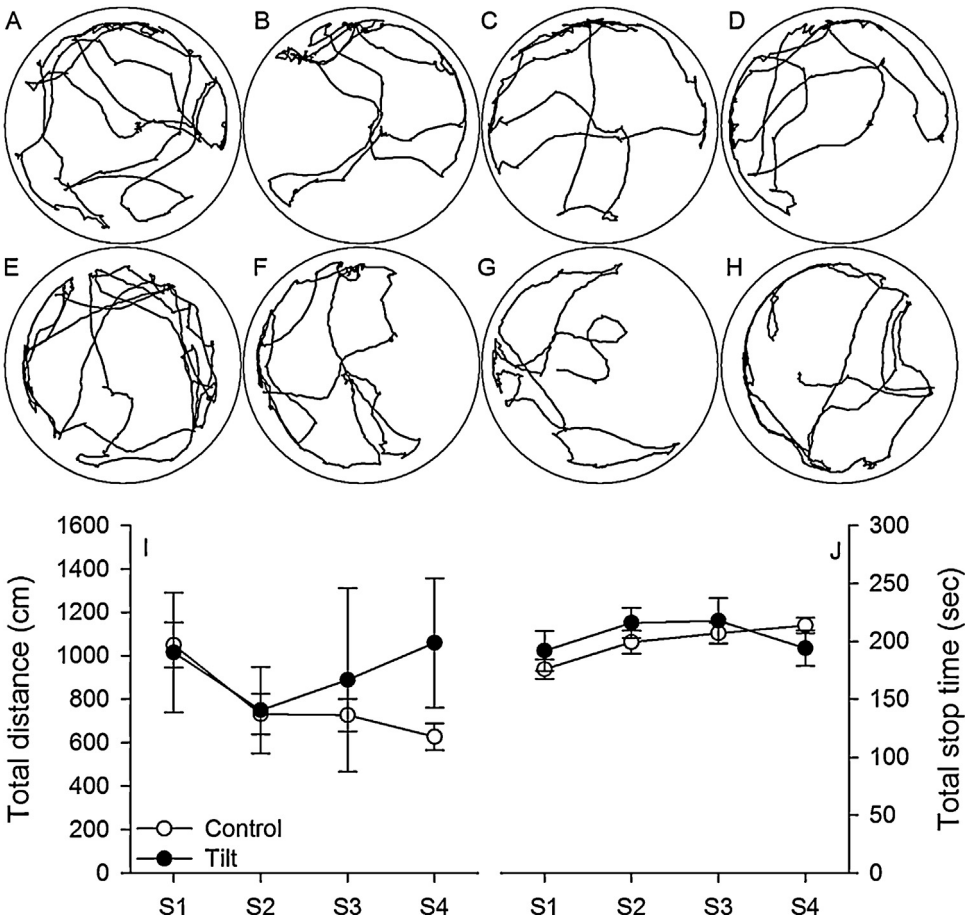

Fig. 5. Circular panels plot paths (black line) followed by representative control (A, B, C, D) and *tilted* (E, F, G, H) mice during each sample under light conditions. Each group's average total distance traveled (I) and stop time (J) are plotted for the four five-minute samples under light conditions.

averaged across the four samples. The *t*-Test conducted on the parameter of concentration for these stops did not reveal significant group difference [T(17) = 0.120, p = 0.906, d = 0.054] (Fig. 8j). No group differences were observed in stop clustering stability across samples under light conditions.

4. Discussion

The current study applied a novel analysis of exploratory behavior to investigate the contribution of otolithic organs to spatial orientation. Control and otoconia-deficient *tilted* mice explored a circular table under dark conditions prior to exploring the same table under light conditions. Both groups exhibited equivalent travel distances and stop times across all samples under dark and light conditions. Both groups tended to engage in similar exploratory behaviors, thus discounting locomotor or anxiety-like factors and their respective influences on exploration. Several observations provide evidence that *tilted* mice have deficits in processing self-movement cues. First, larger changes in heading between progressions were observed in *tilted* mice relative to control mice under dark and light conditions. Next, only under dark conditions were *tilted* mice observed to follow more circuitous progressions relative to control mice. Finally, *tilted* mice were observed to exhibit more variability in stop clustering across samples relative to control mice, but only under dark conditions. These results add to a growing literature for the role of the vestibular system in maintaining spatial orientation during navigation.

