HEADED IN THE RIGHT DIRECTION: START POINT ORIENTATION AND DIRECTION LEARNING IN RATS

GENIEVE PECKFORD

Headed in the right direction: Start point orientation and direction learning in rats

by

Genieve Peckford

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Abstract

When trained to locate a hidden platform in a T-maze moved between two positions, rats appear to adopt a conditional strategy based on start point. To determine if location cues or orientation cues at the start point underlie this discrimination rats were trained from two maze positions to swim in a consistent direction from the choice point to the platform. When the maze was later moved to two new positions, rats required to make the same response based on start point orientation showed no disruption in performance while rats required to make the same response based on start point location did show an initial disruption in performance (Experiment 1). Animals explicitly trained to use start point location cues in Experiment 2 took significantly longer to solve a spatial task than rats trained explicitly to use orientation cues. When the start point location cues were masked, by making the room dark prior to placing the rats in the maze, performance did not deteriorate if rats were required to respond based on orientation of the start point but was disrupted if they were required to respond based on start point location cues (Experiment 3). This sense of direction requires exposure to the room cues to get oriented, as rats brought into an already darkened room (Experiment 4) were disrupted regardless of whether responses were tied to orientation cues or location cues. These findings are consistent with views of spatial learning that attribute a strong role to a rats' sense of direction. However, lesions to the anterior dorsal nucleus of the thalamus, a component of the head direction cell circuit, produced only transient deficits in direction learning in the water maze and small errors in heading on a foraging task.

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ADN: Anterior dorsal nucleus of the thalamus

AP: Anterior-Posterior

DTN: Dorsal tegmental nucleus

DV: Dorsal-Ventral

HD: Head direction

LMN: Lateral mammilary nucleus

ML: Medial-Lateral

NMDA: N-Methyl-D-Aspartate

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Headed in the right direction: Start point orientation and direction learning in rats

Knowledge of one's environment is essential for navigation. From locating food to returning home, most everyday tasks rely on some form of spatial information. Being able to navigate an environment is therefore crucial to an animal's survival. Navigation requires not only knowledge about location but also requires knowledge of directional heading (Taube, 2007). These two key components of navigation are well represented in a 'spatial' network that contains place cells, grid cells, and head direction (HD) cells (Fyhn, Molden, Witter, Moser, & Moser, 2004; Hafting, Fyhn, Molden, Moser, & Moser, 2005; Moser, Kropff, & Moser, 2008; Muller, 1996; O'Keefe & Dostrovsky, 1971; Taube, 2007; Taube, Muller, & Ranck, 1990).

The experimental study of spatial navigation in the brain started over 40 years ago with the discovery of place cells (O'Keefe & Dostrovsky, 1971). O'Keefe and Dostrovsky's report of neurons in the rat hippocampus that fired in a location-specific manner led to the suggestion that the hippocampus was crucial to the formation of spatial maps (O'Keefe & Nadel, 1978). Place cells show spatial selectivity, firing primarily within a small area called a place field (see Muller, 1996 for a review of place cell properties). Neighbouring cells fire in different locations so that the entire environment could be represented in the hippocampus (O'Keefe, 1976; Wilson & McNaughton, 1993). Place cells are strongly tied to distal sensory cues (O'Keefe & Conway, 1978; O'Keefe & Speakman, 1987) but continue to fire in the same location if salient landmarks are removed while an animal is in a familiar environment. Place fields are also influenced by geometric boundaries (O'Keefe & Burgess, 1996). If a boundary changes, such as a

rectangular space becoming larger, place fields will compensate by stretching in the extended direction.

Thirty years after the initial report, new studies revealed that place cells were part of a broader network for location. Another key component to this spatial circuit was the grid cell of the entorhinal cortex (Fyhn et al., 2004; Hafting et al., 2005). The hippocampus receives information from grid cells in layers II and III of the rat medial entorhinal cortex (Fyhn et al., 2004; Hafting et al., 2005). Like hippocampal place cells, these cells show spatially selective firing but unlike place cells they have multiple firing fields. These cells were termed grid cells due to the periodic triangular array, or grid, that covered the entire environment explored by the animal. Like place cells, grid cells are anchored to external landmarks and apparatus boundaries (Hafting et al., 2005). These cells continue to fire after removal of major landmarks, suggesting that grid cells are part of a generalized path integration based representation of spatial environments (Hafting et al., 2005).

As an animal moves around in its environment, it can keep track of its changing position by integrating linear and angular self motion, a process called path integration (Etienne & Jeffery, 2004; Maurer, 1998; Mittelstaedt & Mittelstaedt, 1980). Place specific firing in the hippocampus can be driven by self motion alone, but the activity is soon corrected against external landmarks (Gothard et al., 1996). While the hippocampus is no longer considered the site of the path integrator, the grid cells of the entorhinal cortex are a likely candidate. The persistence of grid fields after removal or replacement of landmarks suggests that self motion might be the primary source of information for the grid representation (see Moser et al., 2008 for review).

Place cells and grid cells may provide a means of perceiving and remembering our position in the environment, but to get from place to place probably requires heading information from HD cells (Kubie & Fenton, 2009; Moser et al., 2008; Muller, Bostock, Taube, & Kubie, 1994; Taube, 1998). HD cells fire when an animal's head is pointed in a particular direction (Taube et al., 1990; Taube & Bassett, 2003) and, unlike place cells or grid cells, are independent of the animal's behavior or location (Taube, 1995). The preferred firing direction of HD cells is dependent on both external cues (e.g., visual and auditory) and internal cues (e.g., vestibular and proprioceptive) (Taube & Bassett, 2003). These cells are prevalent in several areas throughout the brain (Taube & Bassett, 2003) and operate in a hierarchical scheme in order to process directional heading information (Bassett & Taube, 2001). Information travels from the dorsal tegmental nucleus (DTN) to the lateral mammilary nucleus (LMN), to the anterior dorsal nucleus of the thalamus (ADN), and on to the postsubiculum (dorsal presubiculum). Presubiculum axons terminate in layers III and V of the medial entorhinal cortex (Witter & Amaral, 2004). It has been suggested that the HD cells may control grid field orientation (see Moser et al., 2008 review). Direction information then travels from the entorhinal cortex (Van Groen & Wyss, 1990) to the hippocampus. When information reaches the hippocampus, it can be integrated with information about the animal's location and this could provide the animal with a sense of spatial orientation in the environment (see Taube, 2007 for review).

As outlined above, spatial representation engages a wide brain circuit that includes place cells, grid cells and HD cells (see Moser et al., 2008 and Taube, 2007 for reviews). Of the three cell types discussed, HD cells appear to be the first to develop

(Langston et al., 2010; Wills et al., 2010). HD cells show adult-like directional firing on the first exposure to an environment, as early as postnatal day 16. Place cells and grid cells are also present at this time but continue to develop. This presence of adult-like firing of HD cells in the pre- and parasubiculum at such an early stage of development has led to speculation that HD cell signals are instrumental in setting up networks for place and grid cells to function correctly (Langston et al., 2010).

