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NMDA lesions of Ammon's horn and the dentate gyrus disrupt the direct and temporally paced homing displayed by rats exploring a novel environment: evidence for a role of the hippocampus in dead reckoning

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Abstract

Dead reckoning, a form of navigation used to locate a present position and to return to a starting position, is used by rats to return to their home base. The present experiment examined whether dead reckoning is displayed by rats during their first exploratory excursions in a novel environment and also examined whether the behaviour requires the integrity of the cells of the hippocampus. Experimental rats, those with NMDA (*N*-methly D-aspartate) lesions of Ammon's horn and the dentate gyrus, and control rats could leave a cage to explore a large circular table under light and dark conditions. Home base behaviour, use of olfactory cues, and thigmotaxic- based navigation were evaluated. Temporal, topographical and kinematic analyses were conducted on the first three exploratory excursions that extended at least halfway across the table. Groups did not differ in numbers of exits from the home base, lingering near the home base, distance travelled, or the use of surface cues as might be exemplified by thigmotaxic and olfactory behaviour. Temporal, topographical and kinematic reconstructions of homing behaviour, however, indicated that control rats, but not hippocampal rats, made direct high velocity return trips to the home base in both the light and the dark. Peak velocity of the trips occurred at the trip midpoint, independent of trip distance, suggesting the movements were preplanned. These results are discussed in relation to the ideas that dead reckoning is used in the homing of exploring rats and that this form of navigation involves the hippocampus.

Introduction

Dead reckoning is an online form of spatial navigation that involves processing self-movement cues (vestibular cues, somatosensory cues, sensory flow, or efference copy of movement commands) within a temporal context such that an animal can plot a trajectory back to the place where a trip was initiated (Barlow, 1964). Darwin (1873) was the first person to suggest that animals can use dead reckoning to navigate. Subsequent work has demonstrated that dead reckoning is displayed in invertebrates (Muller & Wehner, 1988; Collett & Collett, 2000a, b) as well as vertebrates (Mittelstaedt & Mittelstaedt, 1980; Etienne *et al*., 1986; Seguinot *et al*., 1993; Maaswinkel *et al*., 1999; Whishaw & Gorny, 1999; Mittelstaedt & Mittelstaedt, 2001). The high degree of conservation across species suggests an adaptive value of dead reckoning, but as yet, little is known of the neural or computational bases of the behaviour.

Because the hippocampus is proposed to play a central role in spatial behaviour (O'Keefe & Nadel, 1978), there have been investigations of the contribution of the hippocampus and other limbic structures to dead reckoning. Single unit recording studies show that some hippocampal formation cells respond to self-movement cues (vestibular, optic flow, and proprioceptive related cells), while other cells respond to an animal's directional orientation (Gothard *et al*., 1996; McNaughton *et al*., 1996; Golob & Taube, 1999; Wylie *et al*., 1999; Stackman *et al*., 2002). In addition, a number of studies using different testing procedures report that hippocampal lesions can disrupt dead reckoning (Maaswinkel *et al*., 1999; Save *et al*., 2001; but see Alyan & McNaughton, 1999). Nevertheless, recording studies are not usually performed as an animal dead reckons (but see, Gothard *et al*., 1996), and all of the behavioural studies have involved conditioning paradigms requiring pretraining. Because dead reckoning is an online, unconditioned behaviour, it should not be dependent upon pretraining and should occur as a part of an animal's natural repertoire of behaviours.

The major purpose of the present study was to investigate whether dead reckoning in unconditioned exploratory behaviour is observed upon the first exposure to a novel environment and whether the hippocampus contributes to the behaviour. It is generally thought that exploration is useful, in that once an animal has explored an environment it is subsequently able to use the information it has acquired to travel through that environment again (Whishaw & Brooks, 1999). The animal faces a problem in using this information. In a novel environment, the information that an animal gathers on an outward trip may be of little value in guiding a return trip. Although an animal views and learns about various cues on its outward trip, it does not see those cues, or move in relation to them, from the vantage point of the journey home.

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We made use of the organized exploratory behaviour of rats in which outward trips and return trips are centred around a home base (Eilam & Golani, 1989; Drai *et al*., 2000). We specifically examined the homeward trip, which begins after the final stop of a highly variable outward segment. Although it may begin from any point in the environment on different trips, it is always a direct, rapid return to the home base (Whishaw *et al*., 2001). Because these direct homeward trips are observed when rats are tested under novel light and dark conditions, and thus do not depend upon the availability of environmental cues, we have suggested dead reckoning as the mediating process (Whishaw *et al*., 2001; Wallace *et al*., 2002c).

To produce selective hippocampal lesions, injections of the neurotoxin n-methyl-d-aspartate (NMDA) were made into the cell fields of Ammon's horn and the dentate gyrus (Jarrard & Meldrum, 1993). Subsequent to recovery, the experimental and control rats were permitted to explore a large circular arena from a small home base under light and dark conditions. In addition to a number of aspects of exploratory behaviour, each animal's first three outward and homeward trips under light and dark conditions were analysed using topographical and kinematic reconstructions of the video records of their behaviour.

Materials and Methods

Animals

The subjects were 15 female Long-Evans rats (University of Lethbridge vivarium) weighing approximately 250–300 g. Rats were housed in groups of three in Plexiglas cages in the colony room with the temperature maintained at 20–21 °C and a 12 h light : 12 h dark cycle. Seven rats received NMDA hippocampal lesions two weeks prior to the start of testing, and eight rats served as controls. All experimental procedures in this study were approved by the University of Lethbridge Animal Care Committee, which follows the standards set by the Canadian Council on Animal Care.

Surgery

Rats were anaesthetized with a mixture of isofluorane and oxygen during the surgery. Damage to Ammon's horn and the dentate gyrus was produced by injecting 0.40 μL of 7.5 mg N-methly D-aspartate (Sigma-Aldrich Canada Ltd. Oakville, Ontario L6H 6J8 Canada) in 1 mL of saline at 0.20 μ/min. Injection locations were modified from the sites reported by Sutherland *et al*. (2001) to compensate for using female Long-Evans hooded rats. There were six lesion sites per hemisphere, using coordinates with respect to bregma and the surface of the dura (in mm): −3.1 posterior, 2.0 lateral, and −3.6 ventral; −4.1 posterior, 3.0 lateral, and −3.5 ventral; −5 posterior, 3.5 lateral, and −3.5 ventral; −5.3 posterior, 5.2 lateral, and −5.5 ventral; −5.3 posterior, 5.2 lateral, and −7.5 ventral; −6 posterior, 5.0 lateral, and −7.3 ventral. Subsequent to the surgery, the rats were administered .1 cc of (65 mg/mL) sodium pentobarbital IP and .06 cc of (5 mg/mL) Metcamp SC. Rats were given a week to recover prior to testing.

Feeding

Rats were maintained at their ad lib body weights during the course of exploratory sessions. They were fed measured amounts of Laboratory Diet Laboratory Rodent Pellets (Canadian Laboratory Diets, Inc. Leduc, ALB T9E 6T9) in their home cages subsequent to daily testing.

Apparatus

The apparatus was a wooden circular table without walls measuring 250 cm in diameter. The table was painted white 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 (20 × 29 × 22 cm) with a circular hole (11.5 cm in diameter) in one of the sides was placed on the edge of the table to be used as a refuge by the rat. The surface of the table was approximately 64 cm above the floor. The table was rotated between rats and wiped down after testing each rat.