4.1. Otolith organs and sense of direction

Multiple lines of evidence have suggested that signals from the otolith organs contribute to one's sense of direction, although this otolithic contribution is not well understood at the present time. First, rat head direction cells were observed to lose their directional tuning as animals navigated while in an inverted position [44], possibly because the visual cues and otolith signals associated with inversion were unfamiliar to a terrestrial species. Head direction cells also lost their directional tuning when the animal was moved from a wall to a ceiling during the 0 g phase of parabolic flight [45]. In this condition, the otolith organs would not have detected the change in head position, possibly producing a conflict between visual and vestibular cues. Additionally, relatively normal head direction cells were recorded from *tilted* mice, but the directional tuning became increasingly degraded across trials [22]. The fact that directional tuning was relatively normal in the initial trial suggests that otolith signals are not crucial for the directional tuning of head direction cells but, instead, contribute to the maintenance of directional tuning across changes in vertical orientation. However, it is also possible that the activity of rodent head direction cells is directly influenced by gravity, given the recent discovery of gravity-sensitive neurons in the primate anterior thalamus [46]. Further, it is important to consider the role of linear movements in path integration, given that the acceleration phase of linear movements would be detected by the otolith organs. However, it is unclear whether other self-movement cues

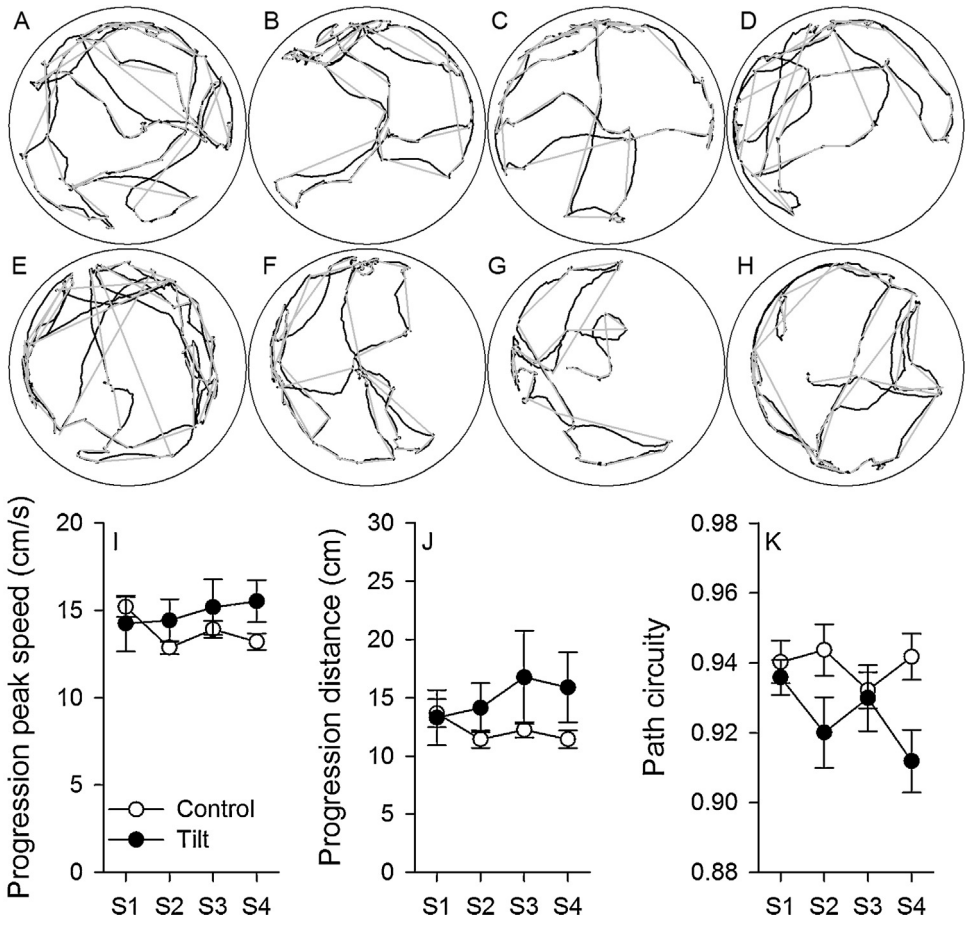

Fig. 6. Circular panels plot the path (black line) and progression Euclidean distances (grey lines) for the same representative control (A, B, C, D) and *tilted* (E, F, G, H) mice under light conditions. Each group's average progression peak speed (I), distance (J), and path circuity (K) is plotted for the four five-minute samples under light conditions.