The behavioral study of spatial navigation in rodents has historically used mazes where the animal must make a particular response at a choice point (or series of choice points) to locate a reinforcer (Blodgett, McCutchan, & Mathews, 1949; Tolman, Ritchie, & Kalish, 1946). While much of the early work focused on which of two strategies, response or place, was dominant (see Restle, 1957 for review), these studies inadvertently demonstrated the key role of direction to spatial learning. In one particular study, Tolman et al. (1946) explicitly trained rats on a place problem or a response problem. Response rats were required to make a right turn when started at both the north and south arms of a plus maze. Place rats were required to make a right turn from the south arm but a left turn from the north arm to end up in the same place relative to extra-maze cues. Tolman et al. (1946) found that rats trained on the place problem learned the task more quickly than rats trained on the response problem, leading the authors to conclude that place learning was simpler and more primitive than response learning. In an early review of these types of maze studies Restle (1957) concluded that place strategies dominated in environments with an abundance of extra-maze cues. Later studies revealed that place learning occurred early in training but that rats switched to response learning with continued training (Packard & McGaugh, 1996).

Many of the earlier dissociations between place and response learning (i.e., Tolman et al., 1946) actually confounded place learning with direction learning since on the place task animals traveled in a common direction from the choice point to the goal on all trials (Blodgett et al., 1949). Blodgett et al. (1949) explicitly separated place learning from direction learning. Rather than rotating the T-maze 180° between trials, as in early studies of place learning, Blodgett & colleagues shifted the maze to the left (or right; see Translation problem in Figure 1). The results showed that rats for which direction of reinforcement was common to the two maze positions made the fewest errors while rats that had place common to the two maze positions made the most errors. Blodgett et al. (1949) concluded that place information provides a negligible contribution to navigation on a simple T-maze in comparison to response and directional information. They suggested that the findings of Tolman et al. (1946) of superior place learning might in fact have been due to direction learning.

Using an open field and a T-maze, Skinner et al. (2003) replicated the finding that direction learning was easier than place learning originally reported by Blodgett et al. (1949). Skinner et al. (2003) also suggested that the poor place learning might be due to the fact that the start points at the two maze positions were less distinguishable in the place group than in the response and direction groups. In the place group the maze was translated (shifted left or right) such that the start position for the rats was on the same side of the room and the view from the maze probably contained many overlapping features (see Translation in Figure 1). In contrast, the start positions for rats in both the direction and response groups were on different sides of the room (180° apart). In an

attempt to address the issue of similar start points in the place problem, the authors designed a new place task where the start points were more distinct but response and direction strategies were not confounded with the place strategy (see Rotation in Figure 1). Rats solved this new place problem as quickly as the response and direction tasks. Because rats had distinct start points in both tasks but the rotation rats solved the problem more quickly, the speculation was that start point orientation was more important than start point location. Skinner, Horne, Murphy, and Martin (2010) later provided a more direct test of this hypothesis. Rats were trained on a place problem on a single plus-maze that was rotated 90° between trials as in the typical Rotation problem. In this version of the place task the start point at the two maze positions was in the same location in the experimental room but the orientation of the maze was changed. Rats solved this task as readily as the Rotation task, outlined in Figure 1, suggesting that orientation of the start arm was more important than start point location.

Orientation, or directional heading, appears to be important to the solution of other spatial tasks as well. Wright et al. (2009) found that changing the orientation of a plus maze in the same room between response tasks facilitated response reversal learning to the same extent as a change in rooms. Rats have difficulty solving place problems if they are disoriented (Dudchenko, Goodridge, Seiterle, & Taube, 1997; Martin et al., 1997). Martin et al. (1997) found that carrying rats to the maze in opaque containers that were slowly rotated prevented the rats from finding food in a fixed location on a plus maze despite an abundance of distal cues.

In the current study, we investigated the influence of orientation cues to behavioural demonstrations of direction learning. The aim of Experiments 1 to 4 was to systemically determine the relative influence of orientation cues versus location cues at the start point. In Experiment 1, two groups of rats were trained to swim in a consistent direction to locate a hidden platform in a water T-maze positioned at two locations throughout training. The maze was then moved to two new locations and the platform was located in the same direction or the opposite direction. Rats in the Same Direction group could locate the platform in the second phase by making the same response based on initial heading/start point orientation as in the first phase. Rats in the Different Direction group could locate the platform in phase two by making the same response based on start point location as in phase one. In Experiment 2, separate groups of rats were explicitly trained to locate a hidden platform using either orientation cues or location cues at the start point. The findings from both experiments revealed that rats' performance was superior in conditions were orientation cues at the start point could be used. The importance of orientation cues was further supported in Experiment 3, where making the room dark prior to placing the rats in the maze masked start point location cues. The sense of direction that was used by rats in Experiment 3 requires exposure to the room cues to get oriented, as rats brought into an already darkened room in Experiment 4 were disrupted regardless of whether responses were tied to orientation cues or location cues. In Experiment 5, we investigated the influence of the HD cell circuit to behavioural demonstrations of direction learning by comparing the performance of rats with bilateral lesions to the ADN and sham controls on two tasks. First, we trained ADN- and Sham-lesioned rats on the direction task in the water maze used in the earlier

experiments. As there was only a transient deficit on this task by the lesioned rats, we then trained them on a food foraging task where directional heading is thought to be important (Bett, Wood, & Dudchenko, 2012; Frohardt et al., 2006; Whishaw & Tomie 1997).

Experiment 1

The basic pattern of impaired performance on the translation place task relative to the rotation place task has been demonstrated using an open field maze in rats (Skinner et al., 2003) and gerbils (Walsh, Harley, Corbett, Skinner & Martin, 2008), using plus mazes in rats (Skinner et al., 2003; 2010) and using a water T-maze in rats (Whyte et al., 2009) and mice (Skinner et al., 2009). More recently, we have shown that rats trained to criterion on the Rotation problem are severely disrupted when switched to the Translation problem, despite the fact that the platform location and one of the maze positions relative to extramaze cues were identical across the two tasks (Peckford, McRae, Thorpe, Martin, & Skinner, in press). This finding demonstrates that distal cues at the goal location do not help rats solve place problems and suggests that cues at the start point might be more important. These start point cues could be distal visual cues at the start point location that control responding at the choice point (i.e., if started at location A, turn right; if started at B, turn left). Alternatively, the rats could use their heading in the start arm, or start point orientation, to control responses at the choice point (i.e., if heading north, make a right turn; if heading south, make a left turn). The aim of this experiment was to determine if cues at the start point are important to the solution of other spatial tasks, namely direction learning. To assess whether cues associated with the start point location or the start point orientation are more important for direction learning, two groups of rats were trained on a

direction problem (Figure 2). Rats were trained to swim in a particular direction from two maze positions for 80 trials and then the maze was shifted to two new locations in the experimental room with the physical location of the start points overlapping. One group was trained to go in the same direction as in the original task (see Same Direction in Figure 2). The second group was trained to go in a direction that was opposite of original training (see Different Direction in Figure 2). Rats in the Same Direction group had to make the same response based on initial heading/start point orientation but a different response based on start point location. Rats in the Different Direction group had to make the opposite response based on initial heading/start point orientation but the same response based on start point location. It was expected that the rats that had consistent orientation cues between training conditions (i.e., Same Direction) would show savings relative to the rats that did not have consistent orientation cues (i.e., Different Direction).

Method

Subjects

Sixteen naïve, male, Long-Evans rats, obtained from Charles River Company (St. Constant, Quebec, Canada) and weighing 220-246 g at the start of the experiment, were used. The rats were singly housed in clear plastic cages (45 x 25 x21 cm) with metal lids in a temperature-controlled room (approximately 20°C) and maintained on a 12-hour light and dark cycle with lights on at 0800. All rats had continuous access to food and water in their home cages. All procedures used in this experiment were approved by Memorial University's Institutional Committee on Animal Care and followed the Canadian Council on Animal Care guidelines.

