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Impairment and Recovery on a Food Foraging Task Following Unilateral Vestibular Deafferentation in Rats

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ABSTRACT: It has been suggested that the vestibular system may contribute to the development of higher cognitive function, especially spatial learning and memory that uses idiothetic cues (e.g., dead reckoning). However, few studies have been done using behavioral tasks that could potentially separate the animals' ability for dead reckoning from piloting. The food foraging task requires the animal to continuously monitor and integrate self-movement cues and generate an accurate return path. It has been shown that bilateral vestibular-lesioned rats were impaired on this task. The present study used the same task to further examine the contribution of vestibular information to spatial navigation by comparing unilateral and bilateral lesions and by testing the animals at different time points following the lesion. The results demonstrated that animals with unilateral vestibular deafferentation were impaired in performing the task in the dark at 3 months after the lesion, and this impairment disappeared at 6 months after the lesion. This supports the notion that vestibular information contributes to dead reckoning and suggests possible recovery of function over time after the lesion. Animals with bilateral vestibular deafferentation were not able to be tested on the foraging task because they exhibited behavior distinct from the unilateral-lesioned animals, with significant hesitation in leaving their home cage for as long as 6 months after the lesion. © 2005 Wiley-Liss, Inc.

KEY WORDS: vestibular system; hippocampus; food hoarding; spatial navigation; dead reckoning

INTRODUCTION

Over the last decade, increasing evidence has suggested that vestibular information may contribute to the development of higher cognitive function. For example, both vestibular stimulation (Matthews et al., 1989; Semenov and Bures, 1989) and peripheral vestibular lesions (Ossenkopp and Hargreaves, 1993; Peruch et al., 1999; Stackman and Herbert, 2002; Wallace et al., 2002; Russell et al., 2003a; Schautzer et al., 2003) disrupt spatial learning and memory in a number of behavioral tasks in both animals and humans.

Spatial learning and memory, which depend on the animal's ability to learn and remember how to get to a specific location in space, are essential forms of higher cognitive processing in mammals. In order to navigate to specific locations, the animal needs to use stable distant stimuli in the environment (visual, olfactory and auditory information, e.g., piloting) and/or information generated from the animal's own movement (proprioceptive feedback and vestibular information, e.g., dead reckoning). It has been suggested that animals are able to use both strategies to navigate (Gallistel, 1990).

By testing the animals in the dark and/or disorienting them, it is possible to investigate the use of vestibular information in dead reckoning and the neural systems involved in different behavioral tasks (Moghaddam and Bures, 1996; de Bruin et al., 1997; Commins et al., 1999; Maaswinkel et al., 1999; Cooper et al., 2001; Whishaw et al., 2001b; Nieto-Escámez et al., 2002; Zheng et al., 2003b). Recently, the importance of the vestibular contribution to spatial navigation was further investigated by Wallace et al. (2002). In their study, vestibular information was eliminated by bilateral intratympanic injections of sodium arsanilate and the animals were tested on a food foraging task that requires the animal to continuously monitor and integrate self-movement cues and generate an accurate return path (Whishaw and Tomie, 1997). The results demonstrated that vestibular-lesioned rats were not only impaired in carrying food back to home base in the dark (dead reckoning), but also impaired in learning a new home location in light (piloting). However, there are several flaws in this study. First, the vestibular lesion was induced by intratympanic injections of sodium arsanilate and previous studies have shown that such injections produce incomplete or reversible lesions to the labyrinth (Jensen, 1983; Saxon et al., 2001). Second, the unilateral vestibular-lesioned rats served only as a baseline to evaluate the extent of the bilateral damage and were not tested on the task. This is unfortunate because unilateral and bilateral vestibular lesions produce distinct behavioral syndromes.

The present study, therefore, compared the performance of unilateral and bilateral vestibular-lesioned animals on a food foraging task and compared complete surgical vestibular deafferentation with its appropriate sham controls. The animals were also tested at 3 and 6 months after the operation to determine the temporal changes in the animals' performance (development of impairment or recovery) that might develop following the lesion.

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MATERIALS AND METHODS

Subjects

Twenty-one male Wistar rats (180–200 g at the beginning of the experiment) were obtained from the animal breeding station, Dunedin, New Zealand. The animals were housed in pairs throughout the entire experiments, and all of the experiments were performed in accordance with regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals.

Food Deprivation

The animals were food deprived during the course of behavioral testing and the body weight was maintained at 85% of their normal feeding weight. Large oat and wheat honey cereal loops were used during behavioral testing. Each day after testing, the rats were supplementally fed with normal laboratory rodent pellets in their home cage to maintain body weight.

Apparatus

The apparatus was similar to that used by other researchers (Whishaw and Tomie, 1997; Maaswinkel et al., 1999; Wallace et al., 2002) with slight modifications. It consisted of a large circular table (140 cm in diameter) with eight holes (10 cm in diameter) equally distributed around the edge and centered 10.5 cm from the edge. The table was located in a room (4.9×3.5 m^2) with plenty of visual cues on the wall and mounted on a center bearing 105 cm above the floor so that the table could be rotated between animals. Twenty-three food cups (4 cm in diameter, 1 cm in height) were attached to the table. A cage that served as the rat's home cage could be placed beneath one of the eight holes. An opaque curtain was hung from the ceiling so that the table could be enclosed for dark conditions. For light conditions, the room was illuminated by two fluorescent lights located on the ceiling; for dark conditions, the fluorescent lights were turned off and an infrared light source was placed inside the curtain. An infrared camera was located above the center of the table so that the behavior of the animals could be video recorded under both light and dark conditions. A speaker continually playing white noise was also mounted above the center of the table.

Preoperation Behavioral Training and Testing

Pretraining

For the pretraining, the animals were required to retrieve four food pellets per session over a maximum period of 10 min for 10 consecutive days. All of the food cups were baited for the first few days and the number of cups baited was gradually reduced over time. By the end of the training, only one cup was baited per trial. After the rat found the food and carried it back home, a new cup was baited while the rat was eating. The food was baited in different cups for different trials, but the same cup was baited for all of the animals on each trial for the purpose of comparison. The home cage was located under the same hole in relation to the room throughout the pretraining phase. The table was cleaned and randomly rotated 135 degrees clockwise or anticlockwise between the animals. The room lights were on and the curtain surrounding the table was drawn back.

Homing accuracy using allocentric cues

After the rats had learned to successfully retrieve four food pellets each day, they were given one probe trial with the home cage located at a novel position under the light condition, with the room setting exactly the same as in the pretraining stage. If a rat could not return back home on the probe trial after 120 s, then it was removed from the table.

Homing accuracy using egocentric cues

Following the probe trial in light, the animals were given one day (four trials) of normal training with the home cage located at the same place as on the pretraining trials. A further 6 days of training was subsequently carried out in the dark with the curtain surrounding the table. The training in the dark consisted of one trial per day with the home cage located at a different position each day. The table was cleaned and rotated between the animals.