(i.e., proprioception, motor efference copy) can compensate for the lack of otolithic detection of linear acceleration.

The effects of otolith signal manipulations on head direction cell function align very well with the deficits observed in both non-visual and visual behavioral tasks. A strain of mice with dysfunctional utricular hair cells, referred to as *headbanger* mice, exhibited hyperactivity but limited spatial exploration of a novel open-field environment in darkness, relative to controls [27]. This limited exploration in *headbanger* mice may have resulted from anxiety, given their increased frequency of behaviors characteristic of anxiety, or from spatial disorientation. The spatial disorientation interpretation is consistent with our recent demonstration that *tilted* mice are impaired at using path integration to guide the homeward segment of a homing task in darkness [4]. Importantly, although the greatest path integration deficit was observed in darkness, the *tilted* mice also showed significantly more circuitous homeward segments in light than control mice. This finding suggests that visual cues can partially compensate for the lack of otolith signals. In fact, rats with complete vestibular lesions were able to perform relatively well on the homing task in light [18], despite the fact that vestibular lesions or inactivation completely abolishes the head direction signal [13,14]. However, *tilted* mice were impaired at performing a radial arm maze discrimination task in light when only distal cues were available to guide navigation [23]. Thus, signals from the otolith organs have an important role in the directional aspect of navigation, and visual information can compensate for these deficits in some tasks. However, it is important to note that some aspects of visual function, such as the vestibulo-ocular reflex (VOR), are attenuated in mice with otolith dysfunction [47]. Whether VOR deficits contributed to *tilted* mice's significantly greater change in heading after a stop is not known at this time. Further, brain development may be somewhat different in *tilted* mice than in controls, given that their otolith dysfunction is congenital [48]. Additional research is therefore warranted to determine whether these changes contribute to deficits on navigation tasks.

The idea that otoconia-deficient *tilted* mice express deficits in using self-movement cues to estimate direction is consistent with the results of the present exploration task. First, the tendency for stops to occur in a limited region, referred to as stop clustering, has been associated with home base establishment [30–32]. In the absence of visual cues, impaired direction estimation would be predicted to disrupt returns to the home base and produce more variable home base locations. This was consistent with the less stable stop clustering observed in the *tilted* mice under dark conditions. In contrast, *tilted* mice were observed to increase stop-cluster stability in light, suggesting they were able to use visual cues to compensate for deficits in self-movement cue based direction estimation. Next, decreases in home base stability would likely enhance deficits in processing self-movement cues. Specifically, returns to the home base function to reset the information processing related to dead reckoning [49,50]. If the home base drifts, then the resetting process may not occur as frequently and errors in direction estimation would continue to accumulate. These errors in direction estimation would make it challenging to maintain direct trajectories while moving through an environment [51,52]. Indeed, *tilted* mice showed impairments in progression path circuity under dark conditions, providing further support for a role of