Apparatus and Materials

The apparatus consisted of a plus maze inserted into a circular metal tank (120 cm in diameter and 31 cm high). Plexiglas walls extended 31 cm above the metal tank. The plus maze was also made of Plexiglas and extended 31 cm above the metal tank. The arms of the maze were 11.5 cm wide and 52.5 cm long. The whole apparatus was placed on a metal frame with wheels. The plus maze was converted into a T-maze using a section of clear Plexiglas which was snapped to the plus maze using butterfly clips, obstructing physical access to the arm opposite the start arm, but not obstructing visual access. The water level was kept approximately 2.5 cm below the top of the metal tank. The water temperature was equilibrated with the room temperature (approximately 20° C) and was made opaque by adding approximately 250 ml of non-toxic white Tempera paint (Rich Art Color Company, Northvale, NJ). The escape platform (11.5 cm in diameter and 26.5 cm high) was constructed from white plumbing tubing filled with sand and attached to a Plexiglas base for stability. The platform was placed approximately 1 to 2 cm below the surface of the water.

The training room (528 x 464 x 267 cm) had windows covering the north wall, and two doors, one located on the south wall and one on the east wall. In the southwest corner of the room was a sink; shelves lined the west wall, and half of the east wall. The southeast corner of the room contained stacked boxes and a coat rack, as well as two garbage cans. Animal cages were arranged on a table below the shelving on the west wall.

Procedure

The sixteen rats were trained to swim in a consistent direction (half went west, half went east) to locate the hidden underwater platform from two maze positions for 80 trials. Between trials, the maze was translated and the start point was rotated 180° as illustrated in Figure 2 (top). Upon completion of the first 80 training trials, the rats were divided into two groups (n = 8/group) and were trained from two novel maze positions for 40 additional trials. The two new maze positions were in different locations in the experimental room; however, the physical locations of the start points were the same. This meant that, at any start point, the orientation of the rat was reversed while the physical location of the rat in the experimental room remained the same. One group was trained to go in the same direction as in the original task (see Same Direction in Figure 2). In order to be successful when the maze was moved, these rats had to make the same response based on initial heading or start point orientation, but a different response based on start point location. For example, rats in the Same Direction group that made a right turn from A and a left turn from C during initial training (shown in white) were required to make a left turn when started at A and a right turn when started at C in the new problem (shown in grey). However, rats were required to make a right turn whenever they headed north and a left turn whenever they headed south. The second group was trained to go in a direction that was opposite of original training (see Different Direction in Figure 2). These rats had to make the opposite response based on initial heading but the same response based on start point location. For example, rats in the Different Direction group that made a right turn from A and a left turn from C during initial

training (shown in white) were still required to make a right turn when started at A and a left turn when started at C in the new problem (shown in grey). However, rats that initially made a right turn when headed north and a left turn when headed south, now had to make a left turn when headed north and a right turn when headed south.

The rats were brought into the training room one group at a time and placed individually in plastic holding cages that were similar to the home cages. The rats that were not being trained were left in their home cages on racks outside the experimental room. On each trial, a rat in its holding cage was carried in a counterclockwise direction to a chair positioned at the start arm. The rat was placed in the start arm facing the wall of the maze. The arms visited by the rat and the time (in seconds) taken to locate the hidden platform were recorded. A rat was considered to have made a choice when the body, minus the tail, was inside the arm. A correct trial was one where the rat entered the arm containing the platform, and successfully climbed onto the platform, without entry into other arms. Once the rat located the platform, it was allowed to sit there for 5 s before being removed from the maze. If the rat did not locate the platform in 60 s, it was placed on the platform by the experimenter. The experimenter remained at the start arm for the duration of the trial. Upon completion of the trial, the rat was placed back in the cage and carried back to the holding table in a clockwise direction and the next rat began its trial. The rats were given eight trials/day for a total of 120 trials. No more than two trials in a row were given from the same maze position.

Rats were given no-platform probe trials after 40, 80 and 120 trials. The probe trials lasted for 60 s and the rats' first choice and the time spent in each arm of the maze were recorded. Start positions for the probe trials were varied between the two training

positions, with the conditions that each rat was released from each start point at least once and on each probe there were equal numbers of rats released from the two start points.

Results

The sixteen rats were divided into two groups based on performance on the original direction problem. To confirm that there were no differences between groups a t-test was conducted and showed that the two groups reached the criterion of 18/20 correct trials in a similar number of trials ($t(14) = 0.19$, $p > .05$; Figure 3, top panel). A two-way (Group x Trial block) ANOVA on the number of trials correct over blocks of eight trials revealed a significant effect of Trial block ($F(14, 196) = 12.93$, $p < .05$) and a significant Group x Trial block interaction ($F(14, 196) = 2.57$, $p < .05$). Bonferroni posttests revealed that the two groups differed only on block 11, the first block after the switch in tasks ($p < .05$), reflecting deterioration in performance in the Different Direction group (see Figure 3, middle panel). A two-way (Group x Trial block) ANOVA on latency to reach the platform over blocks of eight trials revealed significant main effects of Group ($F(1, 196) = 5.47$, $p < .05$) and Trial block ($F(14, 196) = 11.91$, $p < .05$) and a significant Group x Trial block interaction ($F(14, 196) = 2.26$, $p < .05$). Bonferroni posttests revealed that the two groups differed only on block 11, the first block after the switch in tasks ($p < .05$), again reflecting deterioration in performance in the Different Direction group (see Figure 3, bottom panel).

After every 40 training trials the rats were given a no-platform probe trial. Separate two-way (Probe x Arm) ANOVAs on time spent in the correct and incorrect arms over probe trials were conducted for each group. For the Same Direction group this ANOVA revealed only a significant main effect of Arm ($F(1, 21) = 80.03$, $p < .05$),

confirming that the rats spent more time in the correct arm than in the incorrect arm across all probe trials (Figure 4, top panel). The Different Direction group also showed a preference for the arm associated with the correct direction on all probe trials (Figure 4, bottom panel). The ANOVA revealed only a significant main effect of Arm ($F(1, 21) = 80.01$, $p < .05$), confirming the rats spent more time in the correct arm over the incorrect arm across all probe trials.

The number of trials correct and the preference for the correct arm on the last probe trial showed that the disruption in performance in the Different Direction group was limited to the first block of eight trials and the new problem was acquired quickly. As casual observation indicated that the biggest disruption was on the first few trials, we further examined performance on the first block of eight trials by comparing rats' performance on each of two blocks of four trials to chance performance. We also divided the last block of eight trials before the switch into two blocks of four trials and compared rats' performance to chance as well. As can be seen in Figure 5A, rats in the Same Direction group performed better than chance on all four blocks (lowest $t(7) = 3.86$, $p < .05$). For the Different Direction group, performance did not differ from chance on the first four trials after the switch in tasks ($t(7) = -1.53$, $p > .05$) but was better than chance on all other blocks (smallest $t(7) = 7.0$, $p < .05$). Thus, the disruption in performance after the switch in tasks was limited to the first four trials.

Discussion

The findings from Experiment 1 suggest that, as with place learning, start point orientation is important in the occurrence of direction learning. When rats trained on the direction task were switched to the new problem, the Same Direction group showed little

disruption in performance. In this group, rats had to make the same response based on start point orientation, or initial heading, as in the original task. Rats in the Different Direction group had to make the same response based on start point location. The performance of this group deteriorated when switched to the new problem. This suggests that a conditional discrimination based on start point orientation, not start point location, is a likely explanation for successful performance on the direction problem. This conditional argument assumes that rats learn to make a right turn when headed north and a left turn when headed south to locate the goal. Alternatively, rats could learn to travel east at the choice point to locate the goal. We favor the conditional argument, since it explains the superior performance of the Rotation place group over the Translation place group (see Figure 1), where the goal is located in two different directions from the choice point (Skinner et al., 2003; Whyte et al., 2009). The number of trials correct and the preference for the correct arm on the last probe trial showed that the disruption in performance in the Different Direction group was limited to the early trials and the new problem was acquired quickly. This could indicate that rats in the Different Direction group learned a direction reversal where they quickly learned to make the opposite response based on initial heading (as in Wright et al., 2009). Alternatively, the rats may have learned to make responses based on location cues in the second phase.