Grouping and Surgical Procedures

After preoperation training and testing, animals were randomly divided into five groups: anesthetic control ($n = 4$), unilateral vestibular deafferentation (UVD) ($n = 5$), UVD sham ($n = 4$), bilateral vestibular deafferentation (BVD) ($n = 4$), and BVD sham ($n = 4$). Later, the anesthetic, UVD sham, and BVD sham control groups were combined into one larger control group ($n = 12$) for comparison with the UVD and BVD groups (see Results). Pilot studies indicated that these sample sizes provided sufficient power to detect significant differences, and this was borne out by the results of the study. A complete surgical vestibular deafferentation was performed using the method described elsewhere (King et al., 2002). Briefly, animals were anesthetized with 300 μg/kg (i.p.) of fentanyl citrate (1 mg/ml, Mayne Pharma Pty, Australia) and 300 μg/kg (i.p.) of medetomidine hydrochloride (1 mg/ml, Novartis Animal Health Australasia Pty, New Zealand). Xylocaine (1% Lignocaine hydrochloride with 1: 100,000 adrenaline, AstraZeneca, New Zealand) was also injected around the wound margins. A complete unilateral or bilateral surgical labyrinthectomy was performed using an otolaryngological microscope. The tympanic bulla was exposed using a retro-auricular approach and the tympanic membrane, the malleus, and incus were removed. The stapedial artery was cauterized and the horizontal and anterior semicircular canal ampullae were drilled open using a high-speed dental drill with a fine burr. The contents of the canal ampullae and the utricle and saccule were then aspirated and the temporal bone sealed with dental cement. Subcutaneous injection of 5 mg/kg of Carprofen (50 mg/ml, Pfizer Laboratories, UK) was used for postoperative analgesia. The UVD surgery was always performed on the right side. Sham surgery

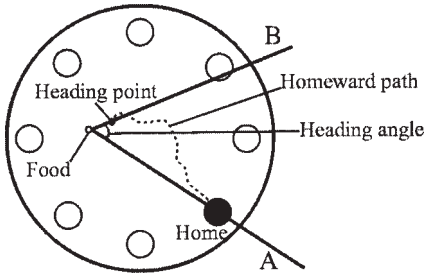

FIGURE 1. Foraging table and the heading angle. The black circle indicates the home location and the small open circle indicates the food location. Heading point (small black circle) was defined as the point 15 cm along the homeward path. Heading angle was calculated as the angle between the line connecting the food location and the home (A) and a line connecting the food location and the heading point (B).

consisted of exposing the temporal bone and removing the tympanic membrane without producing a vestibular lesion.

Postoperation Behavioral Training and Testing

At 3 and 6 months after the operation, all animals were subjected to an object recognition task before beginning the foraging task. The details of the object recognition task have been reported elsewhere (Zheng et al., 2004). Immediately following the last day of the object recognition task, animals were given 10 days of pretraining on the foraging task and tested again under both allocentric and egocentric conditions (see preoperation training stages 2 and 3).

Temporal Bone Histology

At the end of the behavioral tests, animals were decapitated, and the temporal bones were dissected out and fixed in 10% formalin in 0.1 M phosphate buffered saline (PBS) (pH 7.4) for 2 days. Decalcification was carried out in 1.3 N HCl containing 5 mM EDTA for 5 days. The decalcified tissue was embedded in wax and sectioned (5 μm thick) parallel to the horizontal semicircular canal and mounted on slides. The sections were stained with hematoxylin and eosin (H&E). The sensory epithelia of the crista ampullaris in the horizontal, anterior and posterior semicircular canals, and the maculae in the utricle and the saccule were examined using light microscopy to confirm the completeness of the vestibular lesions.

Measurements and Statistical Analysis

The time taken to find the home, the holes chosen by the rats, and the number of errors made before the rat found the correct home were recorded on each trial. The choice for a specific hole was defined as a rat stopping beside the hole and making inspections of it. Video recordings were replayed and the searching distance, searching speed, homing distance, and homing speed were calculated using custom-made tracking software. The rats were spray painted with black stockmarker (Donaghys Industries, Christchurch, New Zealand) between the shoulder blades, and the sampling rate of the software was 20 frames/s. Heading angle was also calculated as the angle between the line connecting the food location and the home (A) and a line connecting the food location and the heading point (a point 15 cm along the homeward path) (B) (Fig. 1). The effects of different treatments and different training sessions were analyzed using two-way ANOVAs with repeated measures. In the cases where the distribution of the data violated the assumptions of this test, a nonparametric Kruskal-Wallis analysis of variance (ANOVA) on ranks was performed. The first choices made by the animals on the light probe trial and on the first and the last of the dark trials were analyzed with circular statistics, and the Rayleigh test was used to determine the significance of the mean-homing direction and the tendency to that direction (Mardia and Jupp, 1999).

RESULTS

Temporal Bone Histology

Compared with the control animals (Fig. 2A), vestibular deafferentation resulted in complete or partial destruction of the supporting cells and extensive loss of the hair cells in the

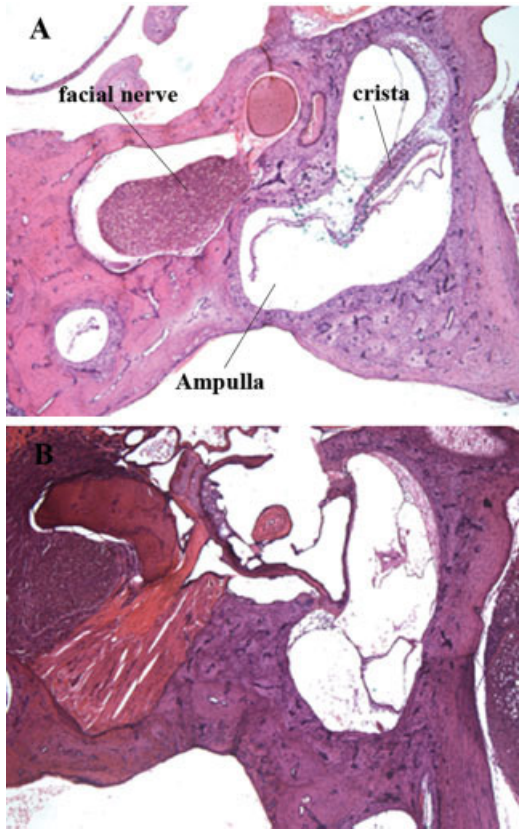

FIGURE 2. Temporal bone histology. A: Representative section of the vestibular inner ear from a control rat showing the facial nerve and normal crista ampullaris. B: Representative section of the vestibular inner ear from a vestibular-lesioned rat showing the ampulla with crista destroyed. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

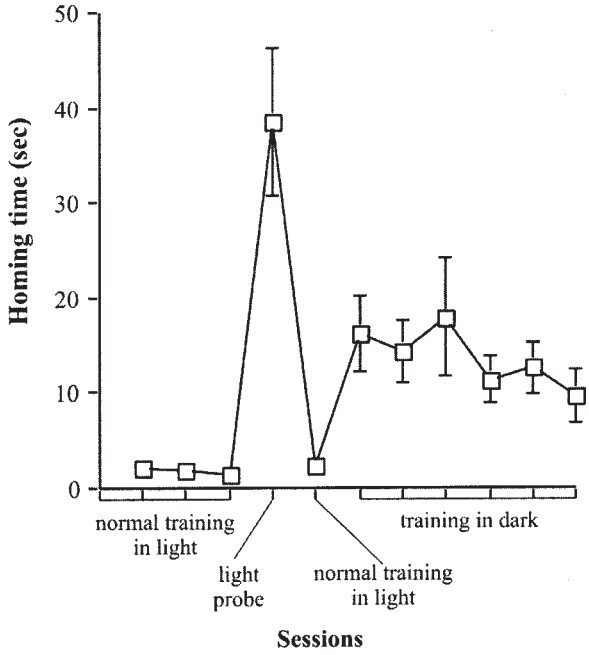

FIGURE 3. Homing time at the preoperation stage. Data reflect the mean of four trials and are expressed as mean ± standard error of mean (SEM).

cristae and the maculae (Fig. 2B). In some cases, a small number of hair cells were still present, but were irregular in shape and detached from the base.