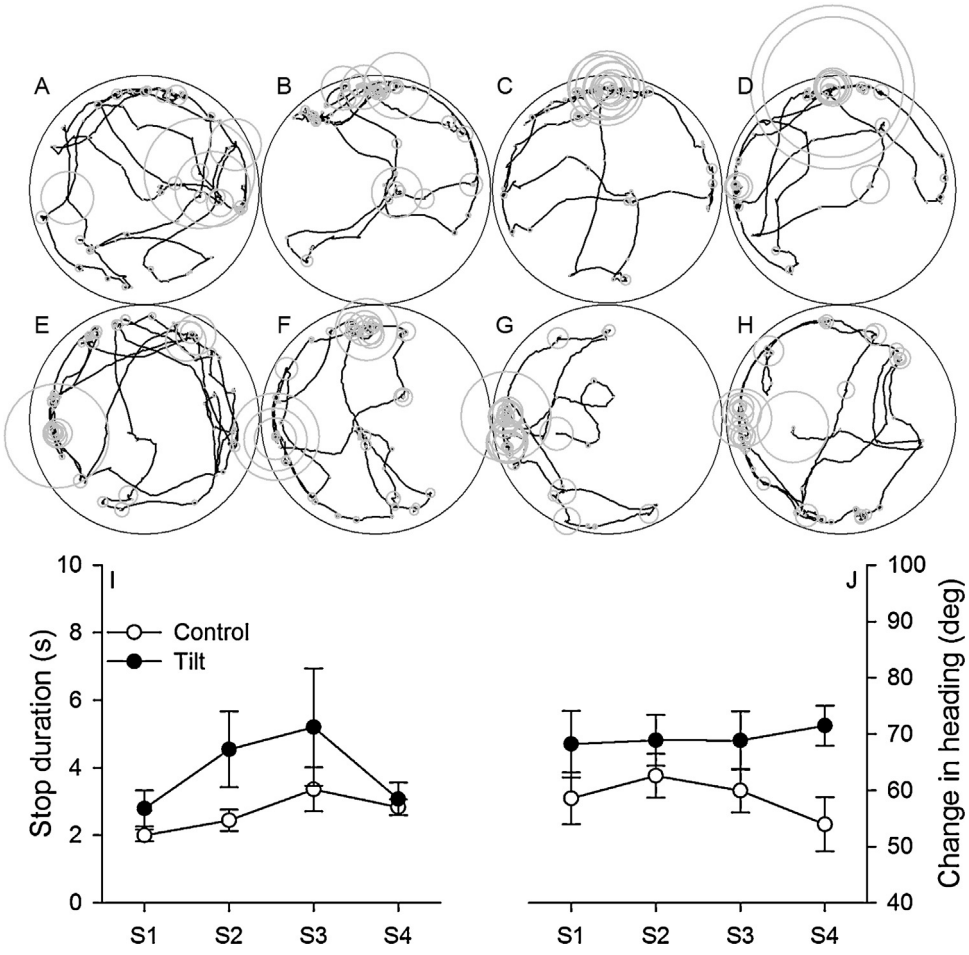

Fig. 7. Circular panels plot the path (black line) and stop location (grey circles) with diameter representing relative stop duration for the same representative control (A, B, C, D) and *tilted* (E, F, G, H) mice under light conditions. Each group's average stop duration (I) and between progression change-in-heading (J) are plotted for the four five-minute samples under light conditions.

otolith organs in direction estimation in darkness. In light, however, these deficits in the use of self-movement information were mostly absent. The available visual cues appear to have allowed *tilted* mice to exhibit progressions and home base stability that were similar to those of control mice. Finally, most of the change in heading during open field behavior typically occurs during stops [33]. Although open field behavior is not typically thought of as goal directed, the organization of exploratory behavior suggests that mice are efficiently sampling the environment. *Tilted* mice exhibited larger changes in heading between progressions under both conditions; however, the effect size was smaller under light conditions ($\eta^2_p = 0.270$) relative to dark conditions ($\eta^2_p = 0.498$). These results thus provide further evidence that visual cues are sufficient to compensate for many of the self-movement cue deficits in direction estimation.

4.2. Advantages of exploration over goal-directed behavior

The sequential analysis of exploratory behavior described in the current study has several advantages over traditional assessments of spatial orientation. First, exploratory behavior is spontaneously generated and does not require extensive training. More protracted training procedures increase the potential of engaging compensatory strategies that may function to confound inferences from performance. For example, performance in the water maze changes across days. Under normal conditions this change in performance reflects the integration of multiple sources of information. However, failure to observe group differences in the water maze after a neural manipulation may reflect the recruitment of a different navigational strategy that is sufficient to guide performance. The acute nature of exploratory behavior limits the potential of mnemonic compensatory mechanisms to confound inferences from performance.

Further, examining exploration behavior under dark then light conditions permits the dissociation of environmental and self-movement cue deficits. Results of the current study add to a growing literature identifying the neural systems involved in processing self-movement cues. For example, separate representations of direction and distance appear to converge on the hippocampus [49]. Accordingly, damage to the hippocampal formation [53,54] or selective hippocampal deafferentation [55,56] has been suggested to impair self-movement cue processing while sparing the use of environmental cues. The sequential analysis of exploratory behavior provides an alternative assessment of spatial orientation that is more acute and does not depend on food deprivation or other motivational factors.