Experiment 2

Experiment 1 revealed deterioration in performance of rats in the Different Direction group when switched to the new problem. This deficit was only apparent in the four trials immediately following the switch and performance on the new problem improved quickly. This rapid improvement could indicate that the rats learned a direction

reversal using orientation cues. It is also possible that the rats used location cues at the start point to control performance in the second task. Such rapid switching to the use of location cues would indicate that rats had some knowledge of these cues in the earlier task but it was overshadowed by the orientation cues at the start point. This seems unlikely given the earlier reports of poor learning on the translation task (Peckford et al., in press; Skinner et al., 2003). However, there are always a subset of rats that do learn the translation task (Peckford et al., in press) and they presumably do so by using location cues at the start point since orientation cues are identical across the two maze positions.

In Experiment 2 we trained two groups of rats using the four maze positions used in the previous experiment (see Figure 2). However, all four trial types were interleaved in the same phase rather than presenting them in two different phases as in Experiment 1. Thus, one group could use orientation of the start arm to locate the platform (see Orientation Cues in Figure 2). For the other group, the platform could be located by using location cues at the start point to control responses (see Location Cues in Figure 2). Hence, interleaving the trials is a more stringent test of whether rats can use location cues at the start point to solve spatial problems. Based on previous findings (i.e., current Experiment 1; Skinner et al., 2010) it was predicted that the Orientation Cues group would solve the task more quickly than rats in the Location Cues group.

Method

Subjects

Seventeen naïve, male, Long-Evans rats, obtained from Charles River Company (St. Constant, Quebec, Canada), and weighing 227-313 g at the start of the experiment, were used. The rats were maintained as in the previous experiment.

Apparatus and Materials

The water maze apparatus and experimental room were the same as in the previous experiment.

Procedure

The seventeen rats were trained to locate a hidden platform in a water maze positioned at four different locations in the experimental room. The maze positions were the same as those used in Experiment 1 but the four trial types were interleaved rather than presented in two distinct phases. The physical location of the start points overlapped in two maze positions, which created two start points at the four maze locations (see Figure 2). In order to complete the task, the Orientation Cues group (n=9) had to make a response based on start point orientation (i.e., turn right when headed north; turn left when headed south). Rats in the Location Cues group (n=8) had to make a response based on start point location to successfully complete the task (i.e., turn right from A; turn left from C).

Due to experimenter error, one rat was stopped before the criterion of 18/20 correct trials was reached. The criterion was then reduced to 16/20 trials in order to include the data from this rat. The rats were given eight trials/day and training was

stopped at 168 trials for those rats that did not meet criterion. All other details of the training procedure were identical to Experiment 1.

Results

All rats in the Orientation Cues group and 7/8 rats in the Location Cues group reached criterion in the 168 trials given. However, the Orientation Cues group reached the criterion of 16/20 correct trials in fewer trials than the Location Cues group ($t(15) = 3.48$, $p < .05$; Figure 6). In fact, the Location Cues group took at least twice as many trials as the Orientation Cues group to solve the task.

Discussion

Rats trained to make a response based on start point orientation performed better than rats trained to make a response based on start point location. Rats in the Location Cues required twice as many trials to learn the task suggesting that while start point location discrimination is possible with extensive training, a conditional discrimination based on start point orientation is a much simpler task. These results suggest that it is unlikely that rats use location cues at the start point to control responding at the choice point, particularly if orientation cues are available.

It remains possible that when rats face two different orientations from the same start point, there are different location cues available. Thus rats in both the Orientation Cues group and the Location Cues group could have learned responses that were tied to four start points (start point A when facing North, A when facing South, start point C when facing North and C when facing South). However, given such an explanation, it is not clear why the two groups were so different during acquisition. In the next experiment we tried to mask location cues at the start point by testing rats in the dark.

Experiment 3

The previous experiments were conducted to assess whether distal visual cues at the start point could control responding at the choice point (i.e., turn right from start point A, turn left from start point B) or alternatively, whether rats use their heading in the start arm to control responses at the choice point (i.e., if heading north, make a right turn; if heading south, make a left turn). Since rats showed less disruption in the Same Direction condition than in the Different Direction condition with the switch in task (Experiment 1), we suggested that they were using orientation cues, not location cues, to control responses at the choice point. However, it remains possible that rats could have used location cues at the start point if they had learned to swim in a particular direction based on those location cues (i.e., if at A, go East; if at C, go East). Rats that had used this strategy would have also shown less disruption in the Same Direction condition than in the Different Direction condition. Interestingly, only rats in the Orientation Cues group from Experiment 2 had this strategy available to them, which might explain why this group was better than the Location Cues group. A better test to distinguish between rats' use of location cues and orientation cues at the start point might be to conduct test trials in the dark.

In Experiment 3 rats were trained on the direction problem in a well-lit room. After this initial training, the maze was moved to two new locations and one group of rats was trained to swim in the same direction and a second group was trained to swim in the opposite direction, as in Experiment 1. For these trials the lights were turned out before the rat was carried to the maze, thus removing location cues at the start point. To ensure that the rats were exposed to distal cues in the room, and that they were not disoriented,

the lights were on when the rats were brought into the room and during part of the inter-trial intervals.

Method

Subjects

Sixteen male, Long-Evans rats, obtained from Charles River Company (St. Constant, Quebec, Canada), and weighing 300-350 g at the start of the experiment, were used. The rats were maintained as in the previous experiment. The rats had been previously trained on a response reversal task in black and white boxes.

Apparatus and Materials

The water maze apparatus and experimental room were the same as in the previous experiments, with the exceptions noted below. All of the doors, windows, electrical outlets and any other source of light were covered with tinfoil. During the trials conducted in the dark, a radio playing static was placed under the maze and a metal receptacle lined with paper towels soaked with 10 ml vanilla extract was placed at the end of each of the four arms. In order to view the rats' behavior in the dark, the experimenter wore night vision goggles (Rigel 3250) obtained from Rigel Optics (DeWitt, IA).

Procedure

The sixteen rats were trained to swim in a consistent direction (half went west, half went east) to locate the hidden platform from two maze positions, as in Experiment 1. This initial training on the direction problem was conducted in a well-lit room and ended when the rats reached a criterion of 18/20 correct trials. All rats received 16 reminder trials (over two days) on the original direction problem before the switch. Since

rats varied on the number of trials required to reach criterion, these extra trials ensured the rats' performance was comparable just prior to the test.

