ELSEVIER

Journal of Neuroscience Methods 113 (2002) 131–145

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Quantification of a single exploratory trip reveals hippocampal formation mediated dead reckoning

Douglas G. Wallace, Dustin J. Hines, Ian Q. Whishaw *

Department of Psychology and Neuroscience, University of Lethbridge, 4401 University Drive, Lethbridge, Alberta, Canada T1K 3M4

Received 6 July 2001; received in revised form 19 September 2001; accepted 19 September 2001

Abstract

A rat's proclivity to explore a novel environment presents a behaviorally rich paradigm to investigate the role of the hippocampus in spatial navigation. Here we describe a novel technique of behavioral analysis that is derived from a single exploratory trip. An exploratory trip was defined as a rat's departure from the home base that ended when the rat returned to the home base. The behavior observed on a single exploratory trip by a control animal is highly organized into outward and homeward segments. An outward segment is characterized by a slow circuitous progression from the home base marked by several stops. A homeward segment is characterized by a rapid direct return to the home base. The velocity attribute of the exploratory trip was quantified by estimating the point of inflection associated with the trip's cumulative moment-to-moment velocity distribution. The heading direction and variance of the homeward trip segment was analyzed with circular statistics. A comparison of the exploratory behavior of control animals and animals with damage to the fimbria-fornix indicated that the velocity and heading direction of the homeward portion of the trip depends upon the hippocampal formation. While control and fimbria-fornix rats had similar outward segments, the return paths of the fimbria-fornix rats were significantly slower, more circuitous, and more variable compared with that of the control rats. This result was also independent of testing in light or dark conditions. The lack of dependence on allothetic cues suggests that rats employ dead reckoning navigational strategies to initiate the homeward portion of exploratory movements. Methods to quantify exploratory behavior in terms of velocity and angular components provide an assessment of control behavior and the assessment of the behavior of rats with hippocampal formation damage that is easy to implement.

Keywords: Dead reckoning; Hippocampus; Hippocampal formation; Path integration; Spatial navigation

1. Introduction

Many current behavioral tests of hippocampal formation function require that the animal be trained for several days or weeks. After training, the animals may be subjected to a variety of probe trials to eliminate performance deficits as the basis for impaired spatial navigation. Generalizations of this protocol have been administered in the water maze (Morris et al., 1982; Sutherland et al., 1982), on the foraging table (Whishaw and Tomie, 1997), and in many different kinds of mazes (see O'Keefe and Nadel, 1978). While damage to the hippocampal formation or selective hippocampal damage has been demonstrated to disrupt performance in these paradigms, a consensus has yet to emerge in the literature as to the nature of the deficit. Some researchers have interpreted the impaired performance of hippocampal rats to a deficit in the construction of a cognitive map (see also O'Keefe and Nadel, 1978; Morris et al., 1982), whereas others emphasize the relevance of performance deficits (Saucier et al., 1996; Hoh and Cain, 1997). Other work emphasizes a role for the hippocampal formation in dead reckoning (path integration) (Worden, 1992; Whishaw et al., 1995; Samsonovich and McNaughton, 1997; Whishaw et al., 1997; Golob and Taube, 1999; Frank et al., 2000; Leutgeb et al., 2000) or working memory (Walker and Olton, 1984; Caramanos and Shapiro, 1994).

More recently, researchers have started to use behavioral tasks that require relatively limited training or

* Corresponding author. Tel.: +1-403-329-2402; fax: +1-403-329-2555.

E-mail address: whishaw@uleth.ca (I.Q. Whishaw).

0165-0270/02/$ - see front matter
PII: S0165-0270(01)00489-7

capitalize on a rat's naturally occurring behaviors to assess behavior (Harley et al., 2001; Mumby et al., 1999; Drai et al., 2000). The current work was prompted by the theoretical prediction that the natural exploratory behavior of rats would be disrupted by hippocampal formation damage (O'Keefe and Nadel, 1978). Rats readily engage in the exploration of a novel environment, and this behavior is an essential first stage in spatial learning. Rats exhibit distinctive patterns of behavior while exploring a novel environment. They rear and turn to mark a 'virtual' home base, progress from the home base in gradually increasing exploratory trips, gradually increase the trip distance over time, and return to the home base with a greater velocity than occurs on the outward segment (Drai et al., 2000; Eilam and Golani, 1989; Tchernichovski and Golani, 1995; Whishaw et al., 1983, 1989). Recently, we have found that not only is travel speed on the homeward portion of an exploratory trip more rapid than on the outward portion, it is initiated with a sudden acceleration, and it is direct. Furthermore, this direct 'homing' behavior occurs in both light and dark and to both real and virtual home bases (Whishaw et al., 2001).

The current study presents new methodological and analytic techniques to quantify the spatial movements employed by rats while exploring an environment and in particular to quantify the distinct homing portion of an exploratory trip. We observe exploratory behavior in control rats and rats with fimbria-fornix lesions under light and dark testing conditions. Rats are provided with a small rectangular home base (that is visible under the light condition) from which they explore a novel large circular table. The rat's behavior during an exploratory session is monitored by a video camera, equipped with infrared sensors for recording under complete dark conditions. After testing, the animal's videotapes are analyzed with the Peak Performance computer system to generate moment-to-moment velocity profiles and raw x–y-coordinates for each exploratory trip. The outward and homeward portions of the trips are then traced onto transparencies for the calculation of circular statistics (Batschelet, 1981). The velocity profiles and circular statistics from control and fimbria-fornix rats provide insight into the navigational strategies employed by each group. These results provide a rapid and incisive method for analyzing hippocampal formation damage to exploratory behavior and may prove useful for the analysis of the contributions of other brain regions to exploratory behavior.

2. Materials and methods

2.1. Animals

The subjects were 12 female Long–Evans rats (University of Lethbridge vivarium) weighing approximately 250–300 g. Rats were housed in groups of four in wire mesh cages in the colony room with the temperature maintained at 20–21 °C and a 12/12 h light/dark cycle. Six rats received fimbria-fornix lesions 2 weeks prior to the start of testing, and six rats served as controls.

2.2. Surgery

Rats were anesthetized with a mixture of isofluorane and oxygen during the surgery. Fimbria-fornix lesions involved passing cathodal current through 00 stainless steel insect pins, insulated with epoxylite except at the surface of their tips, for 40 s. There were two lesion sites per hemisphere, using coordinates with respect to bregma and the surface of the dura: -1.3 mm posterior, ± 1.5 mm lateral, and -3.6 mm ventral; -1.5 mm posterior, ± 0.5 mm lateral, and 3.3 mm ventral. Rats were given 2 weeks to recover prior to testing.

2.3. Feeding

Feeding during habituation training was restricted to maintain the rats at 90% of their ad lib body weight. During a habituation trial, 750 mg food pellets (Bio-Serv, PO Box 450, Frenchtown, NJ) were used as an appetitive stimulus. Subsequent to daily habituation training, rats were fed with LabDiet Laboratory Rodent Pellets in their home cage to maintain their weight restrictions. After rats were successfully retrieving food pellets from random locations on the table they were taken off food deprivation and permitted ad lib food access for the remainder of the experiment.

2.4. Apparatus

Foraging table: the apparatus was a fiberglass circular table without walls measuring 155 cm in diameter (see Fig. 1 panel A). The table was painted green and mounted on ball bearings that permitted the table to be rotated. The table was located in a large room that could either be illuminated or made completely dark. A small dark box (12 cm long, 8 cm wide and 10 cm high) with a circular hole in one of the sides was placed on the edge of the table and used as a refuge by the rat. The surface of the table was approximately 64 cm above the floor.

2.5. Infrared testing

Because some of the testing involved analyzing behavior under completely dark conditions, we used a Sony infrared camera positioned perpendicular to the table. The testing room was light-proofed prior to testing, such that, when the lights were turned off during dark testing conditions the room was completely dark. The experi-

menter had to use an infrared spotter to test the animal under complete dark conditions. Infrared is a wavelength that the rat is not able to detect (Neitz and Jacobs, 1986).

2.6. Digitizing exploratory behavior

An HI-8 Sony video camera with infrared taping abilities was used to record the rat's movements while exploring the table. Exploratory trips to be analyzed were converted from analogue recording to a digital computer file using the Peak Performance system with a sampling rate of 60 Hz. The exploratory trip traveled by the rat is acquired from the digitized file by sampling the x- and y-coordinates of the rat as it moves along its path (see Fig. 1 panel A). Acquiring the path involves manually tracking a single point on the animal (the Peak Performance system can automatically track the rat if a marker is used) by selecting one pixel per frame every ten frames of the digitized video. The x- and y-coordinate velocity is computed from the sampled raw distance data. The resultant velocity (m/s) and cumulative distance (m) are computed from this data. The data reflect moment-to-moment velocities and distance traveled during one exploratory trip.