Infrared testing

An infrared camera was positioned perpendicular to the table. The testing room was light proof, such that when the lights were turned off during dark testing conditions, the room was completely dark. Two infrared emitter banks were attached on different walls. This provided sufficient infrared illumination in the room such that the rat was visible on the camera under dark conditions. The experimenter used an infrared spotter to test the animal under complete dark conditions. Infrared is a wavelength that the rat is not able to detect (Neitz & Jacobs, 1986).

Histology

At the completion of the behavioural studies, animals were deeply anaesthetized 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. Every fifth section was taken and stained with Cresyl violet.

Procedure

Animals were given one week to recover from the surgery. After recovery, animals explored the table under light and dark sessions. A single exploratory session was 50-minutes in duration in which the animals were free to move around the table and were video taped. Light exploratory sessions were conducted in a novel room with the lights on. Dark exploratory sessions were conducted in the same room with all light sources extinguished. During exploratory sessions, a home base was located on top of the table at one edge, with the exit facing toward the middle of the table. Although animals varied in the number of sessions prior to leaving the home base under novel-light conditions (control mean, 3.0 sessions; hippocampal mean, 3.8 sessions), the groups did not differ significantly. Likewise, control (mean, 1.6 sessions) and hippocampal (mean, 1.3) animals were not significantly different in the number of sessions under dark conditions.

Single exploratory trips were defined as a departure from the home base, locomotor activity on the table that displaced the animal at least halfway across the table, and ending when the rat returned to the home base. Golani and coworkers distinguish between different modes of exploration as episodes of lingering or progression with stops (Drai *et al*., 2000). Lingering refers to locomotor behaviour that is restricted to a small area, usually around the home base, whereas movements between areas are classified as progressions. Our criteria for an exploratory trip eliminate exploratory behaviour that would be classified as lingering close to the home base. Therefore, exploratory trips would be characterized as sets of progressions punctuated with brief stops.

Digitizing exploratory behaviour

Exploratory trips were converted from analogue recordings to digital computer files using the Peak Performance system (Peak Performance Technologies, Inc. Englewood, CO 80112 USA) at a sampling rate of 60Hz. Digitizing the path involves manually tracking a single point on the animal by selecting one pixel per frame every 10 frames of the digital computer file. The midline of the rat's back, at the level of the forelimbs, was used as the reference point. Moment-to-moment speeds and scaled *x*- and *y*-coordinates were computed from the raw data.

Analysis of thigmotaxic behaviour

The walls of the home base provide a substrate for thigmotaxic behaviour to occur. If hippocampal lesions produce an increase in the likelihood of thigmotaxic behaviour, then one might expect differences in the amount of time it took for the animals to leave the home base. We calculated the amount of time it took the rat to initially exit the home base on the first day that the rat made exploratory trips under light and dark conditions. An exit from the home base occurred when all of the rat's paws were located outside of the home base. Another possibility is that a hippocampal lesion-induced increase in thigmotaxic behaviour is only observed when the animal explores the table. The first 10 trips observed under both testing conditions for control and hippocampal animals were analysed for the amount of time spent at the perimeter and centre of the table. The perimeter of the table was defined as the area adjacent to the edge of the table that was approximately 30 cm in width (see the top panel of Fig. 1). The resulting central area was a circle, 190 cm in diameter, centred at the middle of the table. Finally, to further characterize the behaviour of control and hippocampal animals at the periphery of the table, three trips under light conditions from both groups were analysed for the number of stops and progressions (walking episodes) observed on the edge of the table.

Analysis of home base behaviour

Control animals naturally set up a home base and use it to organize their exploration. Group differences in trip organization may reflect a general inability to set up a home base, rather than a more specific spatial deficit. Two measures were used to index home base behaviour. First, the percent of time spent in the home base and out on the table was calculated for one exploratory session under light and dark conditions. Second, the number of short trips made prior to the first long trip was recorded. Both of these behavioural measures provide an index of how attached the animal is to the home base.

Analysis of olfactory tracking

It is unlikely that olfactory cues are controlling the organization of exploratory behaviour. First, the differences observed in exploratory trip topography within and between exploratory trips suggest rats are not following a single route to the home (see Wallace *et al*., 2002c). In addition, pilot work with a table rotating at subvestibular thresholds has demonstrated that rats return to the former location of a home base rather than the current location of a home base (unpublished observations). If a rat were employing olfactory cues to navigate, then one would anticipate a different kinematic profile than observed when an animal is using dead reckoning based navigation. Specifically, an olfactory tracking rat would maintain the same speed as long as the odour cue was present. This would contrast with movements guided by dead reckoning in which speeds occur independent of external stimuli and depend on the subjective judgement of distance. We examined the kinematics associated with 10 episodes of tracking from Wallace *et al*. (2002b) and eight dead reckoning episodes (our unpublished data). The spread in moment-to-moment speeds was obtained from each tracking and dead reckoning episode by fitting a three parameter Gaussian curve to the corresponding moment-to-moment speeds. The parameter of spread was then evaluated for group differences.

Analysis of exploratory trips

The first three exploratory trips were selected for analysis. This was done to ensure that the environment was novel, thereby limiting the influence of environmental cues on organizing behaviour. It is important to note that subsequent trips have similar organization to the ones selected for analysis. Figure 1 presents the topography (top panel) and kinematics (bottom panel) for a single exploratory trip. Exploratory trips were broken down into outward and homeward trip segments. The outward segment of the trip was defined as the portion of the path linking the initial departure from the home base to the last stop made prior to returning to the home base (see Fig. 1, the black dots in the top panel and solid line in the bottom panel). Stops were defined as the absence of movement in which the hind feet were stationary for at least