Upon completion of initial training and the reminder trials the rats in the two conditions (n=8) were matched based on the number of trials to reach criterion (Same Direction: 34.88 (±5.29); Different: 34.13 (± 4.74)). As in Experiment 1, the maze was moved to two new locations and one group of rats was trained to swim in the same direction and the second group was trained to swim in the opposite direction to locate the hidden platform. The rats were brought into the room in groups of four with the lights on. The lights were turned out just prior to the rat being carried to the maze. The lights were turned back on briefly (10-20s) when the rat was placed back on the holding table until the next rat was carried to the maze. The darkness should have prevented the rats from using visual cues at the start location once they reached the maze. The radio and vanilla scent were used to mask any auditory or olfactory cues associated with the start location. The rats were given 40 trials using this procedure (eight trials per days for five days). Four of the 16 rats did not reach the criterion of 18/20 correct trials in the 40 trials given. At the end of the 40 trials, the rats were given a second test in the dark with the maze moved back to the original two training locations. Half the rats in each group were trained to go in the same direction and the other half were trained to go in the opposite direction. The rats were assigned to conditions (n=8) based on the number of trials correct in the final eight trials (Same Direction: 7.00 (± 0.50); Different Direction: 7.125 (± 0.40)).

Results

The data of interest include the trials immediately preceding and following the switch in task (i.e., change in maze positions and lighting conditions). As in Experiment 1 we examined performance on the last eight trials before the switch and the first eight trials after the switch by comparing performance in each four-trial block to chance performance. When the maze was moved, and the rats were switched from light to dark conditions, the performance of the Same Direction rats was better than chance on all four blocks (lowest $t(7) = 2.65$, $p < .05$; Figure 5B). For the Different Direction group, performance did not differ from chance on the first four trials after the switch ($t(7) = 0.55$, $p > .05$) but by the second block of four trials the difference approached significance ($t(7) = 2.2$, $p = .06$). Thus, in the dark, as in the light in Experiment 1, rats in the Different Direction group were more disrupted by the switch in task.

When the maze positions were switched after training in the dark and the rats were tested in the dark, rats in the Same Direction condition were better than chance on the two blocks before the switch and the two blocks after the switch (smallest $t(7) = 3.99$, $p < .05$; Figure 5C). Rats in the Different Direction group did not differ from chance in the first four trials after the switch ($t(7) = -1.36$, $p > .05$), but were better than chance on all other blocks (smallest $t(7) = 5.23$, $p < .05$).

Discussion

The findings from Experiment 3 suggest that rats do not use location cues at the start point to control responses (i.e., turn right, or go east) at the choice point. Since the lights were turned out prior to placing the rats in the maze on each trial after the switch in task, the rats could not have identified the start location using distal visual cues. Rats that

had the platform in the opposite direction from previous training performed at chance levels when the task was switched and the lights were turned off while rats in the Same Direction group continued to perform at above chance levels after these changes. Because the lights were turned on when the rats were initially brought into the experimental room and briefly during the inter-trial intervals, rats should have been able to get oriented by taking visual fixes during these periods. This would have enabled the rats to identify when they were swimming north (or south) and to make a response at the choice point based on their heading in the start arm. If this explanation is correct, then depriving the rats of the opportunity to take these visual fixes should impair performance in both conditions since rats should be unable to identify the direction in which they are swimming.

Experiment 4

Experiment 3 revealed that rats trained to swim in the same direction, or make the same response based on start point orientation, performed better than rats that were trained to swim in a different direction when switched to novel maze positions in the dark. We suggest that location cues at the start point cannot control performance on trials conducted in the dark, but that rats' heading in the start arm controls performance at the choice point. The rats in the previous experiment were able to maintain their orientation in the darkened room because of periods of light during the inter-trial intervals. In Experiment 4, we performed the same manipulation but the experimental room remained darkened throughout the second phase of the experiment. As before, the initial direction training was conducted in a well-lit room. Once the rats reached criterion, the maze was moved to two new locations and training was conducted in the dark. Half the rats were

trained to swim in the same direction as in initial training, thus making the same response based on start point orientation, and the other half were trained to swim in the opposite direction.

Method

Subjects

Fourteen male, Long-Evans rats, obtained from Charles River Company (St. Constant, Quebec, Canada), and weighing 289-389 g at the start of the experiment, were used. The rats were maintained as in the previous experiment. The rats had been previously trained on a response reversal task in black and white boxes.

Apparatus and Materials

The water maze apparatus and experimental room were the same as in the previous experiments.

Procedure

The fourteen rats were trained to swim in a consistent direction (half went west, half went east) to locate the hidden platform from two maze positions, as in Experiment 3. This initial training on the direction problem was conducted in a well-lit room and ended when the rats reached a criterion of 18/20 correct trials. All rats received eight reminder trials on the original direction problem before advancing to the dark trials. Since rats varied on the number of trials required to reach criterion, these extra trials ensured the rats' performance was comparable prior to the test.

Upon completion of initial training and the reminder trials the rats in the two groups (n=7) were matched based on the number of trials to reach criterion (Same

Direction: 33.29 (±7.48); Different Direction: 35.14 (± 4.31)). As in Experiments 1 and 3, the maze was moved to two new locations and one group of rats was trained to swim in the same direction and the second group was trained to swim in the opposite direction to locate the hidden platform. The lights were turned off before the rats were brought into the room and all training was conducted in the dark. The rats were given eight trials in one day.

Results

Once again, the data of interest are the trials immediately preceding and following the change in lighting conditions and maze positions. As in the previous experiments, rats in the Different Direction group did not differ from chance on the first block of four trials after the switch ($t(6) = 0$, $p = 1.0$; Figure 5D), but were better than chance on all other blocks (smallest $t(6) = 2.50$, $p < .05$). Unlike in the earlier experiments, rats in the Same Direction group also did not differ from chance on the first four trials after the switch ($t(6) = -0.31$, $p > .05$), but were better than chance on all other blocks (lowest $t(6) = 3.58$, $p < .05$).

Discussion

The results from Experiment 4 suggest that rats need some exposure to the visual cues of the room to maintain a sense of orientation that was used to solve the problem. Rats trained in the dark performed at chance levels when the task was switched regardless of whether they were required to swim in the same or opposite direction. Even here, however, the disruption in performance was brief as rats performed significantly better than chance by the second block of four trials.

The pattern of results from Experiment 3 and 4 are consistent with reports that HD cells maintain their directional firing if the lights are turned out after rats have been placed in an environment (See Taube, 2007 for review) and with Mizumori and Williams' (1993) findings that HD cells in the lateral dorsal nucleus (LDN) were not directional when rats were placed in a darkened room. However, if the room was darkened after the rats had been exposed to the lit room, HD cells continued to show directional activity. HD cells in the LDN required 60s of exposure to the lit room in order for directional activity to occur in the dark. Rats in Experiment 4 quickly re-established their sense of direction, presumably due to the activity of HD cells in other areas that respond to idiothetic sensory inputs.

Experiment 5

Thus far, we have investigated the influence of orientation cues to behavioural demonstrations of direction learning. In the first four experiments we have tried to systemically determine the relative influence of orientation cues versus location cues at the start point. Given that orientation cues, or directional heading, in the start arm seems to control responses at the choice point, in Experiment 5 we investigated the influence of the HD cell circuit to this behavior. As mentioned earlier, HD cells fire when an animal's head is pointed in a particular direction (Taube & Bassett, 2003) and are independent of the animal's location (Taube, 1995). Information in the HD cell circuit travels from the DTN to the LMN, to the ADN, and on to the postsubiculum. Theoretically, lesions to any part of the HD cell circuit would eliminate the use of heading as a spatial strategy. The ADN contains the most abundant proportion of HD cells in the circuit, with 60% of the cells in the ADN firing in response to heading (Taube, 1995). Thus, we compared the

performance of rats with bilateral lesions to the ADN and sham controls on the direction task in the water maze used in earlier experiments. Since only minor deficits were seen in this task, we trained the rats on a direction reversal and on a separate task thought to be sensitive to directional heading; a food-foraging task (Whishaw & Tomie, 1997). The foraging task required the rats to leave a home cage located beneath the surface of a circular table, search for food and then return home. Rats can use distal cues or path integration to accurately return home (Maaswinkel &Whishaw, 1999). In the current experiment, rats had to leave one of three possible start locations to obtain a food reward placed in a variable location (i.e., one of three food cups).