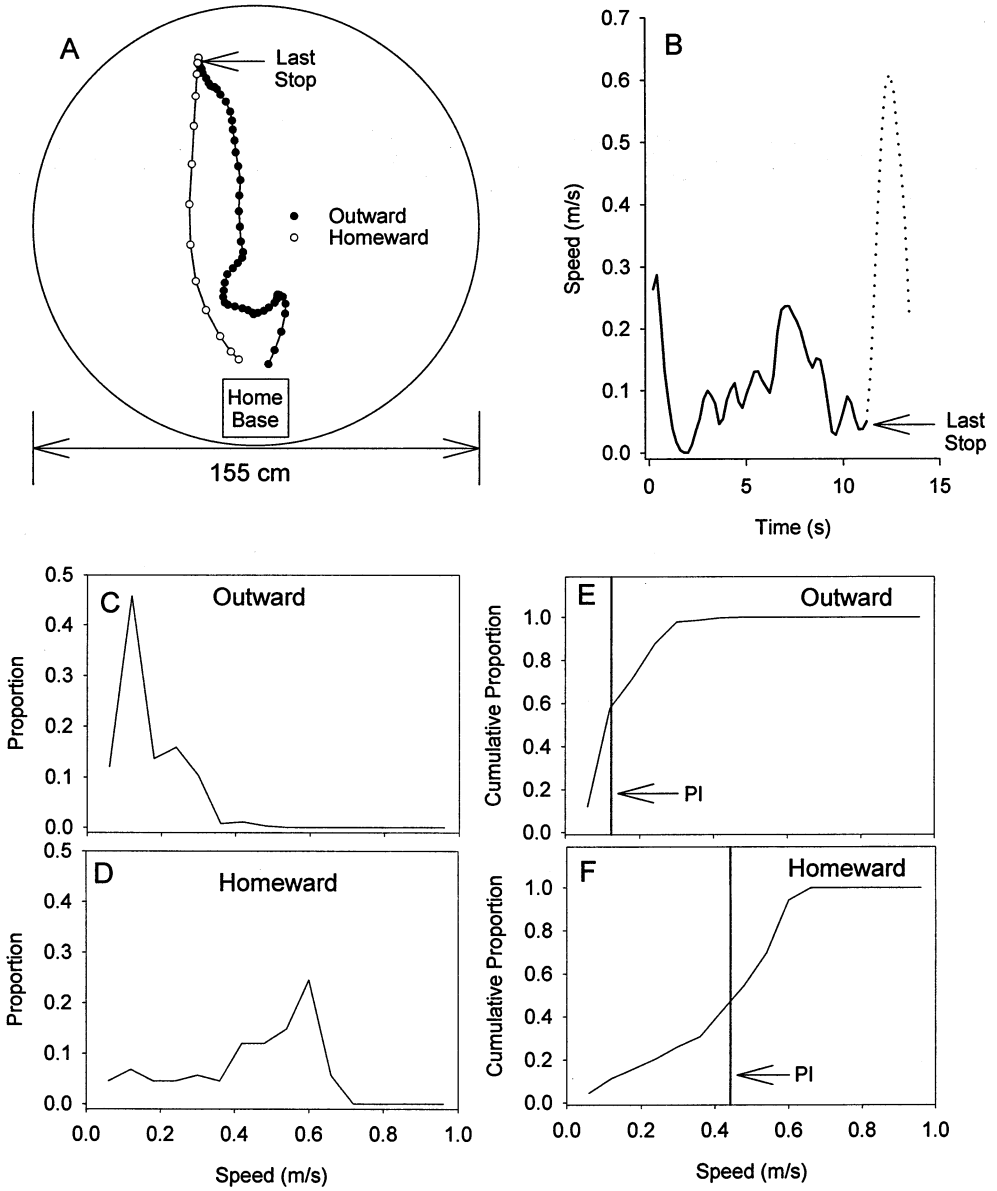

Fig. 1. Diagram of the steps involved in calculating point-of-inflection from the x–y-data and velocity data. The last stop prior to returning to the home base is indicated by the arrow in both A and B panels. Outward and homeward velocity distributions from the moment-to-moment velocity profile in panel B are in panels C and D, respectively. The cumulative velocity distribution for the outward and homeward trip segments, with their corresponding point-of-inflection indicated by the vertical line, are found in panels E and F, respectively.

2.7. Histology

Subsequent to testing, animals were deeply anesthetized and perfused with saline and saline-formalin. Each brain was removed from the skull and stored in 30% sucrose-formalin solution. The brains were frozen and cut at 40 μm slices on a cryostat. Alternate sections were taken and stained with cresyl violet and for acetylcholinesterase.

2.8. Procedure

2.8.1. Habituation

Habituation involved training the rats to search for randomly located food pellets on a different table and return to a visible home cage. Well-handled rats were used in the expectation that they would be motivated to explore. In other work, however, we have found that naïve rats display patterns of exploration similar to that described here, but considerable patience is required because they are slow to leave the confines of the home base.

2.8.2. Exploration

An exploratory session was a 10-min period of taping the animal's movements on the table. No food pellets were present during the exploratory session. Exploratory trips were defined as departure from the home base, locomotor activity on the table that displaced the animal at least halfway across the table, and ended when the rat returned to the home base. Golani and coworkers distinguish between different modes of exploration as episodes of 'lingering' or 'progression' (Drai et al., 2000). Lingering refers to locomotor behavior that is restricted to a small area, whereas movements between areas are classified as progressions. Our criterion for an exploratory trip eliminates exploratory behavior that would be classified as 'lingering' close to the home base. Therefore, exploratory trips would be characterized as sets of progressions punctuated with episodes of lingering. Exploratory sessions were conducted under both light and dark conditions with the home base located on top of the table.

2.9. Analysis

2.9.1. Paths

Exploratory trips were broken down into outward and homeward trip segments. The homeward segment of the trip was defined as the portion of the path linking the last stop made prior to returning home and the home base. Stops were defined by a complete session of movement in which all feet were stationary for 2 s. Stops are also characterized by a near zero velocity on the moment-to-moment velocity profiles. Exploratory trips were copied onto common transparencies based on group membership and testing condition.

2.9.2. Kinematic profiles

One exploratory trip from each rat in every testing condition was randomly selected for kinematic analysis. First, resultant moment-to-moment velocities were plotted by time (Fig. 1 panel B). Second, the moment-to-moment velocities were used to calculate the rat's average time, distance, and velocity for the outward and homeward trip segments. Third, a velocity distribution was generated from each rat's set of moment-to-moment velocities for the outward (Fig. 1 panel C) and homeward (Fig. 1 panel D) segments of the exploratory trip. The frequency for moment-to-moment velocities was calculated by counting the number of velocities that fell within a specific range or bin. The frequency for each bin was then divided by the total number of moment-to-moment velocities yielding the proportion of each trip segment that was spent traveling at velocities that fell within that bin. Each bin was 0.060 (m/s) in size, with the first bin ranging from 0.001 to 0.060 (m/s), the second bin ranging from 0.061 to 0.120, the third bin ranging from 0.121 to 0.180 (m/s), and so on, until the maximum velocity of 0.96 (m/s). Fourth, each rat's outward and homeward velocity distributions were transformed into a cumulative velocity distribution (see Fig. 1 panels E and F). Fifth, points-of-inflection were estimated for each rat's outward and homeward trip segments by fitting a three-parameter sigmoidal function to the corresponding cumulative velocity distribution. The resulting point-of-inflection is a measure of central tendency of the velocity distribution.