Finally, several lines of evidence discount the role of olfactory cues in guiding movement during exploration. First, qualitatively distinct kinematic profiles are observed during self-movement versus odor guided navigation [57]. Moment-to-moment speeds are typically faster and exhibit a leptokurtic profile when guided by self-movement cues during exploration. In contrast, rats trained to track odor trials elicit slower peak speeds and exhibit a platykurtic moment-to-moment speed profile. Next, organization

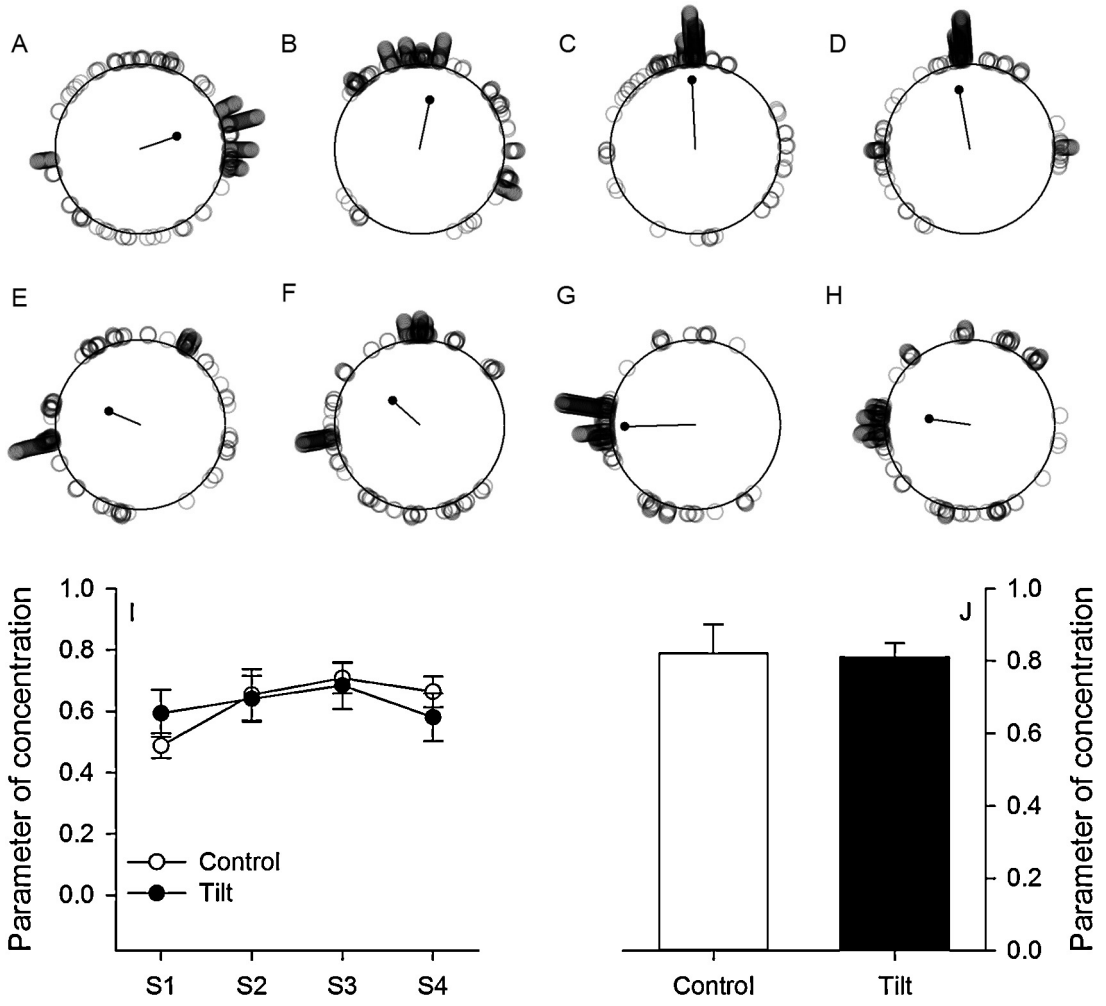

Fig. 8. Circular panels plot stop frequencies relative to polar heading direction for the same representative control (A, B, C, D) and *tilted* (E, F, G, H) mice under light conditions. Average heading (theta) and parameter of concentration (r) are provided for each sample and represented by the polar line graph originating at the center of each plot. Group average parameter of concentration is plotted for each sample under light conditions (I). Average parameter of concentration (calculated from each mouse's four average heading directions) is plotted for both groups (J).

of exploratory behavior was not observed to differ between control and olfactory bulbectomized rats [58]. Nevertheless, a recent study demonstrated that mice readily use any available cues, including airborne odor plumes, when navigating within a novel environment [59]. However, the present study was conducted within a closed chamber with little or no air movement, thus limiting the availability of airborne odors. Overall, rodents typically use a combination of self-movement and available environmental cues to organize exploratory behavior; however, olfactory cues are not necessary to guide performance, and are unlikely to have contributed to the present results.