Preoperation Behavioral Training and Testing

Pretraining

After a period of 10 days pretraining, all animals were reliably carrying the food pellet back to the home cage to eat. This was indicated by the stable homing time over the last three normal training sessions (Fig. 3).

Homing accuracy using allocentric cues

On the probe trial in light when the home cage was located at a novel place, most of the rats carried the food back to the old home location first. Then, once they found that the home cage was not in the old location, approximately half of the animals chose the correct new home location on their second attempt (Fig. 4A,B) and the rest of the animals made more choices before they reached the correct home. This was also evidenced by a significant increase in homing time on the probe trial when compared with other sessions ($F_{10} = 9.151$, $P < 0.001$, Fig. 3). Circular distribution analysis of the animals' first choice of the holes on the probe trial also revealed a strong direction and tendency towards the old home location ($r = 0.887$, $P < 0.001$, Rayleigh test; Fig. 4A).

Homing accuracy using egocentric cues

When the animals were trained in the dark, on the first day, their first choice for home was randomly distributed around the table and approximately half of the animals could not find

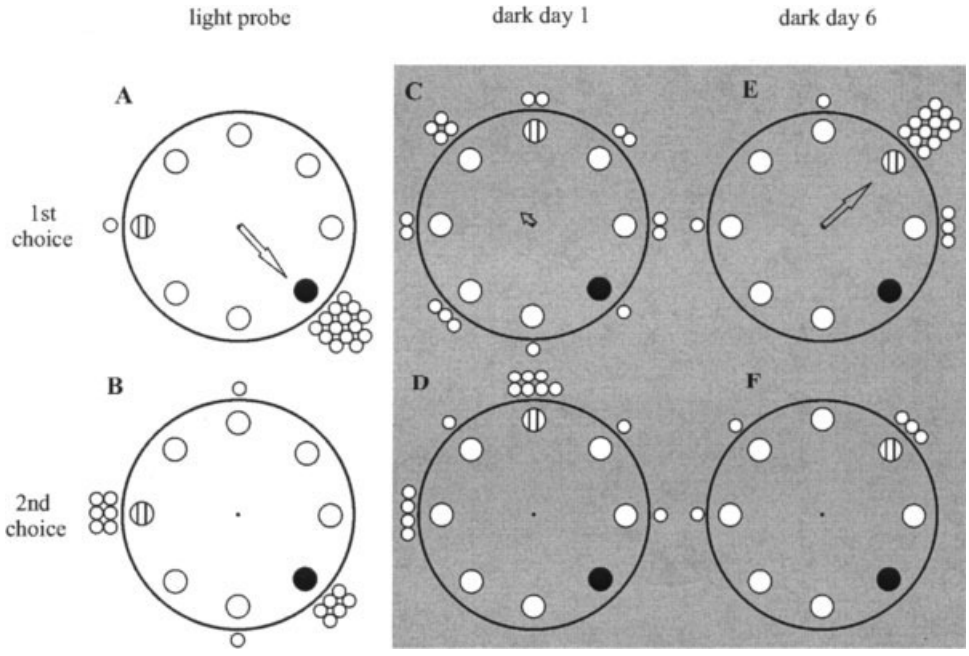

FIGURE 4. Home choices at the preoperation stage. The black circle indicates the old home location and the hashed circle indicates the new home location. The small circle represents each individual animal's home choice. The top panel indicates the animals' first home choices on the light-probe trial (A), the first day of training in the dark (C), and the last day of training in the dark (E). The bottom panel indicates the animals' second home choices on the light-probe trial (B), the first day of training in the dark (D), and the last day of training in the dark (F). The direction of the arrow represents the average direction of the first home choices and the length of the arrow represents the accuracy of the first home choices.

their home on the second choice (Fig. 4C,D). Circular distribution analysis showed that the animals' first choices were randomly distributed around the table ($r = 0.219$, $P = 0.450$, Rayleigh test; Fig. 4C). However, after 5 days of training in the dark, most of the rats went directly back to the correct home on their first attempt on the sixth day (Fig. 4E). Circular distribution analysis also suggested a strong direction and tendency towards the new home location ($r = 0.832$, $P < 0.001$, Rayleigh test; Fig. 4E). Posthoc tests following a one-way ANOVA with repeated measures revealed a significant increase in homing time on the first and the third day in the dark compared with the light sessions, and this difference disappeared on the fourth day in the dark and with no change thereafter (Fig. 3).

Postoperation Behavioral Testing

Preoperative and postoperative comparisons for the three control groups

Two-way ANOVAs with repeated measures were conducted on anesthetic control, UVD sham, and BVD sham groups at preoperation, and at 3 and 6 months postop. time points, and there were no significant differences in the animals' performance in the dark in homing time (preop: $F_{(2,9)} = 0.574$, $P = 0.583$; 3 months: $F_{(2,9)} = 1.525$, $P = 0.269$; 6 months: $F_{(2,9)} = 0.392$, $P = 0.687$), homing distance (preop: $F_{(2,9)} = 0.532$, $P = 0.602$; 3 months: $FF_{(2,9)} = 0.180$, $P = 0.838$; 6 months: $FF_{(2,9)} = 0.360$, $P = 0.707$), heading angle (preop: $F_{(2,9)} = 0.0{,}327$, $P = 0.968$; 3 months: $F_{(2,9)} = 2.333$, $P = 0.153$; 6 months: $F_{(2,9)} = 2.285$, $P = 0.158$), or number of errors (preop: $F_{(2,9)} = 0.0{,}496$, $P = 0.952$; 3 months: $F_{(2,9)} = 2.508$, $P = 0.136$; 6 months: $F_{(2,9)} = 0.215$, $P = 0.810$). Therefore, these three groups were combined to form a new control group, and the performance of lesioned animals was compared against this control group.

Pretraining

At 3 and 6 months after the surgery, UVD animals were hesitant to leave their home cage at the beginning of pretraining; however, after several days of training, they were similar to the control group in finding the food and getting back to home. Nonetheless, UVD animals' homing paths were variable. In some cases, a direct route was achieved, while in other trials, the homeward journey consisted of pauses and turns or incorrect choices. However, when homing time on the last day of the pretraining phase at preop., 3 and 6 months postop. was analyzed, there was no significant difference between control and UVD groups (Fig. 5; $H = 1.25$, $P = 0.263$). BVD animals were reluctant to leave home at both time points. In fact, BVD animals did not leave home in the light condition at either time point although they frequently poked their heads out of the hole to check the table. However, in the dark condition, they did come out from the home, but they made many short trips back home instead of foraging. The behavior of BVD animals will be discussed later in this paper.