2.9.3. Heading direction

Exploratory trips generated by each rat were copied onto a transparency via an HI-8 video player and TV monitor. The homeward segment of the trip was analyzed with circular statistics to determine the basis of group differences in heading direction error (Batschelet, 1981). Heading direction was calculated by measuring the angle between a line that was a direct path to the home base and a line that interpolated the first third of the homeward trip segment. The home base was set at zero with angles increasing in steps of 5° counter clockwise around the perimeter of the table. A group's heading direction is a function of two parameters: (1) mean angle and (2) angular variance. The mean angle reflects the central tendency of a group's heading directions. Mean angles can range from 0 to 360°. Angular variance reflects the spread of a group's heading directions. Angular variance ranges from 0 (in which heading directions are randomly scattered around the

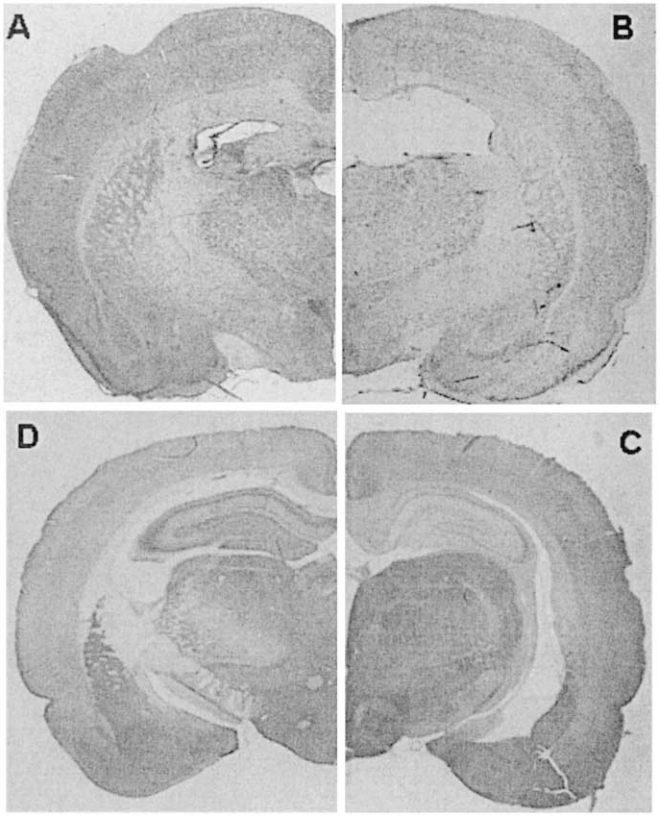

Fig. 2. Photomicrographs are from a control rat and a rat with damage to the fimbria-fornix. Top panels, cresyl violet sections demonstrating the intact fimbria-fornix in a control rat (panel A) and the absence of the fimbria-fornix in a rat with damage to the fimbria-fornix (panel B). Bottom panels, acetylcholinesterase sections demonstrating the high staining density for acetylcholinesterase in the CA1 and dentate gyrus areas of the hippocampus from the control rat and low staining density for acetylcholinesterase in a comparable section from a rat with damage to the fimbria-fornix.

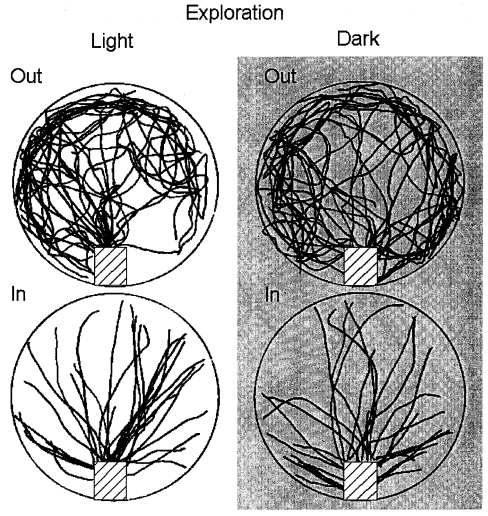

Fig. 3. The pattern of outward (out) and homeward (in) trip segments in the light (left) and in the dark (right). Each panel shows five trips by each of the six control rats. Note that the outward trips were circuitous, whereas homeward trip segments were relatively direct.

perimeter of the table) to 1 (all heading directions are in the same direction). Mean angle and angular variance will be evaluated for group differences under both testing conditions.

2.9.4. Homeward/outward angular deviation ratio

The homeward/outward (H/O) angular deviation ratio is a measure that reflects the circuitousness of an exploratory trip's outward and homeward segments while equating for total trip length. Calculating and summing angles formed by successive sets of three x–y data points along the trip segment result in the angular deviation for a segment of the trip. The homeward angular deviation is then divided by the outward angular deviation resulting in the H/O ratio for a given exploratory trip. H/O ratios that are less than one indicate that the outward trip segment is more circuitous than the homeward trip segment. If both trip segments are equally circuitous then the H/O ratio should be approximately equal to one. When the homeward trip segment is more circuitous than the outward trip segment, the H/O ratio should be greater than one.

3. Results

3.1. Histological results

Fig. 2 presents a coronal hemisection from a control (left) and a fimbria-fornix (right) rat, stained with cresyl violet (top) and AchE (bottom). The dorsal fornix and the fimbria were completely sectioned in all of the rats that were given lesions. The lesions were selective and did not result in damage to the septum, the septal portions of the hippocampus, or the hippocampal commissure. The track produced by the lowering of the electrode to the lesion site did not damage the supracallosal septohippocampal pathways or cortex other than the path made by the penetration. Other work has shown that supracallosal damage has little effect on spatial tasks (Sutherland and Rodriguez, 1989; Jeltsch et al., 1994). Stains for depleted AChE revealed extensive depletion of AChE in the hippocampus. Previous work has demonstrated that electrolytic lesions of the fimbria-fornix result in a 70% loss of cholinergic markers in the dorsal hippocampus (Cassel et al., 1991; Jeltsch et al., 1994).

3.2. Paths

Five exploratory trips observed by each rat under both testing conditions are found in Fig. 3 (control) and Fig. 4 (fimbria-fornix), respectively. We classified homeward trip segments as 'perimeter' if they were restricted to a 10 cm band located around the perimeter of the table. Other homeward trip segments were

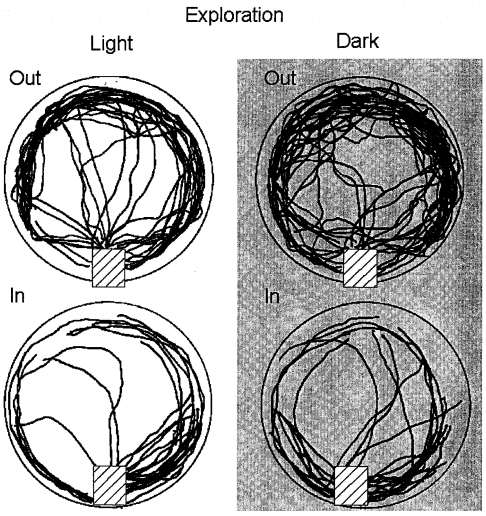

Fig. 4. The pattern of outward (out) and homeward (in) trip segments in the light (left) and the dark (right). Each panel shows five trips by each of the six fimbria-fornix rats. Both outward and homeward trip segments were circuitous under light and dark conditions. It should be noted that only under light conditions did many rats make shortcuts as they approached the home base rather than following the perimeter to the home base.

classified as 'central' if they crossed into the area bound by a 10 cm band. Comparing the number of control and fimbria-fornix homeward trip segments that crossed the central portion of the open field under light conditions revealed a significant group difference (control = 4.6 vs. fimbria-fornix = 0.9, Mann–Whitney $U = 0$, $P < 0.05$). A tendency to travel along the perimeter of the open field was similarly pronounced in the dark for the fimbria-fornix group. Again, very few homeward trips were made across the center of the open field (control = 3.9 vs. fimbria-fornix = 0.5, $U = 0$, $P < 0.05$). It should be noted that as the fimbria-fornix rats approached the home base under light conditions they would often shortcut towards it rather than following the perimeter to the home base, as they did under dark conditions. These group differences in the homeward trip segment will be considered in more detail in Section 3.4.

3.3. Kinematic profiles

The organization of outward and homeward trip segments for individual rat's exploratory trips was consistent within an exploratory session; therefore, we randomly selected one exploratory trip from each rat under both light and dark conditions to analyze with the Peak Performance system. Single exploratory trip velocity profiles for control and fimbria-fornix are presented in Fig. 5 (control) and Fig. 6 (fimbria-fornix), respectively. The top panel in both figures corresponds to testing under light conditions, whereas the bottom (shaded) panel corresponds to testing under dark conditions. Each graph plots a rat's moment-to-moment velocities by time for a representative foraging trip. Trips are broken into outward (solid line) and homeward (dotted line) trip segments. One should recall that the homeward trip segment was designated as the remaining portion of the exploratory trip after the last stop prior to returning to the home base.

Combined single trip velocity profiles and exploratory paths are presented in Fig. 7 for representative control and fimbria-fornix rats under light and dark conditions. Control rats' homeward trip segments are direct paths toward the home base, marked by an increase in velocity, under both light and dark conditions (upper panels of Fig. 7). Under light conditions the fimbria-fornix rat takes a circuitous path towards the home base and accelerates as it approaches the home base (bottom left panels of Fig. 7). The fimbria-fornix rat takes a circuitous route home under dark conditions that is not marked by an increase in velocity as it approaches the home base (bottom right panels of Fig. 7).