4.3. Summary

Otoconia-deficient mice and their control littermates performed a novel open-field exploration task in dark and light conditions. In both conditions, both control and *tilted* mice made numerous progressions that were punctuated by stops, and established a home base to which they regularly returned. In darkness, *tilted* mice differed from control mice on several measures indicative of impaired direction estimation. In light, these differences were absent or attenuated, suggesting visual information was able to compensate for most of the navigational deficits associated with otolith dysfunction. This study adds to a growing body of literature describing a crucial role for the vestibular system in directional orientation, and provides the first empirical evidence that otolithic information contributes to the organization of exploratory behavior.

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References

[1] C.R. Gallistel, The Organization of Learning, MIT Press, Cambridge, MA, 1990.
[2] S. Benhamou, J.-P. Sauve, P. Bovet, Spatial memory in large scale movements: efficiency and limitation of the egocentric coding process, J. Theor. Biol. 145 (1990) 1–12.
[3] A.S. Etienne, R. Maurer, J. Berlie, B. Reverdin, T. Rowe, J. Georgakopoulos, V. Seguinot, Navigation through vector addition, Nature 396 (6707) (1998) 161–164.
[4] R.M. Yoder, E.A. Goebel, J.R. Koppen, P.A. Blankenship, A.A. Blackwell, D.G. Wallace, Otolithic information is required for homing in the mouse, Hippocampus 25 (8) (2015) 890–899.
[5] R.M. Yoder, B.J. Clark, J.E. Brown, M.V. Lamia, S. Valerio, M.E. Shinder, J.S. Taube, Both visual and idiothetic cues contribute to head direction cell stability during navigation along complex routes, J. Neurophysiol. 105 (6) (2011) 2989–3001.
[6] R.U. Muller, J.L. Kubie, The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells, J. Neurosci. 7 (7) (1987) 1951–1968.

