Abstract

Animals use multiple sources of information to maintain spatial orientation, including self-movement and environmental cues. Self-movement cues are processed by the vestibular system, and animals with congenital vestibular defects exhibit impairments in self-movement cue processing but not environmental cue processing. No study currently exists to examine the exploratory behavior of mice following complete bilateral vestibular lesions. The goal of this study was to evaluate the effects of bilateral chemical labyrinthectomies on mouse exploratory behavior under dark and light conditions at two time points. Motion tracking software was used to capture mouse exploratory behavior, such as path circuitry and change in heading. Persistent deficits were observed in mice with vestibular lesions. Mice with bilateral vestibular lesions engaged in more circuitous paths and higher changes in directional heading between progressions at each time point and under dark and light conditions. This study demonstrates the important role that the vestibular system has in organizing the exploratory behavior of mice.
Introduction

The vestibular system encodes head movements and body posture in three-dimensional space with respect to the ground (19). This system consists of two physical apparatuses located in the inferolateral skull and possesses an extensive network of neural correlates in the central nervous system (the thalamocortical vestibular system) [19]. Studies employing electrical stimulation have suggested the cingulate cortex and hippocampus as areas containing some of these vestibular projections [19]. The hippocampus contributes substantially to animal spatial learning and memory, and a considerable amount of work has studied the complex relationship between hippocampus and the vestibular system (e.g. 5,7,8,9,10). As the elderly population has begun to comprise a growing segment of the developed world, the interest in vestibular pathology has begun focusing on patients suffering with neurodegenerative disorders, such as Alzheimer’s Disease. In fact, hypotheses have been made that vestibular pathology might even have a causal role in the development of such disorders (7). Unilateral and bilateral vestibulopathy results from a myriad of other conditions in humans and has been analyzed in the clinical setting (17).

As animals explore their environment, a variety of information is encoded by an array of sensory receptors and processed in the central nervous system. Humans rely extensively on visual information for organizing exploratory behavior and path integration, evidenced by circling behavior under dark or blindfolded conditions, with the diameter of such circular paths reaching minimum values approaching only 20m (20). The vestibular system’s role in encoding information that is vital to self-movement cue processing and exploration under dark conditions (where visual information is unavailable) has been established by previous works assessing rodent exploratory behavior (2,3,12,13,15,16). Such works have also established effective
measures and criteria for analyzing the exploratory behavior of rodents (2,3). Commonly, this is achieved by the use of video data and motion capturing software to divide exploratory movements into a series of stops and progressions (2,3). Extensive work has been performed to assess the behavioral deficits experienced by otoconia-deficient tilted mice (13,15,16). Previous work [2] has provided insight on what exploratory behavior is characteristic of these mice with partially degraded vestibular function. Such studies have identified behavioral differences in tilted mice, including navigational differences under dark conditions (2). Furthermore, the tilted mouse model of vestibulopathy has demonstrated improvements in performance under light conditions, indicating compensation for vestibulopathy via the use of visual stimuli (2). The current project will analyze measures involving the stops and progressions of mice receiving a complete, bilateral vestibular lesion under both dark and light conditions at two separate time points. Measures such as average peak speed, distance traveled, path circuity, average change in directional heading, and stop clustering will hopefully, when combined with histological data, provide a better understanding of the complex relationship between the vestibular system and its extensive neural correlates in the hippocampus.

**Methodology**

**Subjects**

This study used C57BL/6J mice (n=15) received from the Jackson Laboratory in Bar Harbor, Maine. All animals were housed in cohorts of 4 animals in clear, plastic containers with metal wire cage tops. All animals were housed in the same room at approximately 20 degrees Celsius and a constant pressure and humidity. All protocols were approved by NIU IACUC.

**Procedures**
Seven (of fifteen total) mice underwent a bilateral surgical induction of acquired vestibulopathy though the intratympanic injection of an ototoxic substance (Sodium Arsenylate, 100 mg/ml). Following a three-week recovery period, exploratory behavior was assessed. Each exploratory trial consists of first placing the animal in the middle of a circular table (122cm diameter). Each animal is then allowed to explore the table for 40 minutes before it is removed from the table and returned to its home cage. Exploratory behavior of all mice was assessed first under dark conditions. Exactly one week later, the exploratory behavior of all mice was assessed under light conditions. One month from the first day of testing, exploratory behavior was once-again assessed under dark conditions. Exploratory behavior under light conditions was then assessed exactly one-week following this date. All exploratory trials were video recorded.

**Data Analysis**

Motion capture software was used to analyze the video recordings of each exploratory session. Animal movement was sorted into series of stops and progressions. All movements calculated at or above 3 cm/s were defined as progressions. All movements calculated below the 3 cm/s threshold were defined as stops. The video data was assessed for several measures. The total distance traveled, total stop time, and peak speeds achieved for each group in each trial were obtained. A measure of path circuity (distance ratio) was also obtained by calculating a ratio of the Euclidian distance of each progression to the path traveled. Average heading direction change was also obtained by comparing the directional headings of sequential progressions. A stop cluster analysis was also performed in order to assess the topographic consistency of stop groupings within each trail. All mice were then perfused using active perfusion technique for future histological analysis.
Results

Timepoint 1

Results from this study evaluated the role of the vestibular system in the processing of self-movement cues. The general topographic and kinematic measures of the exploratory behavior of mice in each group were plotted for both groups for Timepoint 1 under dark and light conditions. These include distance traveled (Distance), total stop time (Stop time), and peak speeds achieved (Peak speed). No significant differences were observed between groups under dark conditions at Timepoint 1. Under light conditions at Timepoint 1, significant differences between groups were observed in all three general measures: vestibular mice traveled greater distances, spent less time stopped, and achieved higher peak speeds compared to control mice.