Fig. 8 summarizes control and fimbria-fornix mean time (top panel), distance (middle panel), and velocity (bottom panel) for outward and homeward trip segments of the moment-to-moment velocities presented in Figs. 5 and 6. An analysis of variance (ANOVA) was conducted on the rat's outward and homeward trip segment times under light and dark conditions and revealed a significant effect of trip-segment [$F(1, 10) = 29.838$, $P < 0.05$]. All other main effects and interactions were not significant. The ANOVA conducted on outward and homeward trip segment distances under light and dark conditions revealed a significant main effect of group [$F(1, 10) = 7.796$, $P < 0.05$] and trip-segment [$F(1, 10) = 5.411$, $P < 0.05$]. All other main effects and interactions were not significant. The ANOVA conducted on outward and homeward trip segment velocities under light and dark conditions revealed a significant main effect of testing condition [$F(1, 10) = 5.861$, $P < 0.05$], trip-segment [$F(1, 10) = 48.412$, $P < 0.05$], and a significant trip-segment by group interaction [$F(1, 10) = 15.068$, $P < 0.05$]. All other interactions and main effects were not significant. These results justified further examination of the moment-to-moment velocities generated by each group during outward and homeward trip segments.

One way to characterize group differences in moment-to-moment trip velocities would be to analyze the velocity distribution generated on each segment of an exploratory trip. Control and fimbria-fornix outward trip segment velocity distributions are found in the left panel of Fig. 9. Likewise, control and fimbria-fornix homeward trip segment moment-to-moment velocity distributions are found in the right panel of Fig. 9. The upper panels of Fig. 9 correspond to testing under light conditions, whereas the lower panels correspond to

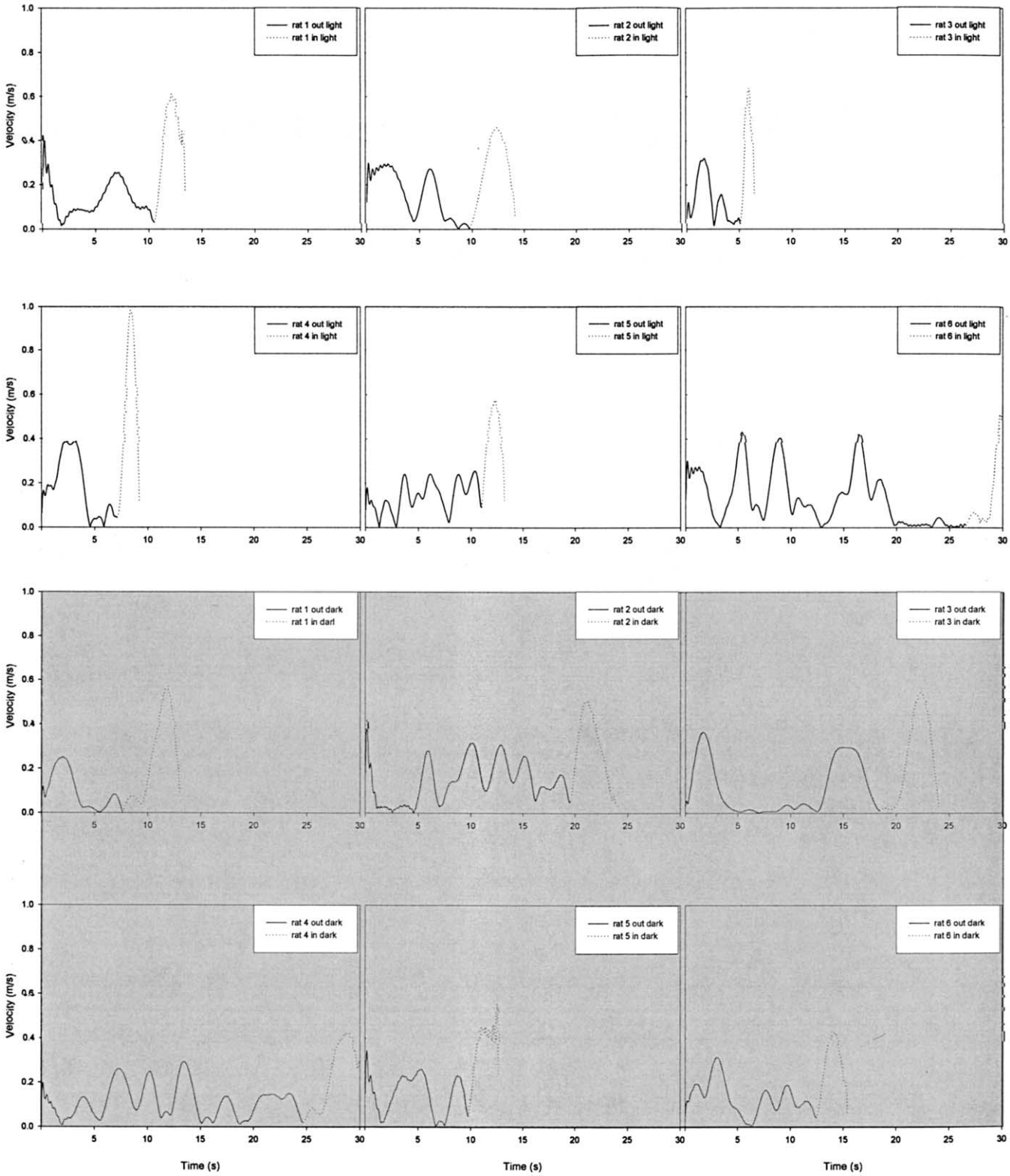

Fig. 5. Elements of movement displayed by control rats exploring a novel circular table in the light (white panels) and dark (gray panels). Each panel presents the moment-to-moment velocities associated with a single exploratory trip. The solid and dotted lines represent the velocities associated with the outward and homeward trip segments of the exploratory trip, respectively. The outward trip segments are characterized as relatively slow variations in movement speed during exploration, whereas the homeward trip segments are marked by a peak in velocity in the last few seconds as the rat returned to the home base. (Kinematic measures made with a Peak Performance system, with digitizing made at the center of the rat's shoulders.)

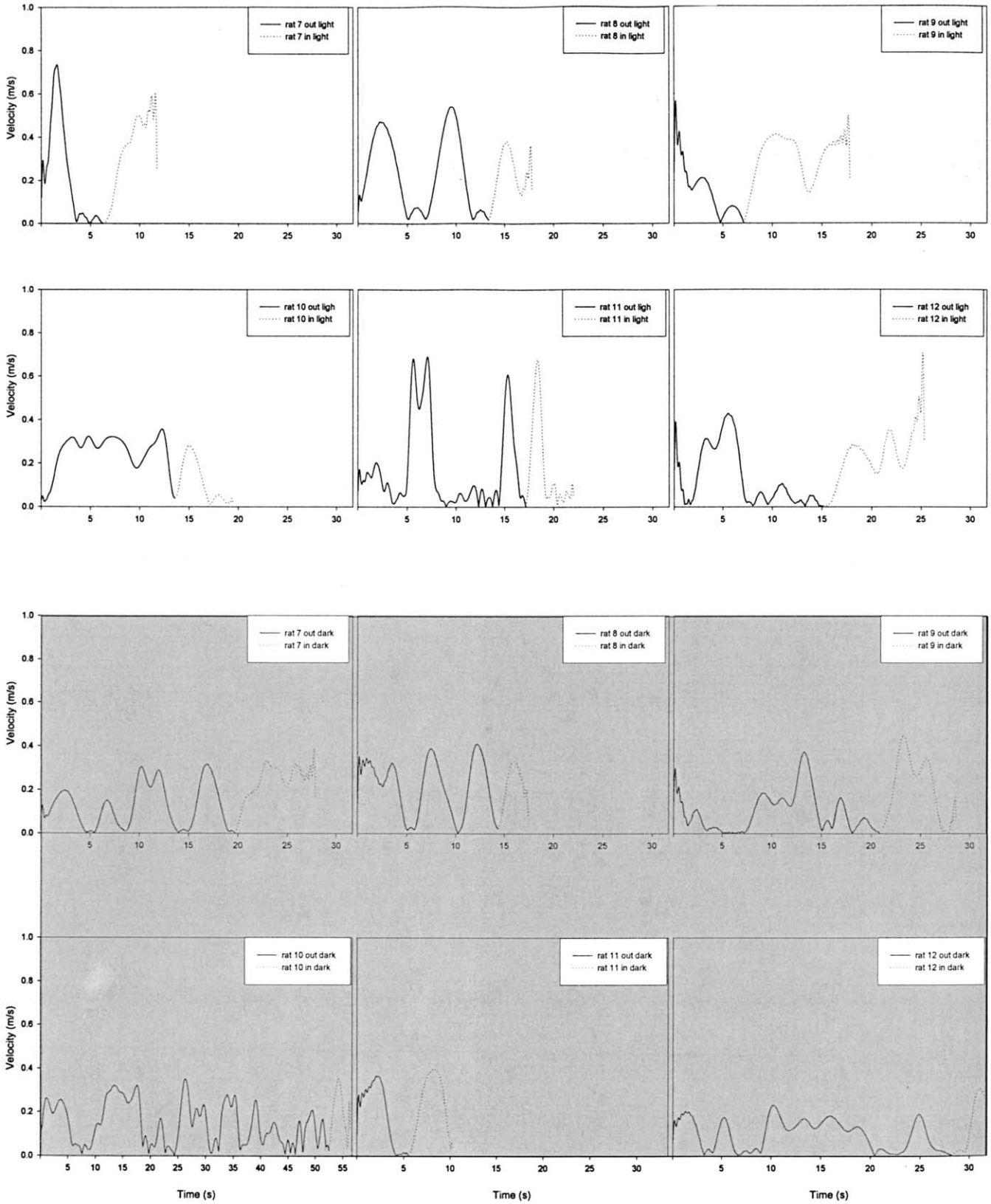

Fig. 6. Elements of movement displayed by fimbria-fornix rats exploring a novel circular table in the light (white panels) and dark (gray panels). Each panel presents the moment-to-moment velocities associated with a single exploratory trip. The solid and dotted lines represent the velocities associated with the outward and homeward trip segments of the exploratory trip, respectively.