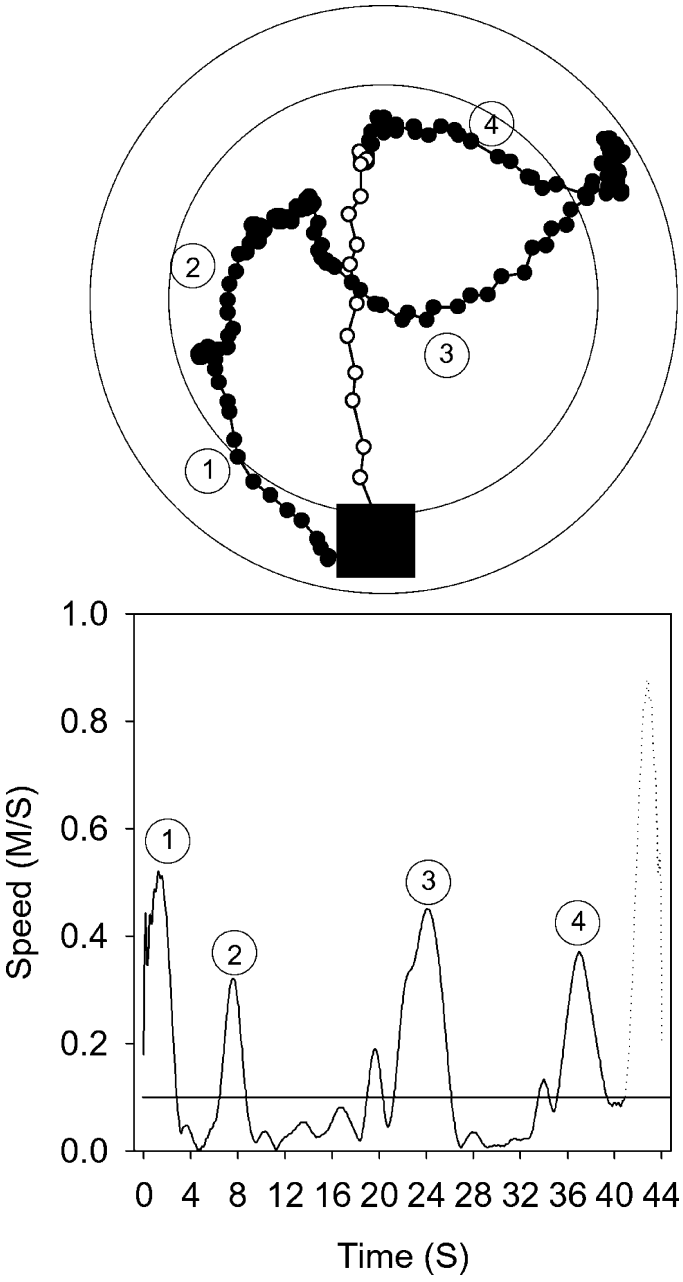

FIG. 1. Circled numbers indicate corresponding time points for the kinematic and topographic representations of the exploratory trip. The top panel plots the topographical characteristics of a single exploratory trip. Solid dots correspond to the outward trip segment; white dots correspond to the homeward trip segment. The inner circle represents the division between periphery and central potion of the table. Note: the dot spacing corresponds to rate of movement during the exploratory trip. The bottom panel plots the moment-to-moment speeds associated with a single exploratory trip. The solid line indicates the outward trip segment; the dotted line indicates the homeward trip segment. The horizontal line (.10 m/s) represents the stop criteria.

2 s. Stops were also characterized by near zero speeds on the moment-to-moment speed profiles. The homeward trip segment was defined as the portion of the path linking the last stop to arrival at the home base (see Fig. 1, the white dots in the top panel and dotted line in the bottom panel).

Several measures were used to quantify exploratory trip organization: spontaneous alternation observed between trips, distance ratio, time, distance, maximal speed, proportion of path dedicated to the homeward trip segment, and spread/mean of the peak speed locations. First, the extent that animals explored different regions of the table between the three exploratory trips was examined. The table was divided into three sections relative to the home base: right, middle, and left. If the rat's outward trip segment started in different regions between trips, then a value of 1 was assigned. If the rat's outward trip segment started in the same region between trips, then a value of 0 was assigned. Summing the values from the two transitions between the three trips provided an index of the animal's tendency to spontaneously alternate. For example, if a rat entered the left, right, and right region of the table it would receive a score of 1, vs. a rat that entered left, right, and left would receive a score of 2.

To determine the extent that a path through the environment was direct or circuitous, the distance of a line that connected the point where the path started to the point where the path ended was divided by the distance that was actually travelled. Direct paths through an environment are associated with distance ratio values of 1.0–0.8. Distance ratio values of 0.7–0.6 correspond to paths that are restricted to the perimeter of the table. As the distance ratio further decreases, paths become progressively more circuitous. Time, distance, and maximum velocity were calculated from the moment-to-moment speeds and the scaled *x*- *y*-coordinates for each trip segment. Time measures were used to calculate the proportion of the exploratory trip composed of the homeward trip segment.

The symmetrical increase and decrease in speed observed over the homeward trip segment was indexed by calculating the location of the peak in speed for each exploratory trip. Several steps were involved in measuring the location of the peak in the speed observed on the homeward trip segment. First, moment-to-moment speeds for the outward and homeward trip segments were normalized to 300 and 100 bins, respectively. Second, the bin number that corresponded to the peak in speed for both trip segments was recorded for each trip under both testing conditions. Third, the peak locations for the outward and homeward trip segments were transformed into a 100-point scale. Finally, each animal's mean and standard deviation in peak location across the three exploratory trips was calculated for the outward and homeward trip segments under both testing conditions. The means and standard deviations from each animal were used to evaluate group differences on trip segments under each testing condition.

Results

Histology

Figure 2 presents representative sections from control and hippocampal animals. A rostral-caudal examination of the extent of the hippocampal lesions indicated that, except for small portions of hippocampal cell fields at the most rostral and caudal extent of the hippocampus, most of the cells of Ammon's horn and the dentate gyrus were killed by the neurotoxin, while the fibers of the fimbria-fornix appeared largely spared. There was only limited damage to the overlying corpus callosum and neocortex at the sites of cannula penetration. The lesion extent was thus consistent with that reported in previous studies involving selective hippocampal cell elimination (Jarrard, 1989; Jarrard & Meldrum, 1993; Maaswinkel *et al*., 1999).

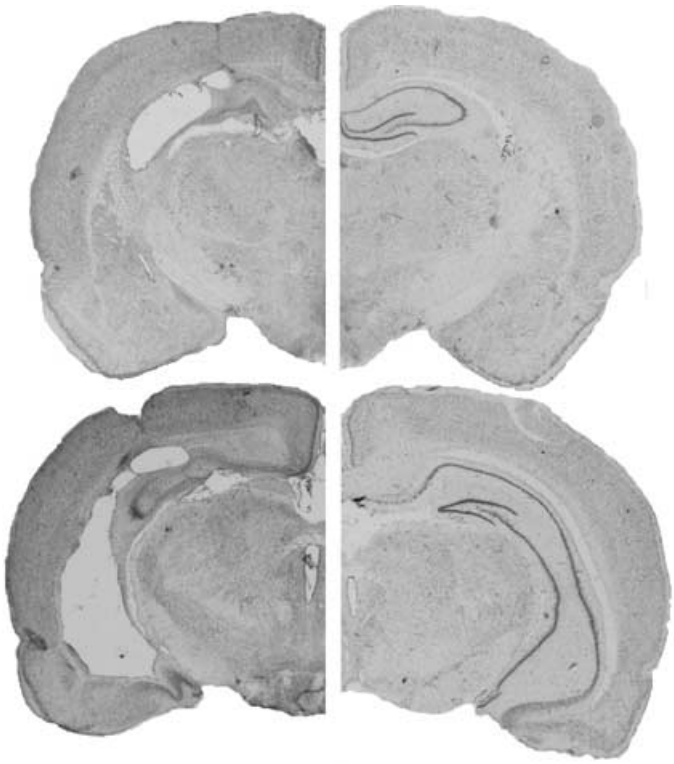

FIG. 2. Photomicrographs are sections stained with Cresyl violet. The left and right panels represent sections through a control rat and a rat with damage to the hippocampus, respectively. Rats receiving NMDA hippocampal lesions had damage that was restricted to the hippocampus.

Thigmotaxic behaviour

Analysis of the amount of time spent in the home base prior to the first exit did not reveal a significant effect of testing condition, group, or a Group–Testing Condition interaction. Both control and hippocampal animals made their first exit approximately 2 min after being placed into the home base under light and dark conditions.

The ANOVA conducted on the average percent time spent at the periphery, across 10 trips under light and dark testing conditions, revealed a significant effect of testing condition ($F_{1,13} = 12.22$, $P < 0.05$). The effect of group and the Group–Testing Condition interaction were not significant. The animals spent approximately 81% of their time at the periphery under light conditions and approximately 68% of their time at the periphery under dark conditions.