Method

Subjects

Twenty-five naïve male Long-Evans rats were obtained from Charles River Company (St. Constant, Quebec, Canada) and weighed 303-357g prior to surgery. One week prior to the foraging task, rats were placed on a food deprivation schedule to maintain them at 85% of their free feeding weight. Otherwise, the rats were maintained as in the previous experiments.

Surgery

Sixteen of the rats were given neurotoxic lesions to the ADN and nine of the rats were used as sham controls. Five rats, three lesions and two shams, were sacrificed before data collection due to post surgery complications. Rats were anesthetized with an injection of a chloral hydrate solution (400mg/kg, i.p.). Lesions to the ADN were produced by injecting two 0.15 µl injections of 100 mM NMDA, mixed with saline, (Sigma Chemical, St. Louis, MO) into each hemisphere using a 1 µl syringe (Hamilton

Company, Reno, Nevada). The coordinates were modified from those used by Calton et al. (2003) (AP coordinates: 1.3 and 1.7 mm posterior to bregma; ML coordinates: ± 1.2; DV coordinates: 5.0 and 4.6 mm below brain surface). The .15 µl of NMDA was injected at a rate of 0.05 µl every 2 min. The needle was left in position for an additional 5 min after the injection to prevent backflow. Sham controls were anesthetized and placed in the stereotaxic instrument. These rats had holes drilled in the skull over the lesion sites; however, they did not receive injections. Rats were sutured and given a least one week to heal. All rats received topical anesthetic, as needed, following surgery (Xylocaine® Jelly 2%, ASTRA, Mississauga, ON).

Apparatus and Materials

The water maze apparatus and experimental room used for the direction task were the same as in the previous experiments.

For the foraging task, the training room (528 x 464 x 267 cm high) had a door on the north, west and south walls and a large window, covered in black curtains, on the east wall. There were cupboards, a counter, and a sink toward the southwest corner and a desk in the northeast corner. There was a metal rack (150 x 50 x 165 cm high), towards the north wall, where rats were held in their wire mesh cages between trials. The maze was situated in the center of the room.

The maze consisted of a large, wooden, black circular table (204 cm diameter) raised 75 cm above the floor. Eight holes (11.5 cm in diameter) were evenly distributed around the perimeter of the table and three food cups were placed on the surface (See Figure 7). A 1 g pellet (BioServ, Frenchtown, NJ) was placed in one of three small wooden cups on the surface of the table. Wire mesh cages (20 × 25 × 19 cm) were used

during training. The wire cages were attached on runners beneath a hole at the periphery of the table. Wooden blocks were placed in the wire cage at the beginning of a trial to allow for easy access to the table. Once the wire cage was placed beneath a hole, a rat could leave the cage by climbing up on the table and return to the cage by climbing back down.

Procedure

The 20 rats (13 lesions and 7 shams) were trained to swim in a consistent direction (half went east, half went west) to locate the hidden platform from two maze positions (see Figure 2). Rats were given eight trials/day until they reached a criterion of 18/20 correct trials. The water maze was then moved to two new positions and the direction of the hidden platform was then reversed (i.e., rats trained to travel East from maze positions A and C were now trained to travel West from maze positions B and D). The rats were given eight trials/day on this new problem until they reached a criterion of 18/20 correct trials.

Five days after the last rat reached criterion, rats began training on the foraging task. Rats were pre-trained in the housing room by being moved from their home cages to the wire mesh cages used in the experiment. Rats were placed beneath a hole that allowed access to a table, half of the apparatus used in the foraging task, upon which the 1 g pellets were placed. Initially, the pellets were placed near the opening of the home location allowing rats to merely reach to obtain the food. The pellets were gradually moved further away from the home location, requiring the rats to leave the hole to obtain the food. Finally, a single pellet was placed in a wooden cup, as in the foraging task, requiring the rats to travel to the cup to obtain the food. This procedure was repeated

daily until rats were consistently leaving the home location to obtain food from the cup.

Rats were trained to leave a hole and find a pellet that was located in one of three small wooden cups on the surface of the training table. The rats were given three trials/day for a total of 60 trials. A trial ended when the rat returned to the wire cage or at the end of 2 min if the animal remained in the wire cage. Upon completion of the trial, the training cage was removed from the training table and the rat was returned to the metal holding rack. The table was rotated 135° every third trial to control for scent tracking. Each hole a rat visited after retrieving the food pellet was recorded. A correct trial was one in which a rat successfully returned directly to the start hole after finding the food pellet (without visiting any other holes). An error occurred if the rat returned to any hole other than the start location. Errors were categorized into three types: memory, adjacent, and other errors. Memory errors occurred when a rat returned to a hole that had afforded escape in the past, but was not the correct start location. These errors are thought to occur due to proactive interference (Martin et al., 2011). An adjacent error was made when a rat returned to a hole adjacent to the start location. Adjacent errors are thought to occur when the path integrator under or over estimates the start location (Martin et al., 2011). These errors indicate impairment in heading. An 'other' error was made when rats returned to a hole that was neither adjacent to the home location nor afforded escape in the past. Upon completion of the 60 trials, the rats were given the same task but with a fixed start location that was one of the three locations used earlier. Rats were given three trials/day until they reached a criterion of 9/10 trials or reached a total of 40 trials.

Histology

Upon completion of the experiment, the rats were euthanized using carbon dioxide and decapitated. The brains were removed, submerged in 2-methylbutane (-70°C), and stored in a -70°C freezer. The brains were sectioned at 30-µm and nissl stained (cresyl violet) for verification of lesion sites.

Results

Two rats were excluded from the behavioural analysis because the ADN damage was restricted to one hemisphere. Of the remaining 11 rats, an absence of neurons in the ADN allowed for lesion verification. Damage extended to other areas including the fimbria ($n = 9$), dentate gyrus ($n = 7$), and the stria medullaris of the thalamus ($n = 4$). In most rats there was also enlargement of the lateral ventricles ($n = 8$) and the dorsal third ventricle ($n = 8$). The injections resulted in minor tissue damage (needle tracks) to the primary motor cortex ($n = 5$). Figure 8 is a picture of a typical ADN lesioned brain and a sham brain. Figure 9 shows the range of damage to the ADN.

ADN lesioned rats were impaired early in training on the direction task. A two-way ANOVA (Group x Phase) on the first block of eight trials revealed a significant effect of Group ($F(1,16) = 10.37$, $p < .05$), confirming that sham controls performed better than ADN lesioned rats early in training (see Figure 10, left panel). There was also a significant effect of Phase, ($F(1, 16) = 9.56$, $p < .05$), showing that rats in both groups performed better in phase 1 than in phase 2. The impairment seen in the ADN rats, on the first block of eight trials, diminished with continued training. A two-way ANOVA (Group x Phase) on trials to criterion did not show a significant effect of Group ($F(1,16) = 2.66$, $p > .05$; Figure 10, right panel. In other words, the ADN lesioned rats did not

differ from sham controls on the number of trials taken to reach the criterion of 18/20 correct trials.