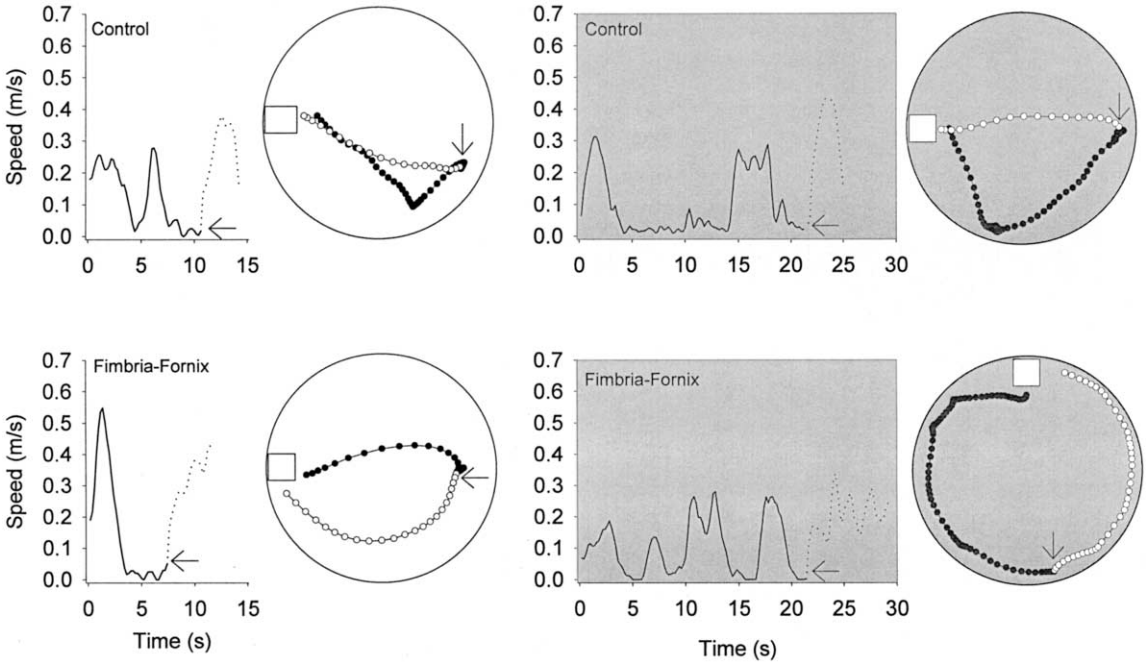

Fig. 7. The upper panels characterize the moment-to-moment velocity profiles and exploration paths for representative control rats under light (white panels) and dark (gray panels) conditions. The lower panels characterize the moment-to-moment velocity profiles and exploratory paths for representative fimbria-fornix rats. The arrows indicate the last stop in corresponding moment-to-moment velocity profiles and exploratory paths.

testing under dark conditions. An ANOVA conducted on outward and homeward trip segment moment-to-moment velocity distributions under light and dark testing conditions revealed the following significant results: a main effect of bin [$F(11, 110) = 33.886$, $P < 0.05$], a bin by trip-segment interaction [$F(11, 110) = 10.151$, $P < 0.05$], a group by bin interaction [$F(11, 110) = 2.488$, $P < 0.05$], and a group by bin by trip-segment interaction [$F(11, 110) = 3.356$, $P < 0.05$]; other effects and interactions were not found to be significant. The left panel of Fig. 10 presents the outward and homeward trip segment moment-to-moment velocity distributions, collapsed across testing condition. The significant group by bin by trip-segment interaction reported in the above ANOVA is consistent with the leftward shift (i.e. overall slower return velocities) of the homeward trip segment moment-to-moment velocity distribution of the fimbria-fornix group, relative to the control group, observed in Fig. 10. The right panel of Fig. 10 presents the outward and homeward trip segment cumulative velocity distributions, collapsed across testing condition and their corresponding points-of-inflection (indicated by the vertical lines in the right panels of Fig. 10). The R-squares associated with estimating each rat's point-of-inflection was significant ($P < 0.001$) and ranged from 0.97 to 0.99. The ANOVA conducted on each group's point-of-inflection revealed a significant main effect of group [$F(1, 10) = 5.601$, $P < 0.05$], main effect of trip-segment [$F(1, 10) = 29.097$, $P < 0.05$], and a significant group by trip-segment interaction [$F(1, 10) = 8.184$, $P < 0.05$]. These results confirm a significant leftward shift in the homeward trip segment moment-to-moment velocity distribution of the fimbria-fornix group relative to the control group.

3.4. Heading direction

Individual and group homeward trip segment heading directions for control and fimbria-fornix rats under both testing conditions are found in Fig. 11. Homeward trip segment heading direction is a function of two parameters: (1) mean angle and (2) angular variance. Circular statistics can be applied to determine whether mean angle, angular variance, or both significantly contribute to group differences (Batschelet, 1981). While groups did not differ in mean angle under light or dark testing conditions, tests for differences in angular variance of homeward trip segment heading direction revealed significant group differences under light (control = 0.034 vs. fimbria-fornix = 0.681, $F(5, 5) = 20.09$, $P < 0.05$) and dark (control = 0.032 vs. fimbria-fornix = 1.023, $F(5, 5) = 32.06$, $P < 0.05$) testing conditions. These results are consistent with a non-systematic error in homeward trip segment heading direction for rats with damage to the fimbria-fornix.

3.5. H/O ratio

Fig. 12 presents each group's H/O ratio under light and dark testing conditions. The ANOVA conducted on each group's H/O ratios under both testing conditions revealed a significant effect of group [$F(1, 10) = 7.036$, $P < 0.05$] while testing condition and the group by testing condition interaction were not significant. These results are also consistent with a non-systematic error in homeward trip segment heading direction observed in rats with fimbria-fornix damage.