The analysis of stops on the edge observed during three exploratory trips under light conditions did not result in a significant effect of group. Under light conditions, both groups made an average of 4.2 stops on the edge of the table. Groups were also not found to be significantly different on the number of progressions observed on the edge of the table. On average, control and hippocampal animals made 1.2 progressions on the edge of the table under light conditions.

Home base behaviour

The top panels in Fig. 3 present ethograms for a representative control and hippocampal animal under both testing conditions. The time spent in the home base is represented as a vertical black bar, and the time spent on the table is represented as a white (light conditions) or grey (dark conditions) vertical bar. The bottom panel of Fig. 3 presents each group's average percent time spent in the home base under both testing conditions. The ANOVA conducted on the percent time spent in the home base revealed a significant group effect ($F_{1,13} = 12.89$, $P < 0.05$)

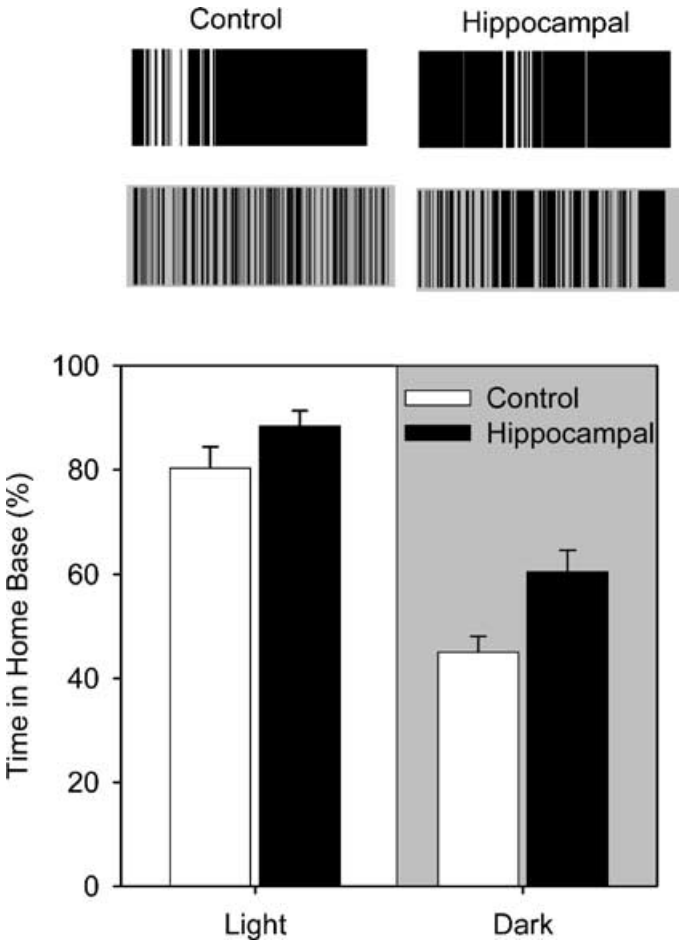

FIG. 3. The ethograms present the amount of time spent in the home base or on the table from a representative control and hippocampal animal under both testing conditions. The vertical black bars represent time spent in the home base; the vertical white (under light conditions) and grey (under dark conditions) bars represent the amount of time spent on the table. The bottom panel presents control and hippocampal average percent of time spent in the home base under both testing conditions.

and a significant effect of testing condition ($F_{1,13} = 64.51$, $P < 0.05$). The Group–Testing Condition interaction was not found to be significant.

The analysis conducted on the number of short trips made prior to the first long trip revealed that hippocampal animals made significantly more short trips relative to control animals ($t_{13} = 2.63$, $P < 0.05$). Hippocampal animals made an average of 3.28 short trips prior to the first long trip. Control animals made an average of 1.62 short trips prior to the first long trip.

Olfactory tracking

The left hand side of Fig. 4 presents the average moment-to-moment speeds for 3 olfactory tracking trips and three dead reckoning trips. The bars compare the parameter associated with the spread of the moment-to-moment curves from the 10 tracking and eight dead reckoning episodes. The *t*-test performed on the parameter of spread revealed a significant difference between tracking and dead reckoning episodes ($t_{16} = 2.164$, $P < 0.05$).

Exploratory trip topography

The analysis conducted on the group spontaneous alternation scores did not reveal a significant effect of group. Both control and hippocampal animals significantly alternated regions on the table between trips ($t_{15} = 5.13$, $P < 0.05$).

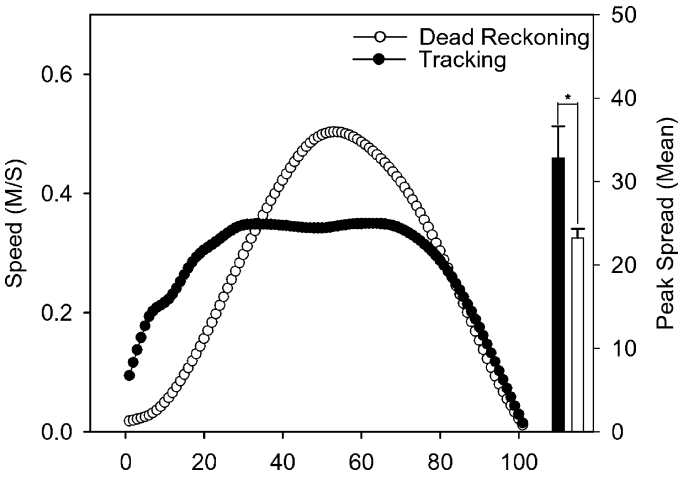

FIG. 4. The line graphs represent three tracking (black dots) and dead reckoning (white dots) episodes. When using an odour to navigate, there is a more gradual onset and offset of speed resulting in a wider spread in moment-to-moment speeds. During dead reckoning episodes, there is a more rapid onset and offset of speed. Using dead reckoning to navigate results in a more narrow spread of moment-to-moment speeds. The bar graphs present the average spread of moment-to-moment speeds for control and hippocampal animals under both testing conditions ($^*P < 0.05$).

Figure 5 presents the topographical representations of the spatial organization of three exploratory trips from control animals under novel-light and dark conditions. The top panels represent the outward trip segments, whereas the bottom panels represent the homeward trip

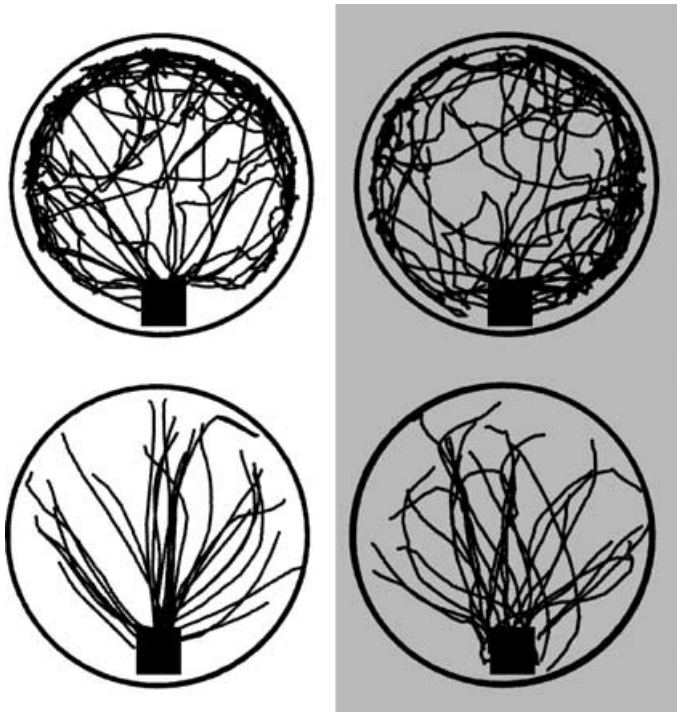

FIG. 5. The black box in each plot represents the normalized home base location. The top panels plot three outward trip segments for each control animal under light (left) and dark (right) conditions. The bottom panels plot three homeward trip segments for each control animal under light (left) and dark (right) conditions.