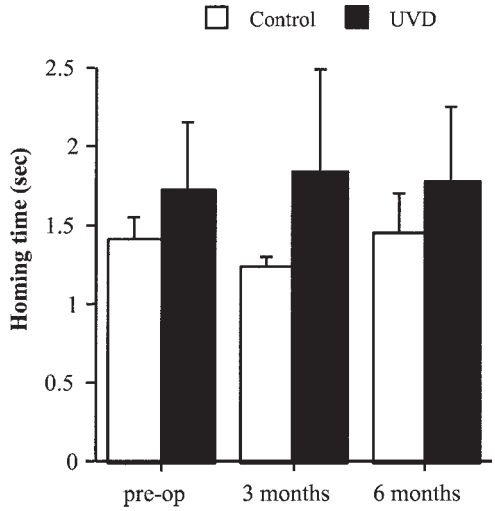

FIGURE 5. Homing time of control and UVD animals on the last day of pretraining in light at the preoperation stage and at the 3 and 6 months postoperation stages. Data reflect the mean of four trials and are expressed as mean ± SEM.

Homing accuracy using allocentric cues

On the probe trial in light, most of the control animals went back to their old home first and this was true for both the 3 and 6 months time points (Figs. 6A and 7A; Rayleigh test: $r = 1$, $P < 0.001$ for 3 months and $r = 0.573$, $P = 0.001$ for 6 months, respectively). UVD animals' first choices at 3 months were close to the old home location and the Rayleigh test of randomness was close to being significant ($r = 0.933$, $P = 0.061$; Fig. 6A). At 6 months, the first choices for UVD rats were away from the old home location, but clustered around the new home location; however, there was no significant preference in the direction as indicated by the Rayleigh test of randomness ($r = 0.8$, $P = 0.069$; Fig. 7A). Then, as they did in the preoperation testing, all of the animals made a number of choices before they reached the correct home. However, by recording the holes they visited before they found the correct one, it was clear that control animals tended to go to the holes that had not been visited previously, while UVD animals persistently went back to the old home location (Fig. 8). Two-way ANOVAs on the average number of returns to the old home location showed a significant group effect ($F_{(1,2)} = 7.103$, $P = 0.012$), a significant session effect ($F_{(2,32)} = 15.698$, $P < 0.001$), and a significant group x session interaction ($F_{(2,32)} = 6.440$, $P = 0.004$). Posthoc tests revealed that UVD animals at 3 months after the lesion produced a significantly higher number of returns to the old home compared with control animals at all time points and compared with UVD animals at either preoperation or the 6 months time point (Fig. 8).

Homing accuracy using egocentric cues

Consistent with the preoperation data, both control, and UVD animals' first choices for home were randomly distributed around the table on the first day of testing in the dark at 3 and

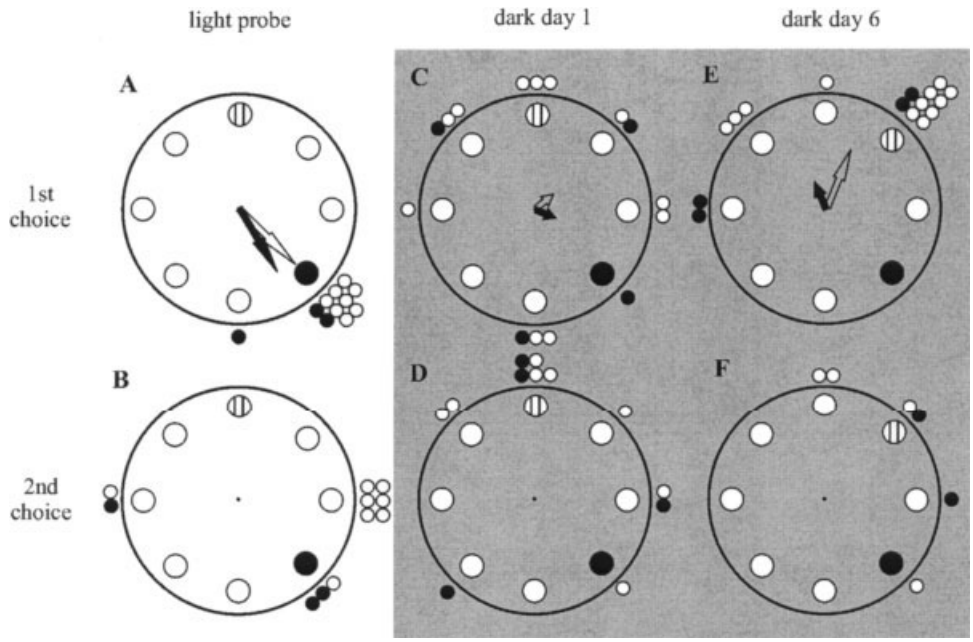

FIGURE 6. Home choices at the 3 months postoperation stage. The black circle indicates the old home location and the hashed circle indicates the new home location. The small circle represents each individual animal's home choice for control (open) and UVD (filled) animals. The top panel indicates the animals' first home choices on the light-probe trial (A), the first day of training in the dark (C), and the last day of training in the dark (E). The bottom panel indicates the animals' second home choices on the light-probe trial (B), the first day of training in the dark (D), and the last day of training in the dark (F). The direction of the arrow represents the average direction of the first home choices and the length of the arrow represents the accuracy of the first home choices for control (open) and UVD (filled) animals.

6 months (Figs. 6C and 7C, Rayleigh test: $r = 0.285$, $P = 0.419$ for 3 months and $r = 0.306$, $P = 0.365$ for 6 months). However, on the last day in the dark, control animals showed a preference towards the new correct home on their first choice (Figs. 6E and 7E, Rayleigh test: $r = 0.789$, $P < 0.001$ for 3 months and $r = 0.844$, $P < 0.001$ for 6 months), while UVD animals had no significant preference in their first choice (Figs. 6E and 7E, Rayleigh test: $r = 0.383$, $P = 0.588$ for 3 months, and $r = 0.663$, $P = 0.108$ for 6 months).

The animals' performance in the dark was further analyzed by comparing their homing time, homing distance, heading angle, and the number of errors they made before reaching the correct home. As shown in Figure 9, there was no difference between control and UVD animals on any of these measurements before the surgery (homing time: $F_{(1,15)} = 0.216$, $P = 0.648$; homing distance: $F_{(1,15)} = 1.981$, $P = 0.168$; heading angle: $F_{(1,15)} = 2.554$, $P = 0.129$, and number of errors: $F_{(1,15)} = 3.184$, $P = 0.095$). However, at 3 months after the surgery, there was a group effect on all four measures (homing time: $F_{(1,15)} = 13.952$, $P = 0.002$; homing distance: $F_{(1,15)} = 15.045$, $P < 0.001$; heading angle: $F_{(1,15)} = 16.924$, $P < 0.001$, and number of errors: $F_{(1,15)} = 15.073$, $P = 0.001$). Posthoc tests showed that UVD animals were impaired on the first and the last pair of sessions. This difference disappeared when the animals were tested again at 6 months (homing time: $F_{(1,15)} = 0.052$, $P = 0.823$; homing distance: $F_{(1,15)} = 0.148$, $P = 0.705$; heading angle: $F_{(1,15)} = 0.293$, $P = 0.595$, and number of errors: $F_{(1,15)} = 0.173$, $P = 0.683$). There was no difference in animals' searching speed, homing speed, or searching distance between the control and UVD groups (data not shown).