[7] J. O'Keefe, D.H. Conway, Hippocampal place units in the freely moving rat: why they fire where they fire, Exp. Brain Res. 31 (4) (1978) 573–590.
[8] L.L. Chen, L.H. Lin, C.A. Barnes, B.L. McNaughton, Head-direction cells in the rat posterior cortex. II. Contributions of visual and ideothetic information to the directional firing, Exp. Brain Res. 101 (1) (1994) 24–34.
[9] J.S. Taube, R.U. Muller, J.B. Ranck Jr., Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis, J. Neurosci. 10 (2) (1990) 420–435.
[10] J.S. Taube, R.U. Muller, J.B. Ranck Jr., Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations, J. Neurosci. 10 (2) (1990) 436–447.
[11] J.S. Taube, H.L. Burton, Head direction cell activity monitored in a novel environment and during a cue conflict situation, J. Neurophysiol. 74 (5) (1995) 1953–1971.
[12] R.M. Yoder, B.J. Clark, J.S. Taube, Origins of landmark encoding in the brain, Trends Neurosci. 34 (11) (2011) 561–571.
[13] R.W. Stackman, A.M. Herbert, Rats with lesions of the vestibular system require a visual landmark for spatial navigation, Behav. Brain Res. 128 (1) (2002) 27–40.
[14] R.W. Stackman, J.S. Taube, Firing properties of head direction cells in the rat anterior thalamic nucleus: dependence on vestibular input, J. Neurosci. 17 (11) (1997) 4349–4358.
[15] N.A. Russell, A. Horii, P.F. Smith, C.L. Darlington, D.K. Bilkey, Long-term effects of permanent vestibular lesions on hippocampal spatial firing, J. Neurosci. 23 (16) (2003) 6490–6498.
[16] M.L. Mittelstaedt, S. Glasauer, Idiothetic navigation in gerbils and humans, Zool. Jb. Physiol. 95 (1991) 427–435.
[17] M.L. Mittelstaedt, H. Mittelstaedt, Homing by path integration in a mammal, Naturwissenschaften 67 (11) (1980) 566–567.
[18] D.G. Wallace, D.J. Hines, S.M. Pellis, I.Q. Whishaw, Vestibular information is required for dead reckoning in the rat, J. Neurosci. 22 (22) (2002) 10009–10017.
[19] N.A. Russell, A. Horii, P.F. Smith, C.L. Darlington, D.K. Bilkey, Bilateral peripheral vestibular lesions produce long-term changes in spatial learning in the rat, J. Vestib. Res. 13 (1) (2003) 9–16.
[20] G.M. Muir, J.E. Brown, J.P. Carey, T.P. Hirvonen, C.C. Della Santina, L.B. Minor, J.S. Taube, Disruption of the head direction cell signal after occlusion of the semicircular canals in the freely moving chinchilla, J. Neurosci. 29 (46) (2009) 14521–14533.
[21] S. Valerio, J.S. Taube, Head direction cell activity is absent in mice without the horizontal semicircular canals, J. Neurosci. 36 (3) (2016) 741–754.
[22] R.M. Yoder, J.S. Taube, Head direction cell activity in mice: robust directional signal depends on intact otolith organs, J. Neurosci. 29 (4) (2009) 1061–1076.
[23] R.M. Yoder, S.L. Kirby, Otoconia-deficient mice show selective spatial deficits, Hippocampus 24 (10) (2014) 1169–1177.
[24] M.L. Machado, N. Kroichvili, T. Freret, B. Philoxene, V. Lelong-Boulouard, P. Denise, S. Besnard, Spatial and non-spatial performance in mutant mice devoid of otoliths, Neurosci. Lett. 522 (1) (2012) 57–61.
[25] R.M. Yoder, J.S. Taube, The vestibular contribution to the head direction signal and navigation, Front. Integr. Neurosci. 8 (2014) 32.
[26] M. Goddard, Y. Zheng, C.L. Darlington, P.F. Smith, Locomotor and exploratory behavior in the rat following bilateral vestibular deafferentation, Behav. Neurosci. 122 (2) (2008) 448–459.
[27] R. Avni, T. Elkan, A.A. Dror, S. Shefer, D. Eilam, K.B. Avraham, M. Mintz, Mice with vestibular deficiency display hyperactivity, disorientation, and signs of anxiety, Behav. Brain Res. 202 (2) (2009) 210–217.
[28] F.E. Harrison, A.H. Hosseini, M.P. McDonald, Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks, Behav. Brain Res. 198 (1) (2009) 247–251.
[29] C.V. Vorhees, M.T. Williams, Morris water maze: procedures for assessing spatial and related forms of learning and memory, Nat. Protoc. 1 (2) (2006) 848–858.
[30] D. Eilam, I. Golani, Home base behavior of rats (Rattus norvegicus) exploring a novel environment, Behav. Brain Res. 34 (3) (1989) 199–211.
[31] B.J. Clark, D.A. Hamilton, I.Q. Whishaw, Motor activity (exploration) and formation of home bases in mice (C57BL/6) influenced by visual and tactile cues: modification of movement distribution, distance, location, and speed, Physiol. Behav. 87 (4) (2006) 805–816.
[32] O. Tchernichovski, I. Golani, A phase plane representation of rat exploratory behavior, J. Neurosci. Methods 62 (1–2) (1995) 21–27.
[33] D.G. Wallace, D.A. Hamilton, I.Q. Whishaw, Movement characteristics support a role for dead reckoning in organizing exploratory behavior, Anim. Cogn. 9 (3) (2006) 219–228.