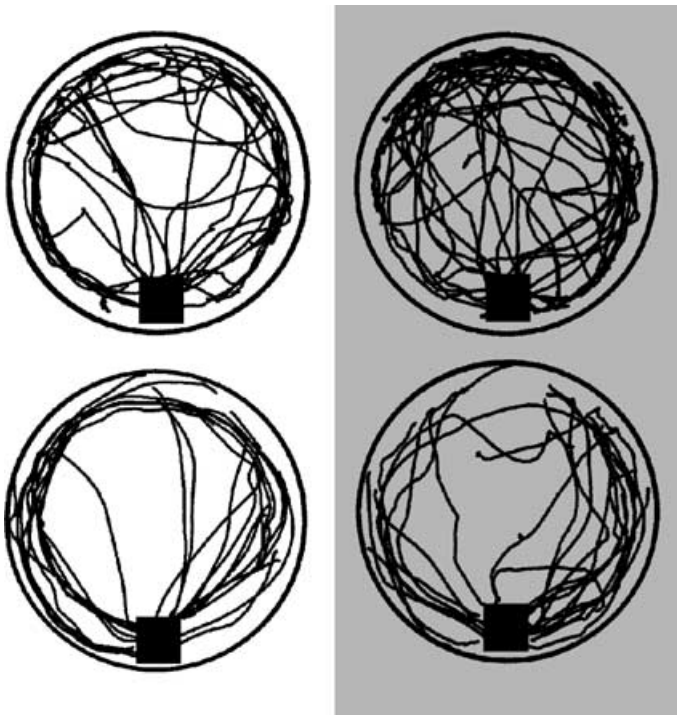

FIG. 6. The black box in each plot represents the normalized home base location. The top panels plot three outward trip segments for each animal with hippocampal damage under light (left) and dark (right) conditions. The bottom panels plot three homeward trip segments for each animal with hippocampal damage under light (left) and dark (right) conditions.

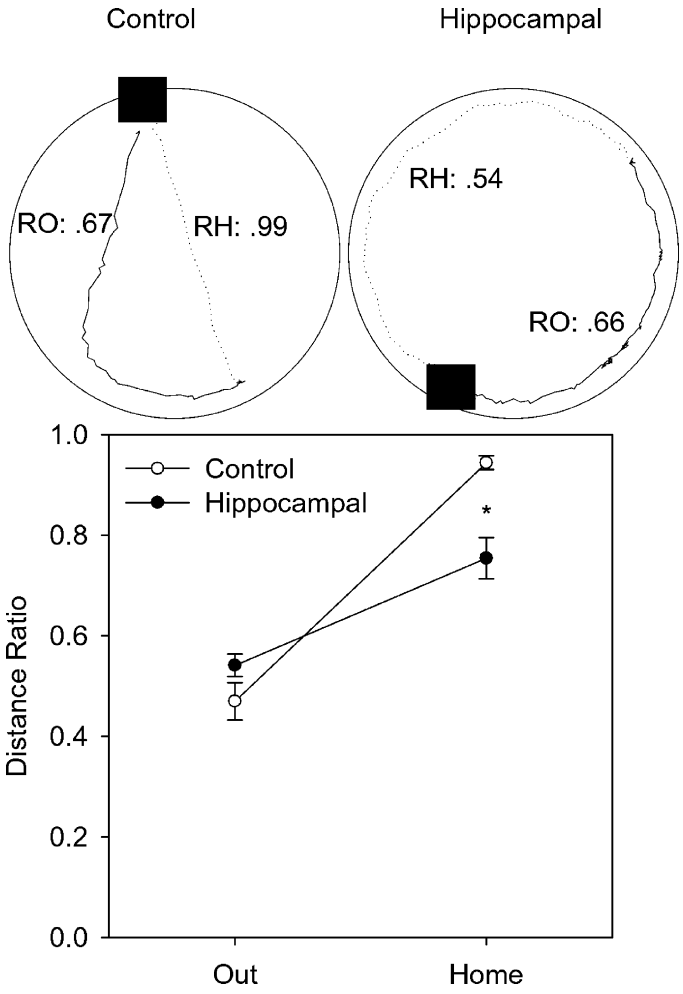

FIG. 7. The top panels plot exploratory trip topography for a control (left) and hippocampal (right) rat under light conditions. The home base is indicated by the black box with the outward trip segment represented by a solid line and the homeward trip segment represented by a dotted line. The distance ratio is provided for the outward (RO) and homeward (RH) trip segments. Distance ratios range in values from 1.0, direct paths through the environment, to 0.0, more circuitous paths through the environment. Distance ratios close to 0.6 are associated with thigmotaxic types of behaviour. The bottom panel plots the outward and homeward distance ratios (mean and standard error) for control and hippocampal groups ($^{*}P < 0.05$).

segments. Control animals' outward trip segments were circuitous and focused on the periphery of the table under both conditions. The homeward trip segments were directed to the home base, noncircuitous, and crossed the centre portion of the table under both conditions. As seen in the top panels of Fig. 6, hippocampal animals' outward trip segments were circuitous and focused on the periphery of the table under both light and dark conditions. In contrast to the control animals' homeward trip segments, hippocampal animals' homeward trip segments were circuitous and did not cross the centre portion of the table under both conditions.

The top panels of Fig. 7 present sample exploratory trips from a control (left) and hippocampal (right) animal. The distance ratio for the outward (RO) and homeward (RH) trip segments is listed for each segment of an animal's trip. The bottom panel plots the control and hippocampal groups' mean distance ratios for the outward and homeward trip segments. The ANOVA conducted for the distance ratio for each group's outward and homeward trip segments under light and dark conditions revealed significant effects of group ($F_{1,13} = 7.47$, $P < 0.05$), trip segment ($F_{1,13} = 66.31$, $P < 0.05$), and Group by Trip segment interaction ($F_{1,13} = 11.5$, $P < 0.05$). The main effect of testing condition and other interactions were not significant. Post hoc tests revealed that the hippocampal group's homeward trip segment was significantly more circuitous under both novel-light and dark conditions (Tukey LSD).