Behavior of BVD animals

All BVD animals displayed increased locomotor activity, walking in circles, and head weaving. These symptoms gradually decreased over time. However, at 6 months after the surgery, some of the symptoms were still prominent. BVD animals were reluctant to leave the home cage in light and when they did come out in dark, they made many short trips back home. To quantify this type of behavior, a foraging score was used. If a rat came out of its home cage and foraged for food on the table on a trial, it was given a foraging score of 1. If a rat did not come out or when it came out, it made short trips back home instead of foraging for food, it was given a foraging score of 0. Figure 10 shows the percentage of animals that had a foraging score of 1 during the 6 days of testing in the dark at 3 or 6 months following the lesion. χ^2 analysis indicated that the percentage of animals that scored 1 was significantly different among the groups at both 3 and 6 months ($\chi^2_{(2)} = 58.24$, $P < 0.0001$ and $\chi^2_{(2)} = 61.90$, $P < 0.0001$, respectively) and that there was a significantly lower percentage of BVD animals scoring 1 compared with the other groups.

DISCUSSION

The present study further examined the effects of vestibular deafferentation on spatial navigation by comparing unilateral and bilateral vestibular lesions and testing the animals at different time points following the lesion. The results demonstrated,

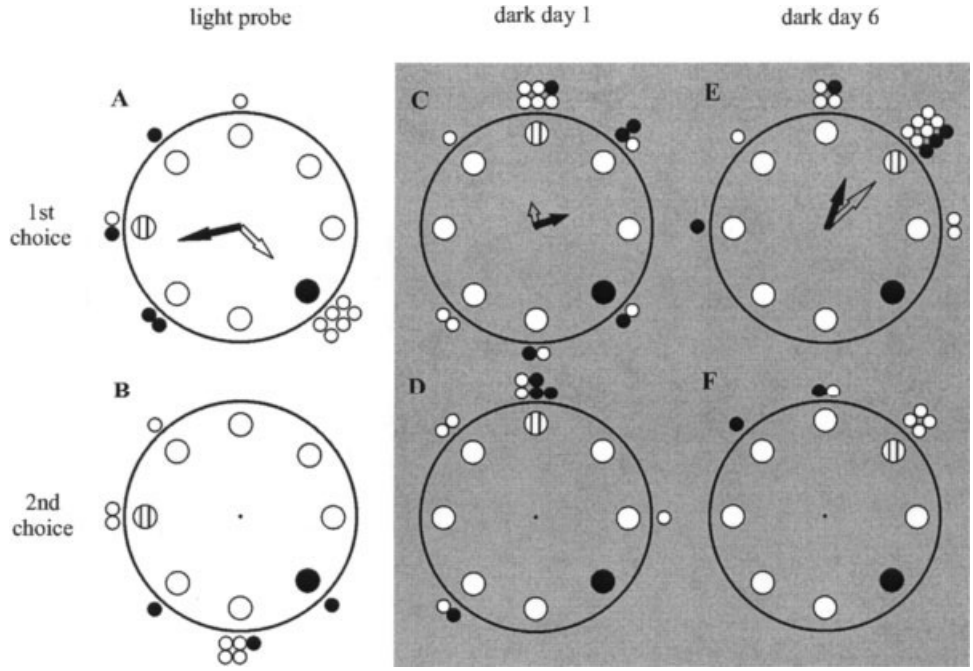

FIGURE 7. Home choices at the 6 months postoperation stage. The black circle indicates the old home location and the hashed circle indicates the new home location. The small circle represents each individual animal's home choice for control (open) and UVD (filled) animals. The top panel indicates the animals' first home choices on the light-probe trial (A), the first day of training in the dark (C), and the last day of training in the dark (E). The bottom panel indicates the animals' second home choices on the light-probe trial (B), the first day of training in the dark (D), and the last day of training in the dark (F). The direction of the arrow represents the average direction of the first home choices, and the length of the arrow represents the accuracy of the first home choices for control (open) and UVD (filled) animals.

for the first time, that: (1) at 3 months following the vestibular lesion, UVD animals were impaired on the foraging task when egocentric cues were required, and this impairment disappeared at 6 months after the lesion; (2) UVD and BVD had distinct effects on animals' behavior for as long as 6 months after the lesion, with BVD animals showing significant hesitation in leaving their home cage.

It has been demonstrated that the foraging task employed in the present study is a suitable task for testing animals' ability in piloting and dead reckoning by manipulating available cues (Whishaw and Maaswinkel, 1998; Maaswinkel et al., 1999; Whishaw et al., 2001a,b; Wallace et al., 2002). In the present study, the animals were pretrained and tested on the task before the operation. After 10 days of training from a familiar home location, all animals were able to carry the food directly back home and when they were released from a new home location on the probe trial in light, their first choice was the old home location. This was in agreement with previous studies (Whishaw and Tomie, 1997; Whishaw and Maaswinkel, 1998; Wallace et al., 2002) and indicated that the animals were using allocentric cues under light conditions. However, when the animals could not find the home cage in the old home location on the light-probe trial, only six of the animals went directly to the new home location and the other eight animals made a few more mistakes before reaching the correct home. It has been suggested by Whishaw and colleagues that normal animals can use both allocentric and egocentric cues to navigate in a visually rich environment; when allocentric cues cannot help them to solve the spatial problem, the animals can flexibly switch to using egocentric cues (Whishaw and Tomie, 1997; Whishaw

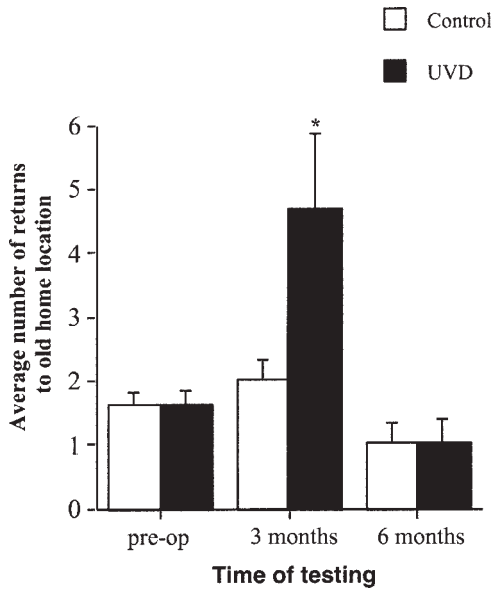

FIGURE 8. Average number of returns to old home location on the probe trial in the light condition for control and UVD animals at the preoperation stage and at the 3 and 6 months postoperation stages. Data are expressed as mean ± SEM, $^{*}P < 0.01$.