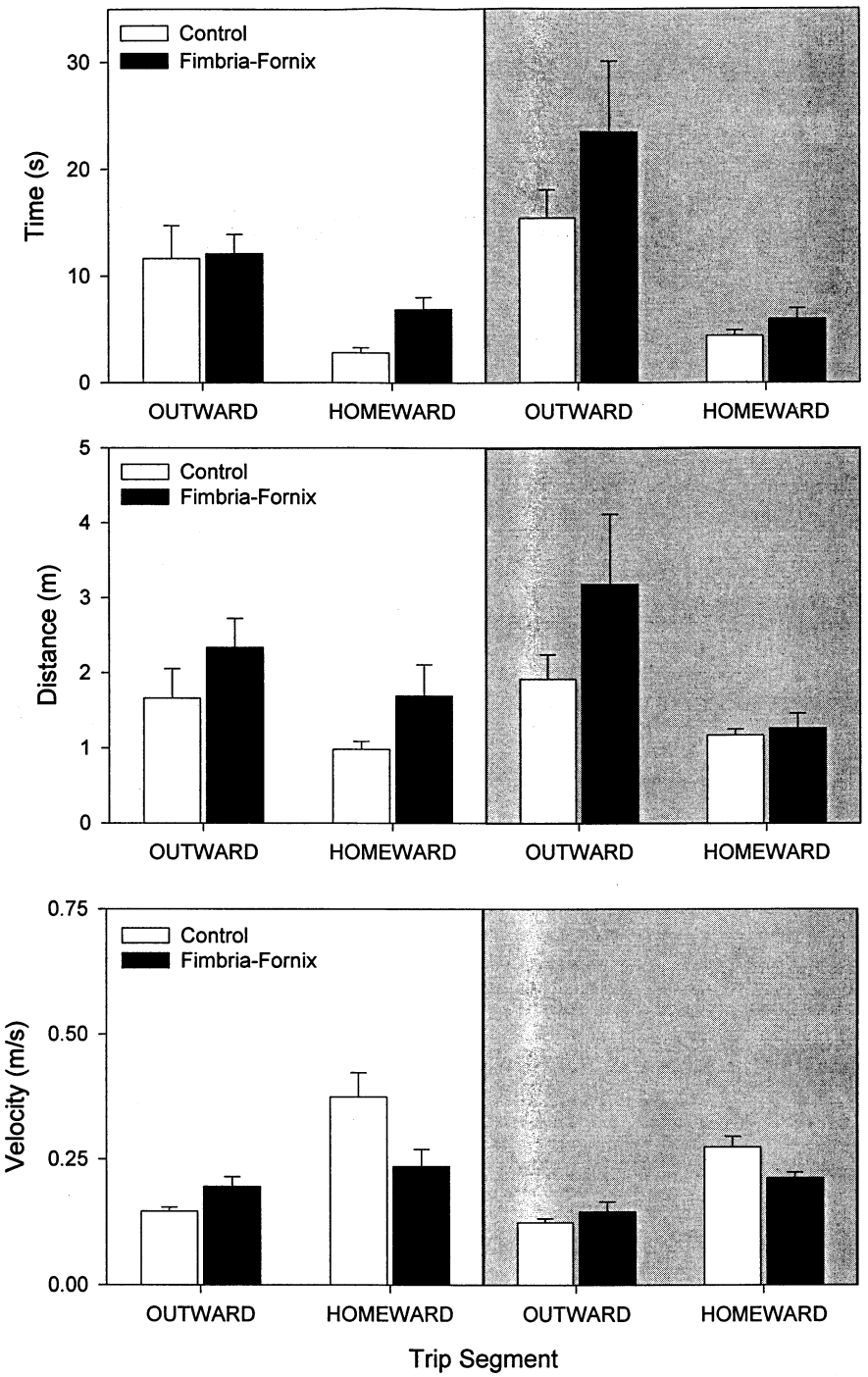

Fig. 8. Mean time (top panel), distance (middle panel), and velocity (bottom panel) associated with the outward and homeward trip segments under light and dark testing conditions for control (white bars) and fimbria-fornix (black bars) rats. Error bars reflect the standard error associated with each group's mean time, distance, and velocity. The asterisk indicates a significant difference between control and fimbria-fornix rats at $P < 0.05$.

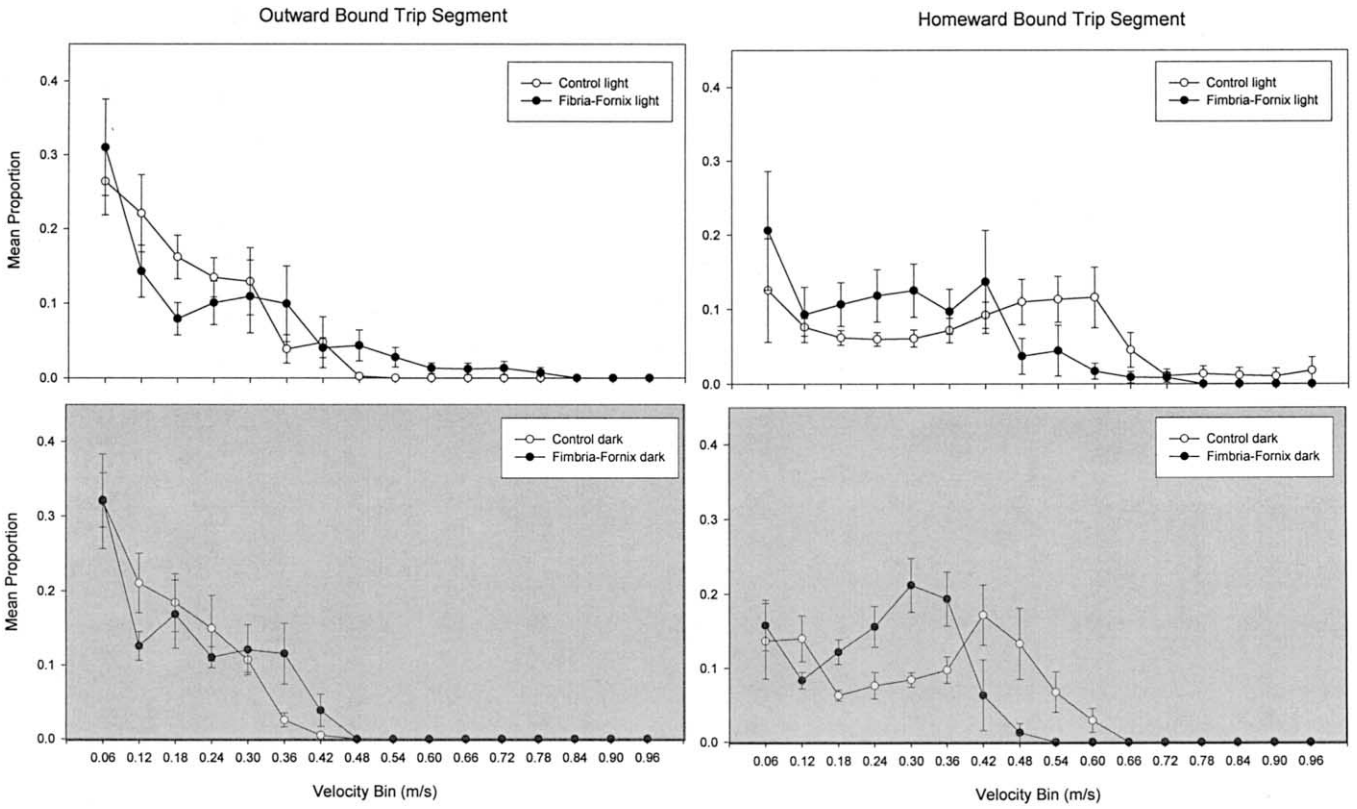

Fig. 9. Control and fimbria-fornix mean velocity distributions for outward (left panels) and homeward (right panels) trip segments under light (white panels) and dark (gray panels) testing conditions. Both groups' outward trip segment mean velocity distributions were equivalent. The fimbria-fornix homeward trip segment mean velocity distribution was shifted to the left under both light and dark conditions relative to the control group. Error bars reflect the standard error associated with each group's mean proportion responding to each bin.

4. Discussion

This work demonstrates that the data obtained from one exploratory trip are sufficient to dissociate animals with damage to the fimbria-fornix from animals with an intact hippocampal formation. Analysis based on the kinematic profiles demonstrated that rats with fimbria-fornix damage return to a home base more slowly than do rats with an intact hippocampus. In addition, rats with damage to the fimbria-fornix return to their home base less accurately, along a more circuitous path, relative to rats with an intact hippocampal formation.

Likewise, analysis of exploratory trips revealed that outward and homeward trip segments differ in their associated lingering and progression components. Drai et al. (2000) defined lingering as locomotor behavior that is restricted to short distances and relatively slow velocities (never exceeding the first gear). Progression episodes are characterized by long distances and fast velocities (second gear and beyond). We observed that the outward trip segment is composed of progressions punctuated by several episodes of lingering that is removed from the home base. The homeward trip segment is a direct, high velocity progression to the home base. The next section will discuss the kinematic and angular analysis in more detail. The following section will consider how the present work adds to our understanding of the mechanisms involved in dead reckoning.

4.1. Kinematic and angular data

Velocity profiles and the point-of-inflection analyses suggest a specific deficit in spatial navigation. The lack of group differences on the average velocities and points-of-inflection observed during the outward trip segment of the exploratory trip discounts a general locomotor impairment. The velocity associated with the homeward segment of the exploratory trip was significantly slower for animals with damage to the fimbria-fornix, as indicated by the point-of-inflection. This pattern of results was observed independently of testing under light or dark conditions. The selective decrease in fimbria-fornix homeward trip segment velocity is consistent with an inability to judge distance of the home base from the animal's current position. In the absence of an accurate estimation of distance, rats may rely on a different navigational strategy to return to a home base. For example, rats with damage to the fimbria-fornix may rely on visual or olfactory cues to locate the

home base. The continual monitoring of the 'piloting' stimulus, relative to the rat's current position, may result in a slower trip towards the home base. This explanation fails to account for the impairments observed in heading direction of the homeward trip segment discussed next.