On the foraging task, a two-way ANOVA (Group x Trial block) on total errors made revealed a significant effect of Group ($F(1, 16) = 7.92$, $p < .05$; Figure 11A), showing that ADN lesioned rats made more errors overall than sham controls. Further analyses were conducted on the types of errors made by the rats. A 2-way (Group x Error type) ANOVA revealed a significant main effect of Error type ($F(2, 36 = 51.27$, $p < .05$) and a significant interaction ($F(2, 36) = 7.36$, $p < .05$). Follow-up Bonferroni tests revealed that both groups made more memory errors than adjacent (*ps* <.05) and other errors (*ps* <.05), but only the ADN group made more adjacent errors than other errors ($p < .05$). A between groups t-test revealed that ADN lesioned rats made more adjacent hole errors than sham controls ($t(16) = 2.86$, $p < .05$; Figure 11B), suggesting an impairment in heading. A between groups t-test revealed that the groups did not differ in terms of trials to criterion from a fixed hole location ($t(16) = 1.15$, $p > .05$); however, five of the ADN lesioned rats did not reach criterion in the 40 trials given (Figure 11C) while all of the sham control rats were able to reach criterion. If rats were given more trials, a significant difference between the groups may have been found.

Discussion

Rats with lesions to the ADN made more errors than sham controls early in training on a direction task in a water T-maze, suggesting that the ADN is involved in heading. This deficit was transient as there was no difference between the groups in total trials to criterion. Once they acquired the initial task, the ADN rats were able to use directional information flexibly, as they were not impaired on the direction reversal in phase 2. On

the foraging task, ADN lesioned rats were more likely than sham controls to return to holes that were adjacent to the start location. In other words, ADN lesioned rats made errors in attempting to return in the direction of the start location. This finding is in line with previous research suggesting that the ADN is involved in heading (Blair, Cho, & Sharpe, 1999; Frohardt et al., 2006; Goodridge &Taube, 1997).

Proactive interference was evident in both ADN lesioned rats and sham control rats on the foraging task. After locating food, the rats tended to return to the correct location for that trial or to one of the two holes that had afforded escape on previous trials. This result was not unexpected as rats make more memory errors when variable start locations are used in combination with variable reward locations (Martin et al., 2011).

General Discussion

Rats appear to use heading, not location, as a conditional cue to control responses at the choice point. Experiment 1 showed that when the two direction groups were trained on a new direction problem, the Same Direction group showed no disruption in performance. In this group, rats had to make the same response based on start point orientation in the two tasks. Rats in the Different Direction group had to make the same response based on start point location in the two tasks. The performance of this group deteriorated when switched to the new problem. Similarly, in Experiment 2, rats in the Location Cues required twice as many trials to learn the task than the Orientation Cues group suggesting that while start point location discrimination is possible with extensive training, a conditional discrimination based on start point orientation is a much easier task.

In a further attempt to distinguish between rats' use of start point orientation and start point location cues we conducted trials in the dark. These experiments revealed that rats were using their sense of direction which they carried to the maze to solve the problem and that this sense of direction required exposure to the room. Rats trained in the light and tested in the dark were not disrupted when required to swim in the same direction provided they were allowed some visual access to the experimental room (Experiment 3). Rats carried into a darkened room were equally disrupted when required to swim in the same or a different direction (Experiment 4), suggesting they needed access to visual cues to get oriented. The rats were able to quickly re-establish a stable sense of direction in the absence of access to visual cues, since the behavioral disruption was transient. As no attempt was made to disorient the rats, other sources of orientation were available, including access to cues outside the experimental room, a stable path and point of entry into the experimental room, and a stable position for the holding table between trials (Hynes et al., 2000; Muir & Taube, 2002).

The present behavioral data are consistent with recent findings on the importance of directional heading to the solution of other spatial problems. Rats trained on the rotation place problem (illustrated in Figure 1) demonstrate superior performance relative to rats trained on the translation place problem across species and motivational conditions (Skinner et al., 2003; Walsh et al., 2008; Whyte et al., 2009). The deterioration in performance that occurred when rats that were successful on the rotation task were switched to the translation task (Peckford et al, in press) is also consistent with a solution based on a conditional discrimination of start point orientation. The importance of directional heading has also been demonstrated in response reversal learning (Wright et

al., 2009). Instability of the direction system, produced by disorientation, produces instability in hippocampal representations (Knierim et al., 1995) and impairs learning of spatial problems (Dudchenko et al., 1997; Martin et al., 1997).

One possible strategy for solving spatial problems of the type described here would be something similar to vector navigation described in insects (Collett, Collett, & Wehner, 1998; Wehner, Michel, & Antonsen, 1996). Ants use path integration to compute the distance and direction from their current location to the nest. This path integration vector, also called a global vector, is updated over the entire journey from nest to food source and back to the nest. In familiar environments, ants and other insects can navigate using visual landmarks and they store short local movement vectors, which are associated with landmarks. A local vector can be recalled at the appropriate landmark. Here landmarks tell the animal what to do rather than where they are (Collett & Collett, 2002). Our manipulations in the dark suggest that this view matching (or using the landmarks as signposts) is not critical; however, rats performed better if allowed some visual access to the room, suggesting they may have been using a global vector.

The present findings are consistent with models of spatial learning that emphasize path integration and an animal's sensitivity to direction (McNaughton et al., 1996; 2006) and recent theoretical work that has applied some of the principles from insect navigation to mammalian navigation (Kubie & Fenton, 2009). In Kubie & Fenton's model, heading-vector navigation is based on the activity of head direction cells (Taube & Burton, 1995; Taube et al., 1990) and not on the sun (birds; Bingman & Jones, 1994) or sky compass (insects; Wehner et al., 1996) as in other species. While the correlation between the activity of head direction cells and behavioral performance is not always strong (Muir &

Taube, 2002), the recent finding that HD cells develop earlier than place and grid cells (Langston et al., 2010; Wills et al., 2010) and could be important in setting up the spatial network has increased interest in their activity (Bett et al., 2012; Vann, 2011).

In the present study we have not disentangled the two ways that direction could play a role in spatial tasks. The conditional argument assumes that rats learn to make a right turn when headed north and a left turn when headed south to locate the goal. Alternatively, rats could learn to travel east at the choice point to locate the goal. The conditional direction strategy might also explain the weak correlations between head direction cell activity and behavioral choice that are often observed (Muir & Taube, 2002). Muir and Taube (2004), for example, trained rats along a fixed route from start to goal. After training, the original path was blocked and multiple paths were made available, one of which pointed directly to the goal as in the sunburst maze originally used by Tolman et al. (1946). The correct response based on direction of the goal would be the shortcut that pointed directly to the goal location. The correct response based on direction as a conditional cue is less clear since the rats would make a response based on their heading. The results obtained by Muir and Taube were ambiguous in that many rats did not take the shortest path even though the head direction system represented direction consistently. A conditional argument would suggest that rats might not pick the shortest path because their choice would be based on a conditional discrimination, in which, when headed in a particular direction, they make a particular response. So the selection of an arm would not be based on the direction that the goal was located but rather would be based on the direction that the rat was pointed.

If direction is important to the solution of spatial tasks, as we suggest, and HD cells represent an animal's sense of direction, then lesions of brain areas known to contain HD cells should disrupt performance on spatial tasks regardless of which of the two putative direction strategies an animal uses. Although rats with lesions to the ADN (Experiment 5) made more errors than sham controls early in training on a direction task in a water T-maze, suggesting a role for the ADN in direction learning, this deficit was transient. The two groups did not differ in total trials to criterion. Lesions to areas containing HD cells have been shown to produce spatial deficits (Bett et al., 2012; Frohardt et al., 2006; Liu, Jarrard, & Bilkey, 2001; Taube, Kesslak, & Cotman, 1992; Vann, 2011) and place cell instability (Calton et al., 2003). However, spatial learning is not abolished and performance often improves with training (Liu et al, 2001; Taube et al., 1992).