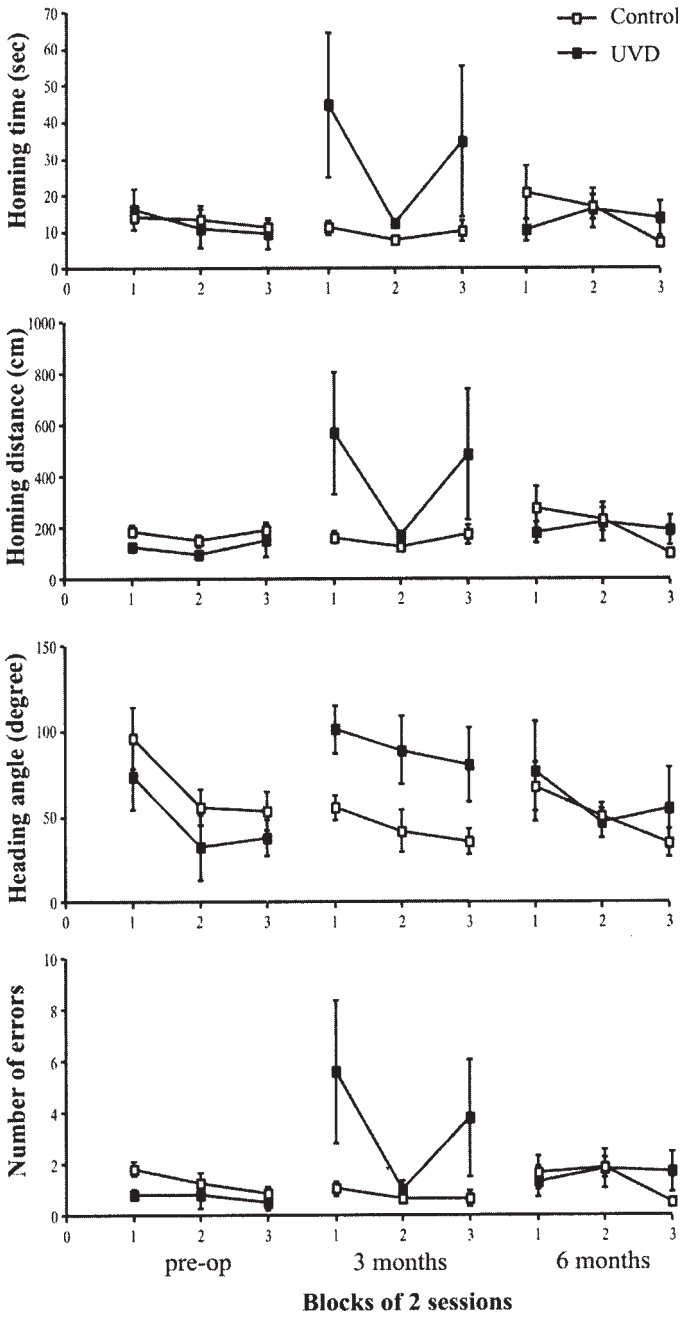

FIGURE 9. Homing time, homing distance, heading angle, and number of errors during the 6 days of training in the dark condition for control and UVD animals at the preoperation stage and at the 3 and 6 months postoperation stages. Data reflect a block of two sessions and are expressed as mean ± SEM.

and Maaswinkel, 1998;) because the animals in their experiments went directly to the new home location on their second choice. There is no doubt that the vestibular system, as well as the proprioceptive receptors, continues sending updated information on animals' movement (e.g., heading angle, velocity, distance) during spatial navigation under either light or dark conditions. The question is whether egocentric information is collected and analyzed by the animals when abundant allocentric cues are available. Our results clearly show that switching to use egocentric cues under light conditions may not be as flexible as suggested previously. Furthermore, we also found that the animals had no preference for the home where they had just emerged from on the first day of dark testing, but could go directly back home after 6 days of training in the dark. This suggested that even in the dark, when allocentric cues were largely eliminated, the animals still needed a number of trials to learn to use egocentric cues.

An important finding in the present study is that UVD animals were impaired on the foraging task. Studies of the vestibular contribution to spatial learning and memory have been largely restricted to bilateral lesions (Ossenkopp and Hargreaves, 1993; Peruch et al., 1999; Stackman and Herbert, 2002; Wallace et al., 2002; Russell et al., 2003a; Schautzer et al., 2003) because it seems an obvious approach to completely eliminate the vestibular input. However, UVD may result in changes that are qualitatively different from BVD because the oculomotor and postural symptoms following unilateral and bilateral lesions are different. It was also reported that UVD produced a decrease in nNOS protein and NMDA receptor expression in the hippocampus (Liu et al., 2003; Zheng et al., 2001), and reduced field potentials, bilaterally, in hippocampal slices (Zheng et al., 2003a). Therefore, it was speculated that UVD might also affect an animal's spatial performance. Indeed, the present results clearly show that UVD animals were impaired in homing under dark conditions. The impairment was evident at 3 months after the lesion as indicated by the increased homing time, homing distances, heading angle and, number of errors, as well as randomly distributed first home choices after 6 days of training in the dark. However, it was notable that except for the heading angle, which was increased consistently throughout the three blocks of ses-

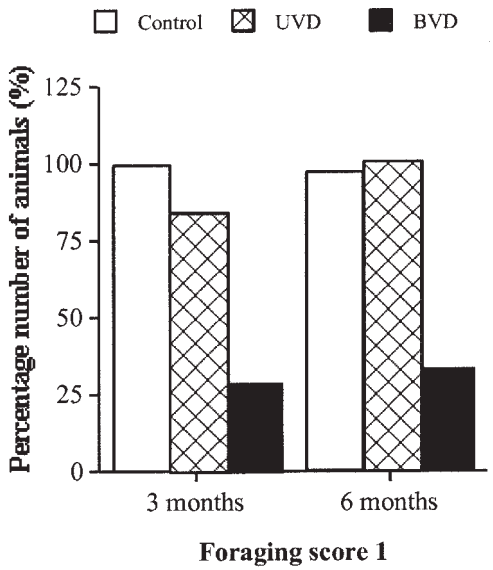

FIGURE 10. Foraging score for the animals. Data are expressed as percentage of animals scoring 1 on the foraging score on the 6 days of training in the dark condition at 3 and 6 months postoperation.

sions, the homing time, homing distance, and number of errors were only increased on the first and the last blocks. The lack of difference between UVD and control animals in the above measurements on the second block should not be taken as a lack of difference in dead reckoning, since the heading angle, a more accurate measurement of the animals' ability to use egocentric cues, was still significantly increased on the second block. Thus, the fact that the UVD animals headed out in a wrong direction, but were then able to reach the correct home with minimum error may explain the discrepancy between the different measurements. When tested again at 6 months, most of the differences between control and UVD animals had disappeared except the mean homing direction. It is noteworthy that there were only five animals in UVD group. Although this sample size was sufficient to detect significant differences on most of the measurements in the present study, a larger group of animals may reveal some more subtle changes, such as in the mean homing direction at the 6-months time point. It was interesting that UVD animals' first home choices were also away from the old home location on the light-probe trial. This does not suggest that UVD animals have not learned the location of the old home. In fact, the persistence in revisiting the old home location by UVD animals at 3 months on the light-probe trial indicated that UVD animals have learned the place and were able to use allocentric cues to guide them there. However, inadequate vestibular information might have prevented them from turning in the correct direction once they collected the food and then caused the subsequent inaccurate first home choice. It was noticed in the pretraining trials in light that UVD animals often paused, looked around, turned, or made incorrect home choices on their homeward trip, although there was no significant difference in their homing time compared with the control animals. Thus, without correct vestibular information, UVD animals tended to make mistakes when initiating the homeward trip even in the light condition but were able to correct themselves using, predominantly, visual cues. However, when the visual cues were eliminated in the dark, UVD animals were impaired in homing. Taken together, the behavior of UVD animals was consistent with the previous study in BVD animals (Wallace et al., 2002) and suggests that vestibular information plays an important role in dead reckoning. Moreover, the behavior of UVD rats in light and light-probe conditions further suggests that vestibular information may also contribute to accurate piloting.