The heading direction of the homeward trip segment was significantly more variable in animals with destruction to the fimbria-fornix relative to animals with an intact hippocampal formation. Animals with damage to the fimbria-fornix also had significantly more circuitous homeward trip segments relative to animals with an intact hippocampal formation. These results suggest that rats with damage to the fimbria-fornix are impaired in estimating the direction to the home base. If rats were tracking odors, piloting towards a visual stimulus, or navigating based on a cognitive map to return to a home base, then heading direction and path circuitousness should not have been affected by damage to the fimbria-fornix. This follows from work demonstrating that while damage to the hippocampal formation or specific lesions of the hippocampus may impair certain forms of spatial learning such as matching-to-place learning, they nevertheless spare the ability to perform olfactory tracking (Whishaw and Gorny, 1999) and simple place learning (Whishaw and Maaswinkel, 1998; Whishaw et al., 1995; Day and Schallert, 1996; McNamara and Skeleton, 1993; Whishaw and Jarrard, 1996).

The results observed during an exploratory trip by animals with damage to the fimbria-fornix are consistent with an impairment in the ability to respond to idiothetic cues while navigating through space or navigation by dead reckoning. This deficit may be due to a loss of an ability to integrate idiothetic cues with respect to a constant time base. A more intriguing possibility is that dead reckoning employed while exploring an environment may reflect multiple modules. One module may process rectilinear displacements, while another module may process angular displacements. In addition, a reverberatory circuit would be necessary to provide a constant time-base with which to analyze the rectilinear and angular components of an exploratory trip. As of yet, the exact nature of the impairment in dead reckoning, subsequent to fimbria-fornix damage, is not known.

4.2. Mechanisms of dead reckoning

Most of the existing research on rat exploration has focused on locomotor behavior recorded during a circadian interval or in brief open field tests under normal light conditions (O'Keefe and Nadel, 1978; Jarrard, 1993). Many of these studies examine directed behavior

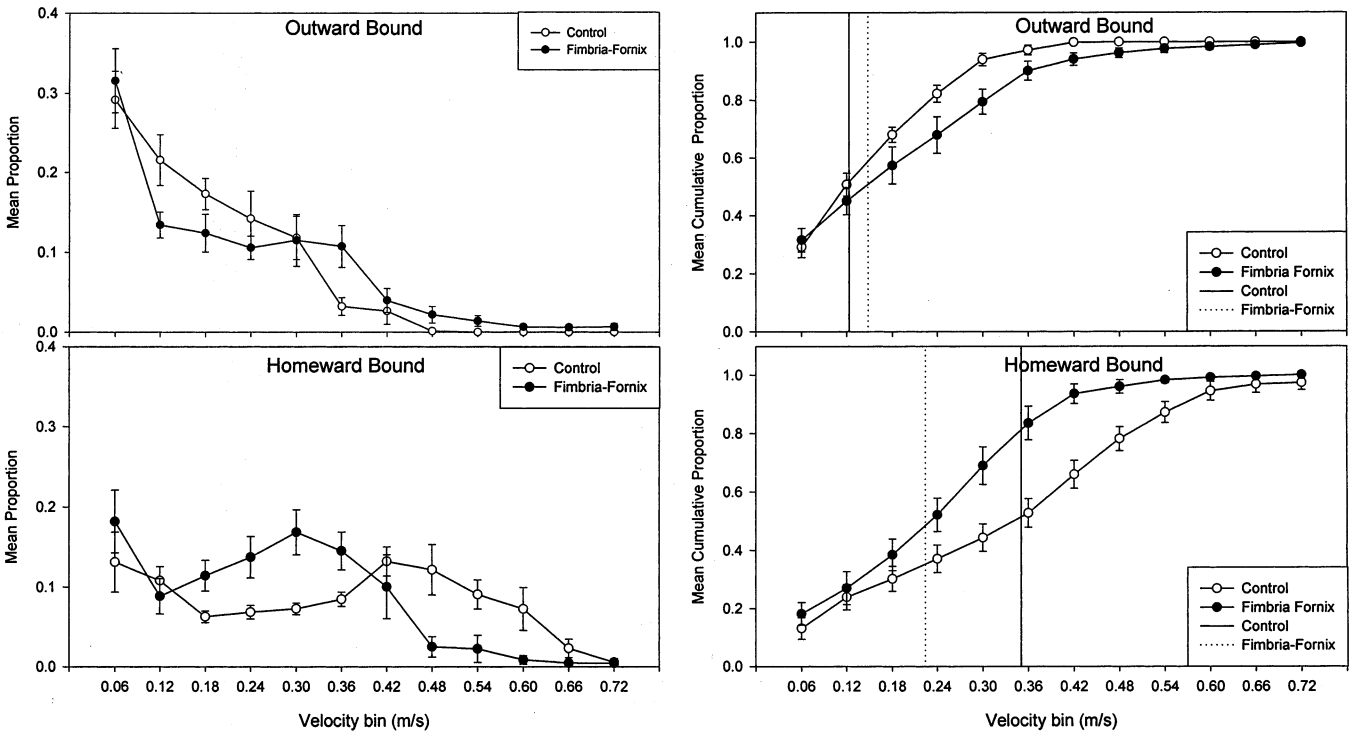

Fig. 10. Control and fimbria-fornix mean velocity distributions for outward (upper left) and homeward (lower left) trip segments collapsed across light and dark testing conditions. Error bars reflect the standard error associated with each group's mean proportion responding to each bin. Control and fimbria-fornix cumulative mean velocity distribution for outward (upper right) and homeward (lower right) trip segments with associated mean points of inflection. Error bars reflect the standard error associated with each group's mean cumulative proportion responding to each bin.

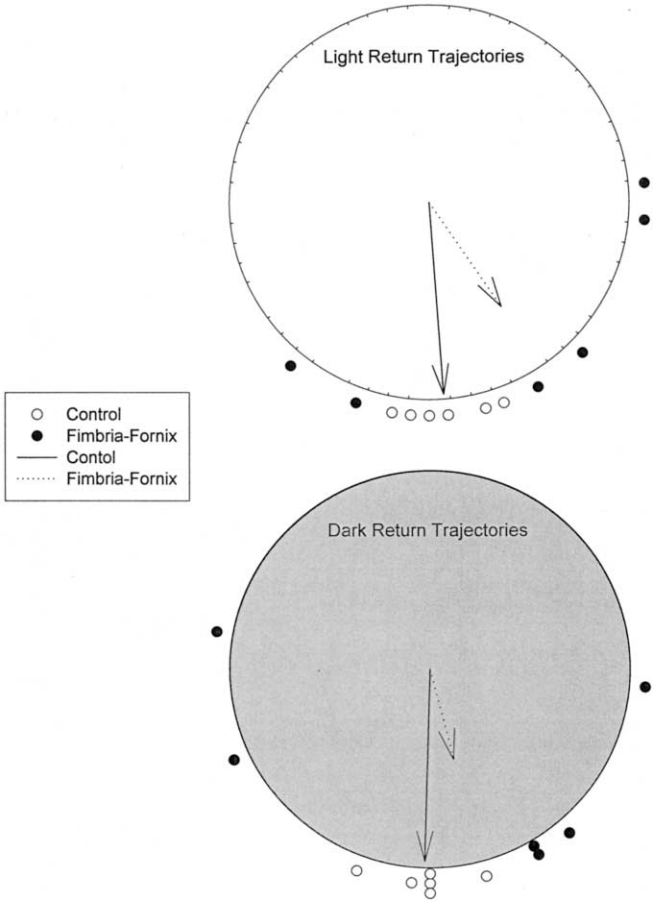

Fig. 11. Control (white circles) and fimbria-fornix (filled circles) heading direction under light (upper panel) and dark (lower panel). Vectors represent control (solid line) and fimbria-fornix (dotted line) mean heading direction. The length of each vector corresponds to *r*, the parameter of concentration or a measure of variability in heading direction.

in a homogenous environment or with a limited set of proximal cues. Our work suggests the importance of including a home base from which an animal can leave and explore an environment then find its refuge. If the home base is removed while the animal is exploring, either under light or dark conditions, a rat will make several short excursions away and then return back to the home base's previous location (Whishaw et al., 2001). This behavior is consistent with the rat's attempt to confirm that the home base was indeed no longer there and/or attempting to confirm that the dead reckoning calculation of where the home base should be was correct. (Some authors suggest that rats have a memory for objects, possibly even a home base, independent of hippocampal damage (Mumby et al., 1999).) The home base is a critical stimulus that organizes the animal's behavior into discrete components that can be quantified. Understanding how the home base organizes exploratory behavior will prove to be an important step in understanding the mechanisms that control dead reckoning.