[34] D.M. Ornitz, B.A. Bohne, I. Thalmann, G.W. Harding, R. Thalmann, Otoconial agenesis in *tilted* mutant mice, Hear. Res. 122 (1–2) (1998) 60–70.
[35] M.M. Martin, K.L. Horn, K.J. Kusman, D.G. Wallace, Medial septum lesions disrupt exploratory trip organization: evidence for septohippocampal involvement in dead reckoning, Physiol. Behav. 90 (2–3) (2007) 412–424.
[36] S.S. Winter, J.R. Koppen, T.B. Ebert, D.G. Wallace, Limbic system structures differentially contribute to exploratory trip organization of the rat, Hippocampus 23 (2) (2013) 139–152.
[37] P.A. Blankenship, S.L. Stuebing, S.S. Winter, J.L. Cheatwood, J.D. Benson, I.Q. Whishaw, D.G. Wallace, The medial frontal cortex contributes to but does not organize rat exploratory behavior, Neuroscience 336 (2016) 1–11.
[38] J.R. Koppen, S.L. Stuebing, M.L. Sieg, A.A. Blackwell, P.A. Blankenship, J.L. Cheatwood, D.G. Wallace, Cholinergic deafferentation of the hippocampus causes non-temporally graded retrograde amnesia in an odor discrimination task, Behav. Brain Res. 299 (2016) 97–104.
[39] I.Q. Whishaw, J.C. Cassel, M. Majchrzak, S. Cassel, B. Will, Short-stops in rats with fimbria-fornix lesions: evidence for change in the mobility gradient, Hippocampus 4 (5) (1994) 577–582.
[40] I. Golani, Y. Benjamini, D. Eilam, Stopping behavior: constraints on exploration in rats (Rattus norvegicus), Behav. Brain Res. 53 (1–2) (1993) 21–33.
[41] B.J. Clark, D.J. Hines, D.A. Hamilton, I.Q. Whishaw, Movements of exploration intact in rats with hippocampal lesions, Behav. Brain Res. 163 (1) (2005) 91–99.
[42] I.Q. Whishaw, B. Kolb, R.J. Sutherland, The analysis of behavior in the laboratory rat, in: T.E. Robinson (Ed.), Behavioural Approaches to Brain Research, Oxford University Press, New York, NY, 1983, pp. 141–211.
[43] E. Batschelet, Circular Statistics in Biology, Academic Press, New York, 1981.
[44] J.L. Calton, J.S. Taube, Degradation of head direction cell activity during inverted locomotion, J. Neurosci. 25 (9) (2005) 2420–2428.
[45] J.S. Taube, R.W. Stackman, J.L. Calton, C.M. Oman, Rat head direction cell responses in zero-gravity parabolic flight, J. Neurophysiol. 92 (5) (2004) 2887–2897.
[46] J. Laurens, B. Kim, J.D. Dickman, D.E. Angelaki, Gravity orientation tuning in macaque anterior thalamus, Nat. Neurosci. 19 (12) (2016) 1566–1568.
[47] C.G. Harrod, J.F. Baker, The vestibulo ocular reflex (VOR) in otoconia deficient head tilt (het) mutant mice versus wild type C57BL/6 mice, Brain Res. 972 (1–2) (2003) 75–83.
[48] M. Beraneck, F.M. Lambert, Impaired perception of gravity leads to altered head direction signals: what can we learn from vestibular-deficient mice? J. Neurophysiol. 102 (1) (2009) 12–14.
[49] D.G. Wallace, M.M. Martin, S.S. Winter, Fractionating dead reckoning: role of the compass, odometer, logbook, and home base establishment in spatial orientation, Naturwissenschaften 95 (11) (2008) 1011–1026.
[50] H. Neven, G. Schöner, Dynamics parametrically controlled by image correlations organize robot navigation, Biol. Cybern. 75 (4) (1996) 293–307.
[51] A.A. Schaeffer, Spiral movement in man, J. Morphol. Physiol. 45 (1928) 293–370.
[52] J.L. Souman, I. Frissen, M.N. Sreenivasa, M.O. Ernst, Walking straight into circles, Curr. Biol. 19 (18) (2009) 1538–1542.
[53] H. Maaswinkel, L.E. Jarrard, I.Q. Whishaw, Hippocampectomized rats are impaired in homing by path integration, Hippocampus 9 (5) (1999) 553–561.
[54] J.H. Gorny, B. Gorny, D.G. Wallace, I.Q. Whishaw, Fimbria-fornix lesions disrupt the dead reckoning (homing) component of exploratory behavior in mice, Learn. Mem. 9 (6) (2002) 387–394.
[55] M.M. Martin, D.G. Wallace, Selective hippocampal cholinergic deafferentation impairs self-movement cue use during a food hoarding task, Behav. Brain Res. 183 (1) (2007) 78–86.
[56] J.R. Koppen, S.S. Winter, S.L. Stuebing, J.L. Cheatwood, D.G. Wallace, Infusion of GAT1-saporin into the medial septum/vertical limb of the diagonal band disrupts self-movement cue processing and spares mnemonic function, Brain Struct. Funct. 218 (5) (2013) 1099–1114.
[57] D.G. Wallace, I.Q. Whishaw, NMDA lesions of Ammon's horn and the dentate gyrus disrupt the direct and temporally paced homing displayed by rats exploring a novel environment: evidence for a role of the hippocampus in dead reckoning, Eur. J. Neurosci. 18 (3) (2003) 513–523.
[58] D.J. Hines, I.Q. Whishaw, Home bases formed to visual cues but not to self-movement (dead reckoning) cues in exploring hippocampectomized rats, Eur. J. Neurosci. 22 (9) (2005) 2363–2375.
[59] D.H. Gire, V. Kapoor, A. Arrighi-Allisan, A. Seminara, V.N. Murthy, Mice develop efficient strategies for foraging and navigation using complex natural stimuli, Curr. Biol. 26 (10) (2016) 1261–1273.