The fact that UVD animals performed better in the dark at 6 months than at 3 months was interesting. Temporal bone histology has shown that these UVD animals had their vestibular labyrinth completely destroyed and therefore, there is no possibility that the behavioral recovery was due to the recovery of the sensory receptors. However, UVD animals still have one half of the vestibular system intact, and it is possible that the animals developed a compensatory strategy by correctly using vestibular information from one labyrinth. Following UVD, the static symptoms, including spontaneous ocular nystagmus and head tilt, undergo recovery through a process of "vestibular compensation" and this is partially due to the recovery of resting activity in neurons in the vestibular nucleus complex (see Smith and Curthoys, 1989; Curthoys and Halmagyi, 1995 for reviews). It is possible that a similar "compensation" process might occur in the hippocampus, affecting the way that the hippocampus processes vestibular information. Another explanation for the recovery could be that the repeated training over time may serve as a rehabilitation exercise, since it has been reported that vestibular rehabilitation improves performance in path integration tasks in patients with vestibulopathies (Cohen and Kimball, 2002). It could be argued that the UVD animals could potentially use the odour they left on their outward trip to guide them home, because the table was not cleaned or rotated between the outward and homeward trips. However, if the odour cues were used, the UVD animals would be expected to follow the outward path home and this was not the case. Moreover, the impairment at 3 months would not have occurred if the UVD animals used odour cues. Therefore, our results demonstrate, for the first time, a time-dependent recovery of dead reckoning following UVD. Although the mechanisms underlying the recovery are not clear, the present study suggests the possibility of using vestibular rehabilitation to help patients with vestibular disorders to recover accurate spatial navigation.

It has been suggested by numerous studies that the hippocampus is one of the key structures involved in spatial learning and memory (see O'Keefe and Nadel, 1978; Poucet, 1993; O'Mara, 1995; Wiener, 1996 for reviews). More recently, animals with fimbria-fornix or hippocampal lesions have been shown to be impaired in dead reckoning on the food foraging task (Whishaw and Tomie, 1997; Maaswinkel et al., 1999), which suggests that the hippocampus plays an important role in navigation using idiothetic cues. The vestibular system, on the other hand, provides a continuous update of angular and linear velocity information to the limbic system, including the hippocampus, for spatial navigation (Etienne, 1980; Mittelstaedt and Mittelstaedt, 1980; McNaughton et al., 1996). The most direct evidence for the vestibular system influencing hippocampal function was reported by Cuthbert et al. (2000) and Hicks et al. (2004). They found that electrical stimulation of one vestibular labyrinth could evoke field potentials and theta activity, bilaterally, in the hippocampus. Horii et al. (2004) showed that electrical stimulation of the medial vestibular nucleus could evoke single unit activity in complex spiking CA1 neurons. It has also been reported that electrical stimulation of the vestibular labyrinth can increase acetylcholine (ACh) release in the hippocampus (Horii et al., 1994, 1995). Moreover, lesion studies further confirmed the effects of the vestibular system on hippocampal function by showing that peripheral vestibular lesions disrupted hippocampal place cell firing (Stackman et al., 2002; Russell et al., 2003b) and caused neurochemical changes in the hippocampus (Zheng et al., 1999a,b, 2001). Our results, together with those of Wallace et al. (2002), support the notion that the vestibular system plays an important role in maintaining hippocampal function in spatial navigation, and especially in dead reckoning.

It has been reported by Wallace et al. (2002) that BVD animals displayed "agoraphobia"-like behavior with "extreme reluc-

tance to perform" on the foraging task. However, the BVD animals in their study did perform the task after additional training at about 2 weeks after the lesion. In contrast, the BVD animals in the present study did not leave their home cage in the light even at 6 months after the lesion. While in the dark, they made many short trips out of the home cage. Failure to perform the task by our BVD animals could not be explained by the animals' abnormal locomotor activity following the vestibular lesion, because there was no significant difference in the number of squares traversed between BVD animals and other groups when tested in the open field (Zheng et al., 2004). However, BVD animals did show less exploratory activity and impaired object recognition (Zheng et al., 2004), which may have contributed to their inability to perform the foraging task. The discrepancy in the BVD animals' performance between the present study and the study by Wallace et al. (2002) may be due to the differences in the completeness of the vestibular lesions as discussed earlier in this paper. Nevertheless, our results suggest that bilateral vestibular lesions may produce qualitatively different behavior from unilateral vestibular lesions. Since patients with bilateral vestibular damage have been suggested to have a high incidence of conditions such as agoraphobia, other anxiety disorders, and depression (Herdman et al., 1994; Jacob et al., 1996; Furman and Jacob, 2001), further studies are necessary to address this issue.

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REFERENCES

Cohen HS, Kimball KT. 2002. Improvements in path integration after vestibular rehabilitation. J Vestib Res 12:47–51.

Commins S, Gemmell C, Anderson M, Gigg J, O'Mara SM. 1999. Disorientation combined with bilateral parietal cortex lesions causes path integration deficits in the water maze. Behav Brain Res 104: 197–200.

Cooper BG, Manka TF, Mizumori SJ. 2001. Finding your way in the dark: the retrosplenial cortex contributes to spatial memory and navigation without visual cues. Behav Neurosci 115:1012–1028.

Curthoys IS, Halmagyi GM. 1995. Vestibular compensation: a review of the ocular motor, neural and clinical consequences of unilateral vestibular loss. J Vestib Res 5:67–107.

Cuthbert PC, Gilchrist DPD, Hicks SL, MacDougall HG, Curthoys IS. 2000. Electrophysiological evidence for vestibular activation of the guinea pig hippocampus. NeuroReport 11:1443–1447.

de Bruin JP, Swinkels WA, de Brabander JM. 1997. Response learning of rats in a Morris water maze: involvement of the medial prefrontal cortex. Behav Brain Res 85:47–55.

Etienne AS. 1980. The orientation of the golden hamster to its nest-site after the elimination of various sensory cues. Experientia 36: 1048–1050.

Furman JM, Jacob RG. 2001. A clinical taxonomy of dizziness and anxiety in the otoneurological setting. J Anxiety Disord 15:9–26.

Gallistel CR. 1990. The organization of learning. Cambridge, MA: MIT Press.

Herdman SJ, Borello-France DF, Whitney SL. 1994. Treatment of vestibular hypofunction. In: Herdman SJ, editor. Vestibular rehabilitation. Philadelphia: FA Davis. p 287–313.

Hicks SL, Gilchrist DPD, Cuthbert PC, Curthoys IS. 2004. Hippocampal field responses to direct electrical stimulation of the vestibular system in awake or anesthetised guinea pigs. Proc Aust Neurosci Soc 15:87.

Horii A, Takeda N, Mochizuki T, Okakura-Mochizuki K, Yamamoto Y, Yamatodani A. 1994. Effects of vestibular stimulation on acetylcholine release from rat hippocampus: an in vivo microdialysis study. J Neurophysiol 72:605–611.

Horii A, Takeda N, Mochizuki T, Okakura-Mochizuki K, Yamamoto Y, Yamatodani A, Kubo T. 1995. Vestibular modulation of the septo-hippocampal cholinergic system of rats. Acta Otolaryngol Suppl 520:395–398.