The idea that dead reckoning can be broken down into several components has received limited attention. Etienne et al. (1986) have demonstrated that hamsters compensate for angular components of passive transport but not linear components. This dissociation of cue sensitivity is consistent with multiple stimuli controlling different aspects of dead reckoning. In addition, Miller et al. (1983) explored the role of the vestibular system on learning a passive transport task subsequent to enucleation. They found that rats with damage to the vestibular system were impaired in learning the task relative to enucleated controls. Both of these studies demonstrate that the vestibular system contributes to

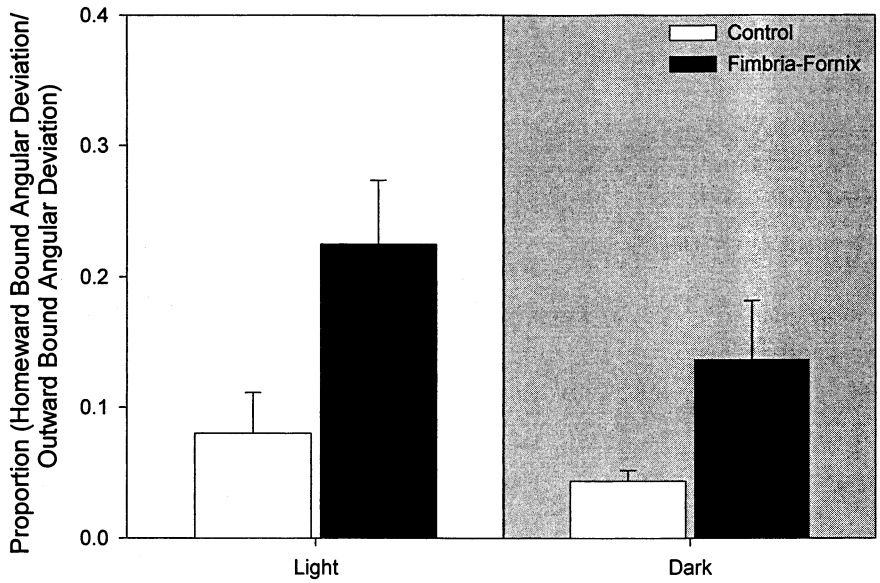

Fig. 12. Control and fimbria-fornix mean homeward angular deviation/outward angular deviation ratio under light (left panel) and dark testing (right panel). Error bars reflect the standard error associated with each group's mean H/O ratio.

dead reckoning. From our work and others (Etienne et al., 1986), it is clear that the vestibular system is not sufficient for accurate dead reckoning. A minimal dead reckoning system would require vestibular, proprioceptive (muscle sense) or kinesthetic (joint sense), and temporal input to accurately navigate through space.

5. Conclusion

The current work provides a foundation for the quantitative analysis of naturally occurring exploration. The combined kinematic and angular data provide a robust method for assessing the navigational abilities of a rat. Researchers interested in the effects of various lesions or pharmacological manipulations may apply this procedure as a useful technique in dissociating general locomotor deficits from specific disruption of spatial navigation strategies. The wide availability of tracking systems, or activity monitors, that measure moment-to-moment velocities, makes this a relatively simple procedure to implement with only limited behavioral testing sessions. Future work should be directed at gaining a better understanding of the mechanisms that control dead reckoning.

Acknowledgements

This research was supported by grants from the Canadian Institute of Health Research.

References

Batschelet E. Circular statistics in biology. London: Academic Press, 1981.

Caramanos Z, Shapiro ML. Spatial memory and *N*-methyl-D-aspartate receptor antagonists APV and MK-801: Memory impairments depend on familiarity with the environment, drug dose, and training. Behav Neurosci 1994;108:30–43.

Cassel JC, Jackisch R, Duschek M, Hornsperger JM, Richards MH, Kelche C, et al. Long term effects of septohippocampal lesions and intrahippocampal grafts on acetylcholine concentration, muscarinic stimulated formation of inositol phospholipids and electrically evoked release of neurotransmitters in the rat hippocampus. Exp Brain Res 1991;83:633–42.

Day LB, Schallert T. Anticholinergic effects on acquisition of place learning in the Morris water task: Spatial mapping deficit or inability to inhibit nonplace strategies? Behav Neurosci 1996;110:998–1005.

Drai D, Benjamini Y, Golani I. Statistical discrimination of natural modes of motion in rat exploratory behavior. J Neurosci Meth 2000;96:119–31.

Eilam D, Golani I. Home base behavior of rats (*Rattus norvegicus*) exploring a novel environment. Behav Brain Res 1989;34:199–211.

Etienne AS, Maurer R, Saucy F, Teroni E. Short-distance homing in the golden hamster after a passive outward journey. Animal Behav 1986;34:696–715.

Frank LM, Brown EN, Wilson M. Trajectory encoding in the hippocampus and entorhinal cortex. Neuron 2000;27:169–78.

Golob EJ, Taube JS. Head direction in rats with hippocampal or overlying neocortical lesions: evidence for impaired angular path integration. J Neurosci 1999;19:7198–211.

Harley CW, Martin GM, Skinner DM, Squires A. The moving fire hydrant experiment: Movement of objects to a new location reelicits marking in rats. Neurobiol Learn Mem 2001;75:303–9.

Hoh TE, Cain DP. Fractionating the nonspatial pretraining effect in the water maze task. Behav Neurosci 1997;111:1285–91.

Jarrard LE. On the role of the hippocampus in learning and memory in the rat. Behav Neural Biol 1993;60:9–26.

Jeltsch H, Cassel JC, Jackisch R, Neufang B, Greene PL, Kelche C, et al. Lesions of supracallosal or infracallosal hippocampal pathways in the rat: behavioral, neurochemical, and histochemical effects. Behav Neural Biol 1994;62:121–33.

Leutgeb S, Ragozzino KE, Mizumori SJ. Convergence of head direction and place information in the CA1 region of hippocampus. Neuroscience 2000;100:11–9.

McNamara RK, Skeleton RW. Effects of intracranial infusions of chlordiazepoxide on spatial learning in the Morris water maze. I. Neuroanatomical specificity. Behav Brain Res 1993;59:175–91.

Miller S, Potegal M, Abraham L. Vestibular involvement in a passive transport and return task. Physiol Psychol 1983;11:1–10.

Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. Nature 1982;297:681–3.

Mumby DG, Astur RS, Weisend MP, Sutherland RJ. Retrograde amnesia and selective damage to the hippocampal formation: memory for places and object discriminations. Behav Brain Res 1999;106:97–107.

Neitz J, Jacobs GH. Reexamination of spectral mechanisms in the rat (*Rattus norvegicus*). J Comp Psychol 1986;100:21–9.

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

Samsonovich A, McNaughton BL. Path integration and cognitive mapping in a continuous attractor neural network model. J Neurosci 1997;17:5900–20.

Saucier D, Hargreaves EL, Boon F, Vanderwolf CH, Cain DP. Detailed behavioral analysis of water maze acquisition under systemic NMDA or muscarinic antogonism: Nonspatial pretraining eliminates spatial learning deficits. Behav Neurosci 1996;110:103–16.

Sutherland RJ, Rodriguez AJ. The role of the fornix/fimbria and some related subcortical structures in place learning and memory. Behav Brain Res 1989;32:265–77.

Sutherland RJ, Whishaw IQ, Kolb B. Spatial mapping: Definitive disruption by hippocampal or medial frontal cortical damage in the rat. Neurosci Lett 1982;31:271–6.

Tchernichovski O, Golani I. A phase plane representation of rat exploratory behavior. J Neurosci Meth 1995;62:21–7.

Walker JA, Olton DS. Fimbria-fornix lesions impair spatial working memory but not cognitive mapping. Behav Neurosci 1984;98:226–42.

Whishaw IQ, Cassel J-C, Jarrard LE. Rats with fimbria-fornix lesions display a place response in a swimming pool: A dissociation between getting there and knowing where. J Neurosci 1995;15:5779–88.

Whishaw IQ, Gorny B. Path integration absent in scent-tracking fimbria-fornix rats: evidence for hippocampal involvement in "sense of direction" and "sense of distance" using self-movement cues. J Neurosci 1999;19:4662–73.

Whishaw IQ, Hines DJ, Wallace DG. 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 2001, in press.

Whishaw IQ, Jarrard LE. Evidence for extrahippocampal involvement in place learning and hippocampal involvement in path integration. Hippocampus 1996;6:513–24.

Whishaw IQ, Kolb B, Sutherland RJ. The analysis of behavior in the laboratory rat. In: Robinson TE, editor. Behavioral approaches to brain research. New York: Oxford University Press, 1983:141–211.

Whishaw IQ, Maaswinkel H. Rats with fimbria-fornix lesions are impaired in path integration: a role for the hippocampus in "sense of direction". J Neurosci 1998;18:3050–8.

Whishaw IQ, McKenna JE, Maaswinkel H. Hippocampal lesions and path integration. Curr Opin Neurobiol 1997;7:228–34.

Whishaw IQ, Nicholson L, Oddie SD. Food-pellet size directs hoarding in rats. Bull Psychon Soc 1989;27(1):57–9.

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

Worden R. Navigation by fragment fitting: a theory of hippocampal function. Hippocampus 1992;2:165–87.