Figure 1: Distance traveled (top), Stop time (middle), and Peak speed (bottom) under dark (shaded) and light (unshaded) conditions at Timepoint 1
Path circuity (Dist ratio) and Change in directional heading (Change in heading) were plotted for each group at Timepoint 1 under dark and light conditions. Significant differences between groups were observed for both measures under both conditions at Timepoint 1: Vestibular mice engaged in more circuitous paths (evidenced by a lower distance ratio), as well as higher changes in directional heading compared to control mice.

Figure 2: Path circuity/distance ratio (top) and Peak speed (bottom) under dark (shaded) and light (unshaded) conditions at Timepoint 1

The results of the stop cluster analysis for each trial (First order r) were plotted for each group at Timepoint 1 under dark and light conditions. Significant differences were observed between groups under both dark and light conditions at Timepoint 1: Vestibular mice engaged in stops with a tighter clustering within each trial compared to control mice.
Figure 3: First order r under dark (shaded) and light (unshaded) conditions at Timepoint 1

**Timepoint 2**

The general topographic and kinematic measures of the exploratory behavior of mice in each group were plotted for both groups for Timepoint 2 under dark and light conditions. These include distance traveled (Distance), total stop time (Stop time), and peak speeds achieved (Peak speed). No significant differences were observed between groups under dark conditions at Timepoint 2. Under light conditions at Timepoint 2, significant differences between groups were observed in all three general measures: vestibular mice traveled greater distances, spent less time stopped, and achieved higher peak speeds.
Figure 1: Distance traveled (top), Stop time (middle), and Peak speed (bottom) under dark (shaded) and light (unshaded) conditions at Timepoint 2

Path circuity (Dist ratio) and Change in directional heading (Change in heading) were plotted for each group at Timepoint 2 under dark and light conditions. Significant differences between groups were observed for both measures under both conditions at Timepoint 2:

Vestibular mice engaged in more circuitous paths (evidenced by a lower distance ratio), as well as higher changes in directional heading compared to control mice.
The results of the stop cluster analysis for each trial (First order r) were plotted for each group at Timepoint 1 under dark and light conditions. Significant differences were observed between groups under dark conditions at Timepoint 2: Vestibular mice engaged in stops with a tighter clustering within each trial compared to control mice. No significant differences were observed between groups under light conditions at Timepoint 2.

Figure 2: Path circuity/distance ratio (top) and Peak speed (bottom) under dark (shaded) and light (unshaded) conditions at Timepoint 2.

Figure 3: First order r under dark (shaded) and light (unshaded) conditions at Timepoint 2.
Discussion

Acquired bilateral vestibular pathology (modeled via bilateral chemical labyrinthectomy surgery) had specific deficits in the exploratory behavior of mice. Mice in both groups engaged in highly-organized exploratory behavior consisting of series of stops and progressions. Under dark conditions at both time points, no significant differences were observed for the general measures obtained for exploratory behavior (consisting of distance traveled, stop time, and peak speed). Under light conditions, the observed differences in these measures at both time points could be due to the vestibular pathology’s role in amplifying the anxiety produced by light conditions, which, to begin with, are usually considered slightly aversive for rodents. Under both dark and light conditions at both time points, mice that received bilateral chemical labyrinthectomies showed increases in Path circuitry and Change in heading. This is indicative of less-organized exploratory behavior, most likely due to disruption in self-movement cue processing and spatial orientation.

The persistence of these deficits under light conditions as well as dark conditions (i.e. with or without access to visual cues) is markedly different than results from partial vestibular dysfunction seen in studies analyzing the Tilted Mouse model of otoconia deficiency. A significant difference was observed in First order r under both conditions at Timepoint 1 and under dark conditions at Timepoint 2. Because this difference was observed, statistical analysis of Second order r (i.e. stop cluster analysis across all four trials) is prohibited. Mice with acquired vestibular pathology engaged in more clustered stops than control mice, however this might be an artifact of the movement characteristics of the mice with vestibular pathology (e.g. greater path circuitry). In other words, a vestibular mouse might travel as far as a control mouse
in between stops, however a more circuitous path will take the mouse a shorter Euclidian distance from the previous stop, giving the appearance of a more-clustered set of stops.

Given these results, future studies could investigate the role of vestibular pathology in amplifying anxiety brought on by certain environmental conditions (e.g. light acting as an aversive stimulus). Such studies could employ the use of mild anxiolytics in order to analyze the role of vestibular pathology and amplified anxiety in creating differences in the general measures of exploratory behavior in mice (such as the ones that were observed in this study under light conditions). Altogether, the data suggest that self-movement cue processing and, therefore, the organization of spatial orientation and exploration were disrupted by acquired vestibular pathology. This study adds to a growing literature illustrating a role for the vestibular system in maintain spatial orientation and processing self-movement cues.
References