Horii A, Russell NA, Smith PF, Darlington CL, Bilkey DK. 2004. Vestibular influences on CA1 neurons in the rat hippocampus: an electrophysiological study in vivo. Exp Brain Res 155:245–250.

Jacob R, Furman J, Perel J. 1996. Panic, phobia and vestibular dysfunction. In: Yates BJ, Miller AD, editors. Vestibular autonomic regulation. New York: CRC Press. p 197–227.

Jensen DW. 1983. Survival of function in the deafferented vestibular nerve. Brain Res 273:175–178.

King J, Zheng Y, Liu P, Darlington CL, Smith PF. 2002. NMDA and AMPA receptor subunit protein expression in the rat vestibular nucleus following unilateral labyrinthectomy. NeuroReport 13:1541–1545.

Liu P, King J, Zheng Y, Darlington CL, Smith PF. 2003. Long-term changes in hippocampal NMDA receptor subunits following peripheral vestibular damage in rat. Neuroscience 117:965–970.

Maaswinkel H, Jarrard LE, Whishaw IQ. 1999. Hippocampectomized rats are impaired in homing by path integration. Hippocampus 9: 553–561.

Mardia KV, Jupp PE. 1999. Directional statistics. Chichester, UK: Wiley.

Matthews BL, Ryu JH, Bockaneck C. 1989. Vestibular contribution to spatial orientation. Evidence of vestibular navigation in an animal model. Acta Otolaryngol Suppl 468:149–154.

McNaughton BL, Barnes CA, Gerrard JL, Gothard K, Jung MW, Knierim JJ, Kudrimoti H, Qin Y, Skaggs WE, Suster M, Weaver KL. 1996. Deciphering the hippocampal polyglot: the hippocampus as a path integration system. J Exp Biol 199:173–185.

Mittelstaedt ML, Mittelstaedt H. 1980. Homing by path integration in a mammal. Naturwissenschaften 67:556–567.

Moghaddam M, Bures J. 1996. Contribution of egocentric spatial memory to place navigation of rats in the Morris water maze. Behav Brain Res 78:121–129.

Nieto-Escámez FA, Sánchez-Santed F, de Bruin JPC. 2002. Cholinergic receptor blockade in prefrontal cortex and lesions of the nucleus basalis: implications for allocentric and egocentric spatial memory in rats. Behav Brain Res 134:93–112.

O'Keefe J, Nadel L. 1978. The Hippocampus as a cognitive map. Oxford, UK: Clarendon.

O'Mara SM. 1995. Spatially selective firing properties of hippocampal formation neurons in rodents and primates. Prog Neurobiol 45:253–274.

Ossenkopp K-P, Hargreaves EL. 1993. Spatial learning in an enclosed eight-arm radial maze in rats with sodium arsanilate-induced labyrinthectomies. Behav Neural Biol 59:253–257.

Peruch P, Borel L, Gaunet F, Thinus-Blanc G, Magnan J, Lacour M. 1999. Spatial performance of unilateral vestibular defective patients in nonvisual versus visual navigation. J Vestib Res 9:37–47.

Poucet B. 1993. Spatial cognitive maps in animals: new hypotheses on their structure and neural mechanisms. Psychol Rev 100:163–182.

Russell NA, Horii A, Smith PF, Darlington CL, Bilkey DK. 2003a. Bilateral peripheral vestibular lesions produce long-term changes in spatial learning in the rat. J Vestib Res 13:9–16.

Russell NA, Horii A, Smith PF, Darlington CL, Bilkey DK. 2003b. Long-term effects of permanent vestibular lesions on hippocampal spatial firing. J Neurosci 23:6490–6498.

Saxon DW, Anderson JH, Beitz AJ. 2001. Transtympanic tetrodotoxin alters the VOR and FOS labelling in the vestibular complex. NeuroReport 12:3051–3055.

Schautzer F, Hamilton D, Kalla R, Strupp M, Brandt T. 2003. Spatial memory deficits in patients with chronic bilateral vestibular failure. Ann N Y Acad Sci 1004:316–324.

Semenov LV, Bures J. 1989. Vestibular stimulation disrupts acquisition of place navigation in the Morris water tank task. Behav Neural Biol 51:346–363.

Smith PF, Curthoys IS. 1989. Mechanisms of recovery from unilateral labyrinthectomy: a review. Brain Res Rev 14:155–180.

Stackman RW, Clark AS, Taube JS. 2002. Hippocampal spatial representations require vestibular input. Hippocampus 12:291–303.

Stackman RW, Herbert AM. 2002. Rats with lesions of the vestibular system require a visual landmark for spatial navigation. Behav Brain Res 128:27–40.

Wallace DG, Hines DJ, Pellis SM, Whishaw IQ. 2002. Vestibular information is required for dead reckoning in the rat. J Neurosci 22: 10009–10017.

Whishaw IQ, Maaswinkel H. 1998. Rats with fimbria-fornix lesions are impaired in path integration: a role for the hippocampus in "sense of direction". J Neurosci 18:3050–3058.

Whishaw IQ, Tomie J. 1997. Piloting and dead reckoning dissociated by fimbria-fornix lesions in a rat food carrying task. Behav Brain Res 89:87–97.

Whishaw IQ, Hines DJ, Wallace DG. 2001a. Dead reckoning (path integration) requires the hippocampal formation: evidence from spontaneous exploration and spatial learning tasks in light (allothetic) and dark (idiothetic) tests. Behav Brain Res 127:49–69.

Whishaw IQ, Maaswinkel H, Gonzalez CL, Kolb B. 2001b. Deficits in allothetic and idiothetic spatial behavior in rats with posterior cingulate cortex lesions. Behav Brain Res 118:67–76.

Wiener SI. 1996. Spatial, behavioral and sensory correlates of hippocampal CA1 complex spike cell activity: implications for information processing functions. Prog Neurobiol 49:335–361.

Zheng Y, Smith PF, Darlington CL. 1999a. Noradrenaline and serotonin levels in the guinea pig hippocampus following unilateral vestibular deafferentation. Brain Res 836:199–202.

Zheng Y, Smith PF, Darlington CL. 1999b. Subregional analysis of amino acid levels in the guinea pig hippocampus following unilateral vestibular deafferentation. J Vestib Res 9:335–345.

Zheng Y, Horii A, Appleton I, Darlington CL, Smith PF. 2001. Damage to the vestibular inner ear causes long-term changes in neuronal nitric oxide synthase expression in the rat hippocampus. Neuroscience 105:1–5.

Zheng Y, Kerr DS, Darlington CL, Smith PF. 2003a. Unilateral inner ear damage results in lasting changes in hippocampal CA1 field potentials in vitro. Hippocampus 13:873–878.

Zheng Y, Pearce JM, Vann SD, Good M, Jenkins TA, Smith PF, Aggleton JP. 2003b. Using idiothetic cues to swim a path with a fixed trajectory and distance: necessary involvement of the hippocampus, but not the retrosplenial cortex. Behav Neurosci 117:1363–1377.

Zheng Y, Darlington CL, Smith PF. 2004. Bilateral labyrinthectomy causes long-term deficit in object recognition in rat. NeuroReport 15:1913–1916.