Exploratory trip kinematics

Figure 8 presents the time, distance, and maximum speed observed on the outward and homeward trip components. The ANOVA conducted on each group's mean time observed on outward and homeward trip segments under light and dark conditions revealed significant effects of group ($F_{1,13} = 11.89$, $P < 0.05$), trip segment ($F_{1,13} = 139.56$, $P < 0.05$), and Group by Trip segment interaction ($F_{1,13} = 16.65$, $P < 0.05$). Post hoc tests revealed that the control animals spent more time on the outward trip segment relative to hippocampal animals under both testing conditions (Tukey LSD). The ANOVA conducted on each group's mean distance observed on outward and homeward trip segments under light and dark conditions revealed a significant effect of trip segment ($F_{1,13} = 46.67$, $P < 0.05$) and Group by Trip segment interaction ($F_{1,13} = 5.8$, $P < 0.05$). Post hoc tests revealed that the hippocampal animals' homeward trip segments were significantly longer, relative to the control animals' homeward trip segments, under both testing conditions (Tukey LSD). The ANOVA conducted on each group's mean maximum speed observed on outward and homeward trip segments under light and dark conditions revealed significant effects of trip segment ($F_{1,13} = 14.91$, $P < 0.05$), testing condition ($F_{1,13} = 21.19$, $P < 05$), Group by Trip segment interaction ($F_{1,13} = 5.04$, $P < 0.05$), and Trip segment by Testing condition interaction ($F_{1,13} = 7.37$,$P < 0.05$). Post hoc tests revealed that the hippocampal animals had significantly slower maximal speeds on the

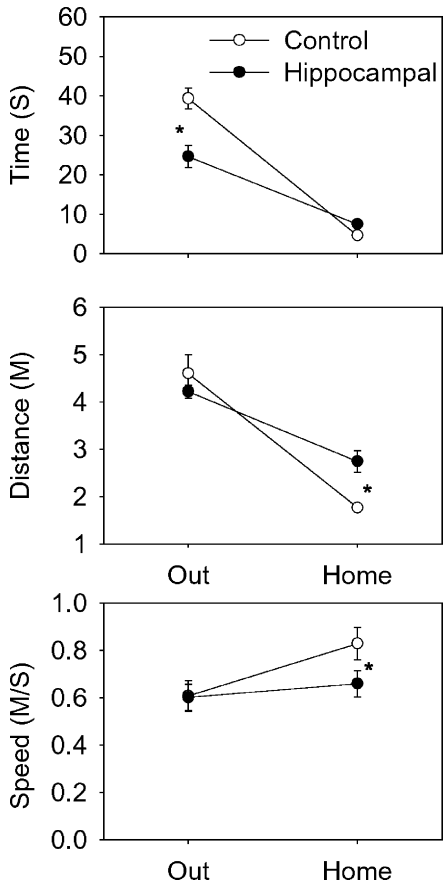

FIG. 8. The top panel plots the amount of time (mean and standard error) spent on the outward and homeward trip segments. The middle panel plots the distance travelled (mean and standard error) on the outward and homeward trip segments. The bottom panel plots maximum speed (mean and standard error) observed on the outward and homeward trip segments ($^{*}P < 0.05$).

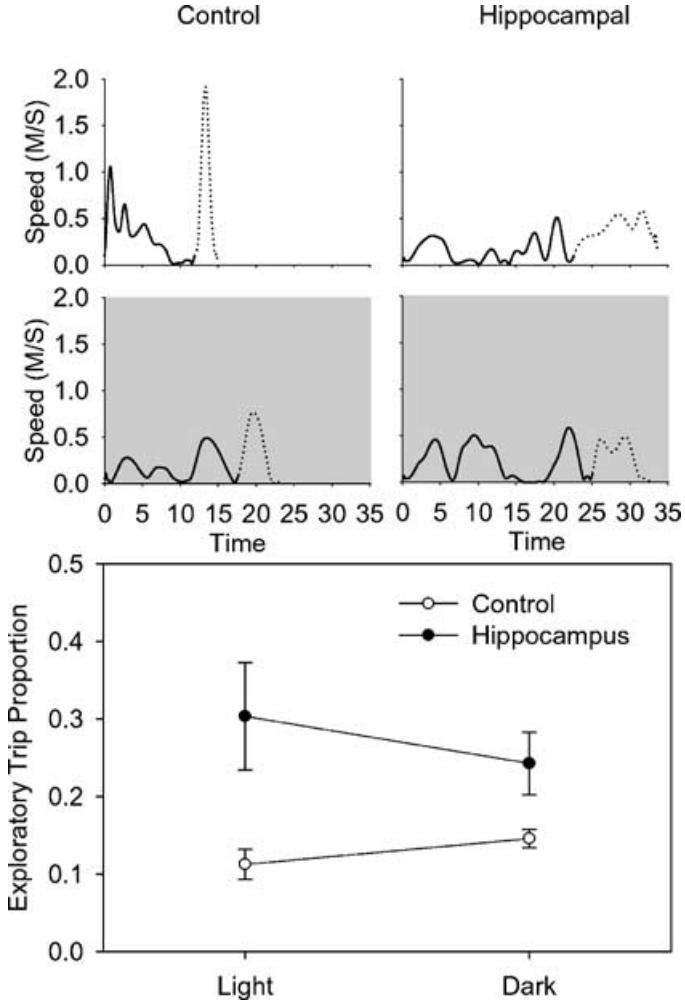

FIG. 9. The top and middle panels plot exploratory trip kinematics for a control (left) and hippocampal (right) rat under light (top) and dark (middle) conditions. The solid lines represent the outward trip segment; dotted lines represent the homeward trip segment. The bottom panel plots each group's proportion (mean and standard error) of the exploratory trip dedicated to the homeward trip segment ($^{*}P < 0.05$).

homeward trip segment, relative to control animals, under both testing conditions (Tukey LSD).

Figure 9 presents the moment-to-moment speeds for control and hippocampal animals under light (top panels) and dark (middle panels) conditions. The bottom panel of Fig. 9 plots the proportion of the exploratory trip composed of the homeward segment. The ANOVA conducted on each group's mean proportion of the exploratory trip composed of the homeward trip segment under light and dark conditions revealed a significant effect of group ($F_{1,13} = 10.65$, $P < 0.05$), while the effect of testing condition and the Group by Testing condition interaction were not significant.

Each panel of Fig. 10 plots the outward and homeward trip kinematics for three exploratory trips from an animal under light (white) and dark (grey) testing conditions. The open circles indicate the location of maximal speed for an exploratory trip segment. It is important to note that the controls' homeward mean and spread for the location of the maximal speed do not change under light and dark conditions. In contrast, the hippocampals' homeward mean and spread for the location of the maximal speed depend on the testing condition.

The top panel of Fig. 11 presents the average standard deviation observed in the peak location of the homeward trip segment under light and dark conditions. The ANOVA conducted on the standard deviation of peak locations revealed significant effects of group ($F_{1,13} = 5.36$, $P < 0.05$), testing condition ($F_{1,13} = 7.79$, $P < 0.05$), and Group–Testing Condition interaction ($F_{1,13} = 4.93$, $P < 0.05$). Post hoc tests revealed that the hippocampal animals had significantly more variability in peak location only under dark testing conditions (Tukey LSD). The bottom panel of Fig. 11 plots each group's mean peak speed location for the homeward trip segments under light and dark conditions. Considering that group differences in variability of peak location depended on the testing condition, we elected to evaluate group differences in mean peak location separately for each testing condition. Under light conditions, the hippocampal animals' peaks in speed were shifted significantly closer to the home base relative to the control animals ($t_{13} = 3.24$, $P < 0.05$). Groups were not significantly different in the mean peak location under dark conditions.

Discussion

This study investigated the role of the hippocampus in the dead reckoning based navigation displayed by rats spontaneously exploring a novel environment in the light and in the dark. Beginning with the first outward trip, both control and hippocampal animals' exploratory trips were organized into outward and homeward trip segments. Outward trip segments were circuitous sets of slow progressions

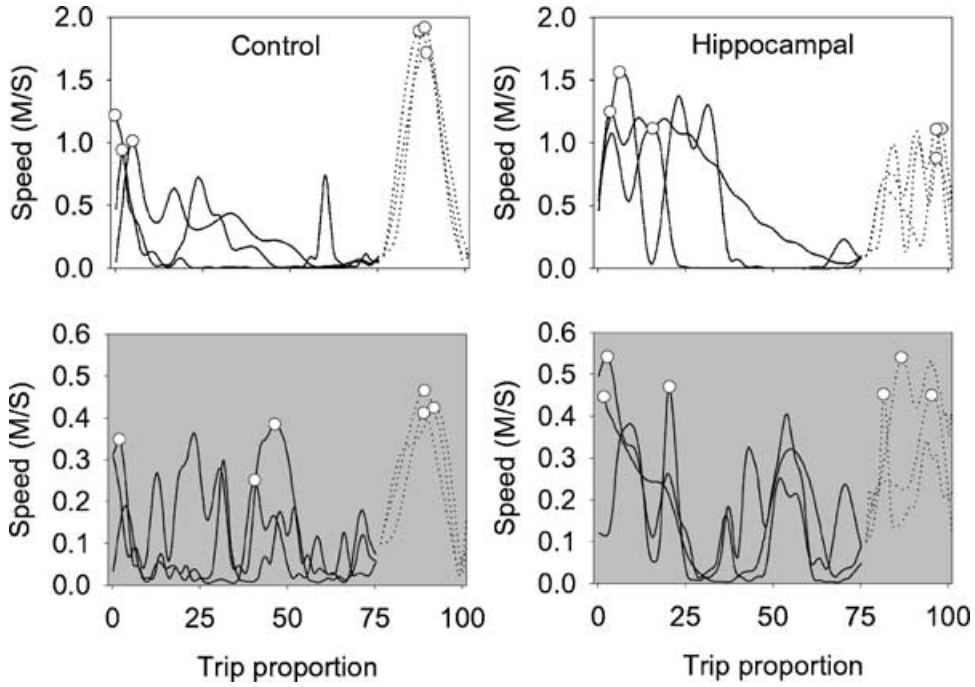

FIG. 10. The top panels plot three normalized exploratory trips from a control (left) and hippocampal (right) animal. The solid lines represent the outward trip segments; the dotted lines represent the homeward trip segments. The white dots indicate the location of the peak in speed for each exploratory trip's outward and homeward segments.

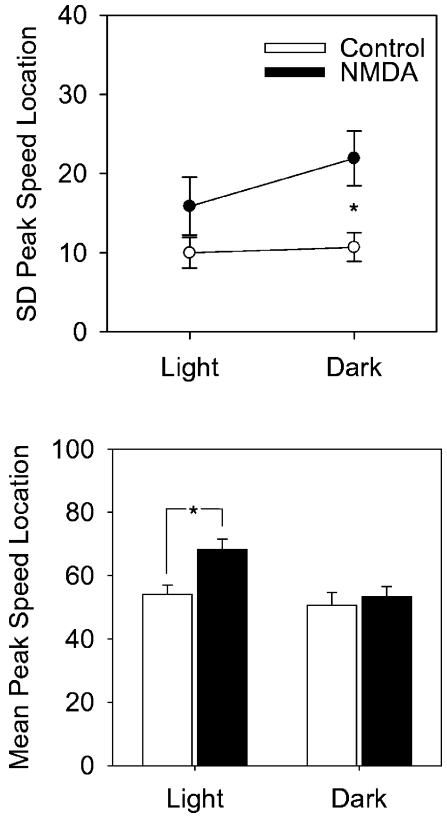

FIG. 11. The top panel plots the homeward standard deviation in peak speed location (mean and standard error) for control (left) and hippocampal (right) groups under light and dark testing conditions. The bottom panel plots the homeward mean in peak speed location (mean and standard error) for control (left) and hippocampal (right) groups under light and dark testing conditions ($^{*}P < 0.05$).

punctuated by stops. In control rats, homeward trip segments were noncircuitous paths directed toward the home base, associated with an increase and then a decrease in speed, with the velocity peak occurring at the midpoint of the trip. In hippocampal rats, however, the topographic and kinematic components of the homeward trip segment were disrupted. The use of dead reckoning in spontaneous exploration suggests that dead reckoning is an unconditioned behaviour, and its disruption by selective hippocampal lesions provides evidence that the hippocampus proper is involved in dead reckoning.

Dead reckoning in control rats

The central finding of the present study is that beginning with the first exploratory trip in a novel light or dark environment, control animals made direct homeward trips with a temporal pattern in which the velocity peak occurred at the midpoint of the trip irrespective of trip distance. Thus, both the temporal characteristic of the trips as well as their independence from visual guidance suggest that the homeward trips were internally produced, presumably from calculations derived from self-movement cues generated during the outward trip. The behaviour of the control rats is consistent with the idea that the homeward trips represent a form of dead reckoning.

Previous studies have reported that rats and other rodents make direct homeward trips when foraging for food (Maaswinkel & Whishaw, 1999) or when exploring a familiar environment (Wallace *et al*., 2002c), but the strengths of the present findings are that: (i) the direct homeward trips occur on a rat's first exploration of a novel environment and (ii) they are temporally paced. The temporal pacing is reminiscent of work examining the kinematics of planar reaching movements in humans that are executed independent of visual cues (Gordon *et al*., 1994). For example, Gordon *et al*. (1994) demonstrated that hand trajectory velocities were identical across varying distances when normalized for magnitude and time. Such velocity profiles suggest that subjects are generating movements based on internal rather than external guidance. Thus, the temporal patterns of the

homeward trip contribute to the suggestion that this portion of exploration is guided by dead reckoning.

Although the temporal pacing of the homeward trips suggests that they are based on calculations derived from self-movement cues generated on the outward trip, the complement of cues that are used and the way that the dead reckoning calculations are made is not known. It is likely that vestibular cues are important because the linear and angular acceleration information, generated by stimulation of the vestibular system, contributes to a rat's ability to dead reckon during foraging behaviour. Vestibular damage as the result of intratympanic injections of arsenic acid produced a deficit in carrying food directly home when visual cues were removed or conflicted with previous experience (Wallace *et al*., 2002a).

The unconditioned behaviour of exploration provides an ethologically valid and potent behaviour for examining spatial guidance, but it is none-the-less a complex behaviour. Therefore, we thought it important to demonstrate that the control rats' behaviours were not guided by surface or directional cues, especially in the dark condition. We found it unlikely that homing was guided by olfactory cues. First, the rats' outward trips were circuitous and thus it seems improbable that a direct trail would be present to guide homeward movements. Second, it seems unlikely that the rats could smell the home base because the homeward trips commenced at a distance beyond which the animals would be able to detect home base cues. Third, the velocity profiles of rats that are tracking a scent and rats that are homing are dissimilar. Fourth, tracking rats have a distinctive posture in which the nose is down and the back arched (Whishaw & Gorny, 1999; Wallace *et al*., 2002b), and this posture was not displayed by the homing control rats. It is possible that auditory or geomagnetic cues could provide the rats with homeward guidance, but the test environment provided no constant sound sources, and there is no evidence that rats use geomagnetic cues for guidance in small environments (Cain *et al*., 1997). It is also unlikely that visual cues were essential, as homing was displayed by the rats in the dark test, and the infrared lighting used for filming this test lies beyond the spectral sensitivity of the rat's retinal receptors (Neitz & Jacobs, 1986).

Absence of dead reckoning in hippocampal rats

The topography and kinematics observed across outward trip segments from hippocampal rats were not significantly different from that observed in controls. These observations in hippocampal rats are consistent with work demonstrating that hippocampal lesions spare an animal's ability to respond to proximal and distal external cues (Sutherland, 1985; Save *et al*., 1992; Whishaw *et al*., 1995; Gaffan *et al*., 2000; Mumby, 2001; Astur *et al*., 2002; Mumby *et al*., 2002) and so might display normal exploratory behaviour toward distal cues.

Hippocampal rats did not display the direct and temporal paced returns either in the light or dark. At first glance, the result that hippocampal damaged rats failed to return directly to the home base from a distance in light conditions (in which the home base was visible) may appear puzzling. Hippocampal animals are clearly able to return directly to a home base under light conditions after training, as is reported elsewhere (Maaswinkel *et al*., 1999). Presumably, under novel-light conditions, rats do not use distal cues to guide navigation and must rely on dead reckoning to return to a general location of the home base. Thus, we suggest that in the absence of dead reckoning, the ability to periodically make direct homeward trips is also disrupted.

The temporal pacing observed during hippocampal rats' homeward trip segments under novel-light and dark conditions also supports impaired dead reckoning with preserved proximal cue use. The hippocampal rats' peak in speed was reliably shifted towards the home base, relative to control animals, under novel-light conditions. The hippocampal rats accelerated as they approached the home base. Thereby, suggesting that the cues associated with the home base did control their behaviour, but only when they were close to the home base. While the hippocampal and control rats' mean peak locations were not different under dark conditions, hippocampal rats' variability in the location of the peak in speed was significantly greater under dark conditions relative to control rats. This observation indicates that under dark conditions, behaviour is no longer controlled by the cues associated with the home base. These results showing the absence of normal temporal pacing of the homeward trip segment supports the suggestion that the hippocampal animals are not using dead reckoning based navigation.

If dead reckoning is dependent upon vestibular signals, then it follows that the hippocampus must be involved in processing vestibular signals. Indirect support for the role of the hippocampus in vestibular-based computations comes from studies demonstrating that changes in vestibular system function produce changes in the hippocampus. Zheng *et al*. (2001) demonstrate that damage to the vestibular system produces changes in nitric oxide synthase in the rat hippocampus. In addition, temporary inactivation of the vestibular system by intratympanic injections of tetrodotoxin disrupts the firing patterns of place and head direction cells (Stackman *et al*., 2002). Together, these results support the hypothesis that the hippocampus processes vestibular information that may contribute to dead reckoning.

We were concerned that the failure of the hippocampal rats to make direct return trips from a distance may have been due to a general disruption of exploratory behaviour rather than a specific deficit in dead reckoning. For example, we noted that the hippocampal rats tended to confine much of their return trip to the periphery of the table, and so it seemed possible that they were displaying a 'release' of thigmotaxic behaviour, which may have interfered with their ability to otherwise display dead reckoning. This hypothesis was not supported. First, if hippocampal rats are excessively thigmotaxic, they should be less likely to exit the confines of the home base, but there were no differences between control and hippocampal rats in exits. Second, although hippocampal rats did return to the home base around the periphery of the table, the total time spent around the periphery did not differ between the control and hippocampal rats. In addition, when near the periphery, neither control nor hippocampal rats were in constant contact with the table edge. Rather, after contacting the edge and stopping, they walked away from the edge until they again encountered it. Third, other aspects of the pattern of exploration of the hippocampal rats were very similar to that of control rats, especially in that they made variously directed, circuitous outward trips, they made a number of stops, and the home base was the focal point of their behaviour. Thus, in terms of surface features, we were unable to detect obvious differences between the control and hippocampal rats, except for the profile of their homeward trips.

Caveats

We are aware that rats can use a variety of navigational strategies. In the present study we suggest that control rats use dead reckoning as a part of their normal exploratory behaviour in both the novel-light and dark conditions. It might be viewed as surprising that the rats would rely on dead reckoning in the light, when other guidance cues are available. This finding is nevertheless consistent with substantial literature on dead reckoning in ants (Wehner & Srinivasan, 1981), bees (Collett & Collett, 2000a, b), hamsters (Etienne *et al*., 1986), and rats (Whishaw & Maaswinkel, 1998). Given that dead reckoning is a normal aspect of navigation in the light in so many species of animals, it appears to be an 'action pattern' as the term is applied by ethologists

(Hinde, 1982). As such, the strategy would take precedence over other navigational strategies.

Understanding that dead reckoning takes the form of an action pattern may help explain its absence in the hippocampal rats. Even though they can navigate to a visual cue (Whishaw, 1998) or use the relational properties of distal cues (Whishaw & Tomie, 1997) they fail to do so from a distance within the context of their normal exploratory behaviour. It is important to note, nevertheless, that the hippocampal rats did orientate to the home base in the light, but did so only when they came quite close to it. This observation reinforces the notion that dead reckoning is a strategy designed to bring an animal from a distance to the proximity of the home base (Wehner & Srinivasan, 1981; Whishaw & Gorny, 1999) and that this strategy is affected by hippocampal damage.

Despite the persuasive evidence presented here and elsewhere (Maaswinkel *et al*., 1999) that the hippocampus can disrupt dead reckoning, we are not arguing that this is the only or even the primary function of the hippocampus. It is possible that the hippocampus is required for certain calculations related to spatial behaviour more generally, that the lesions disrupt the motivation to dead reckon, or even that hippocampal lesions disrupt some form of retrospective memory process that would allow an animal to calculate a first homeward trip. Although these alternate proposals could be examined in future research, we suggest that parsimony favours the more moderate conclusion that dead reckoning is a spatial behaviour shared by rats, and it can be disrupted by hippocampal lesions.

Conclusion

Although previous work using conditioning tasks has produced contradictory results concerning the role of the hippocampus in dead reckoning (Alyan & McNaughton, 1999; Maaswinkel *et al*., 1999), the present results make a more compelling case for its role in dead reckoning because the behaviour examined during exploration is an online unconditioned response, properties that are essential to a dead reckoning system. This result is also consistent with a body of research that demonstrates that dead reckoning is impaired after limbic system damage in food hoarding tasks and in exploratory tasks given in familiar environments (Whishaw & Maaswinkel, 1998; Wallace *et al*., 2002c). The topographical and kinematic reconstructions of the rats' exploratory behaviour demonstrate that rat exploration in a light or dark environment is highly structured. Both the topographical trip reconstructions and kinematics and topography of the homeward trip segment provide evidence that dead reckoning plays a critical role in trip organization. The results also indicate that hippocampal lesions disrupt the kinematics and topography of the homeward trip. These results provide further evidence that the hippocampus contributes to dead reckoning based navigation.

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