On the foraging task, ADN lesioned rats were more likely than sham controls to return to holes that were adjacent to the start location, again suggesting a mild impairment in heading. As in the water maze task, the lesioned rats were not totally disoriented as they did not make more other errors than sham controls. This finding is consistent with an earlier report showing that lesions to the ADN produced only a mild impairment on a similar foraging task (Frohardt et al., 2006). More recently, Bett et al. (2012) reported that lesions of the postsubiculum did not impair performance on a foraging task that they suggest requires the use of a path integration strategy. In both of these earlier studies, rats were trained on the task prior to the lesions. In the present study, lesions to the ADN were made prior to any training. This suggests that these areas are not critical for the development or maintenance of heading in the foraging task.

Although Frohardt, et al. (2006) showed that rats with ADN lesions were only mildly impaired on the foraging task, rats with lesions to the dorsal tegmental nucleus (DTN) were more severely impaired. While such comparisons between lesion sites cannot be drawn from this experiment, it is likely that lesions earlier in the HD cell circuit would produce greater impairment on the direction and foraging tasks. Since, as mentioned earlier, HD cells operate in a hierarchical scheme in order to process directional heading information, lesions to the DTN would presumably eliminate HD cell activity in the ADN, LMN, and postsubiculum as well. Indeed, it has been shown that lesions to the DTN eliminate HD cell activity in the ADN (Bassett & Taube, 2001), that lesions to the ADN eliminate HD cell activity in the postsubiculum (Goodridge &Taube, 1997), and that lesions to the LMN abolished HD cell activity in the ADN (Blair, Cho, & Sharpe, 1999). The hierarchical scheme of directional information flow does not appear to be completely unidirectional as lesions to the postsubiculum change HD cell activity in the ADN (Goodridge & Taube, 1997). Lesion to the postsubiculum caused ADN HD cells to expand their directional firing range with the preferred firing direction substantially less influenced by visual landmarks within the recording environment.

The present set of experiments demonstrated the importance of orientation or directional heading to the solution of spatial problems. However, the neural underpinnings of this strategy remain to be elucidated. The built-in redundancy of the HD system and the bidirectional flow of information along the most prominent HD cell circuit add to the difficulty of understanding this critical component of navigation.

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Figure captions

Figure 1. A schematic representation of the maze positions used in the Translation and Rotation place problems from earlier studies. The arrows represent correct paths to the hidden platform from the two start positions (designated by letters). The black bars indicate the barrier used to convert the plus maze to a T-maze and X marks the location of the goal (hidden platform or food reward, depending on the experiment). Maze positions are differentiated using grey or white. The grey maze (start position A) and the goal location are in the same location in the rotation and translation task.

Figure 2. A schematic representation of the maze positions used in the direction tasks. In phase 1 of Experiment 1 (top panel), half the rats were trained to go east from maze positions A and C. The other half was trained to go west from positions B and D (not indicated on the diagram). In phase 2 (bottom panel) the maze was re-positioned to two new locations, with the start points overlapping in space; the original positions are shown in white and the new positions are shown in grey. The arrows represent correct paths to the hidden platform from the two start positions (designated by letters). The black bars indicate the barrier used to convert the plus maze to a T-maze. In Experiment 2, rats were given trials from four maze positions during acquisition (bottom panel). Rats in the Orientation Cues group had to make a response based on start point orientation (i.e., turn right when headed north, turn left when headed south). The Location Cues group had to make a response based on start point location (i.e., turn right at A and turn left at C). In Experiment 5, ADN lesioned rats and sham controls were trained on the direction task shown in the top panel. In phase 1, half the rats were trained to go east from maze positions A and C. The other half was trained to go west from positions D and B (not

indicated on the diagram). In phase 2, the direction was reversed. For example, rats trained to go east from maze positions A and C had to travel west from maze positions D and B.

Figure 3: The top panel shows the mean (+SEM) trials to criterion for the Same and Different direction groups in Experiment 1. The middle panel shows the mean (±SEM) trials correct across blocks of eight trials for the Same and Different direction groups. The vertical dotted line denotes the switch from phase 1 to phase 2. The lower panel shows the mean (±SEM) latency (s) to locate the hidden platform for the Same and Different direction groups across blocks of eight trials.

Figure 4: The mean (+SEM) time (s) spent in the correct and incorrect arms for the three probe trials, one given after every 40 trials, for the Same Direction (upper panel) and Different Direction (lower panel) groups. The vertical dotted line denotes the switch from phase 1 to phase 2.

Figure 5: Panel A shows the mean (+SEM) trials correct in blocks of four trials for the eight trials immediately preceding and following the switch in task and maze positions in Experiment 1. The dashed line indicates chance performance. All trials were conducted in a well-lit room. Panel B shows the mean (+SEM) trials correct in blocks of four trials for the eight trials immediately preceding and following the switch in task, lighting conditions and maze positions in Experiment 3. On dark trials the lights were turned out just prior to carrying the rat to the maze. Panel C shows the mean (+SEM) trials correct in blocks of four trials for the eight trials immediately preceding and following the second switch in task and maze positions in Experiment 3. Again, the lights were turned out just prior to carrying the rat to the maze on dark trials. Panel D shows the mean (+SEM) trials

correct in blocks of four trials for the eight trials immediately preceding and following the switch in task, lighting conditions and maze positions in Experiment 4. In this experiment the rats were brought into an already darkened room during the dark trials and the lights were not turned on between trials.

Figure 6: The mean (+SEM) trials to criterion for the Location Cues and Orientation Cues groups of Experiment 2.

Figure 7: A schematic diagram of the foraging task used in Experiment 5. The black circles represent holes in the table under which a metal cage could be inserted. Only holes 1, 3, and 5 were used as variable start points. ADN lesioned rats and sham controls had to locate a food reward, positioned in one of three food cups (indicated in grey), and return to the variable home location.

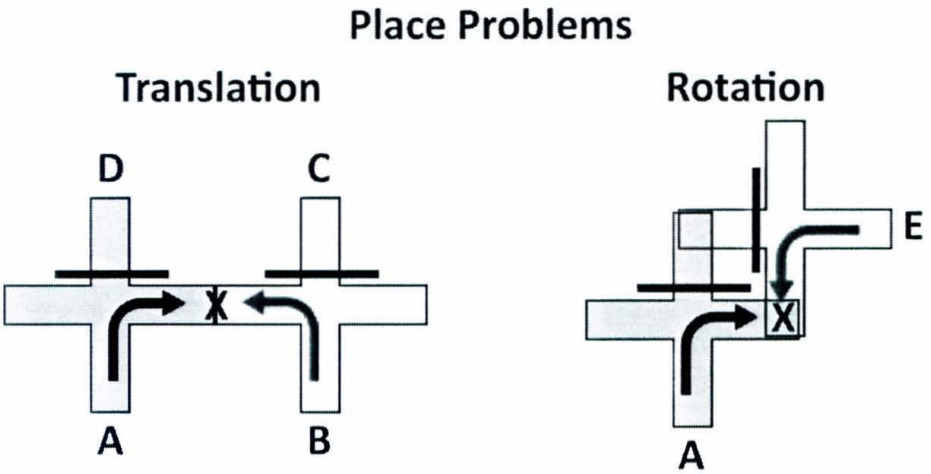
Figure 8: A picture of a typical ADN-lesioned brain and a sham comparison brain. The striped region of the top picture represents the ADN.

Figure 9: A diagram showing the smallest (stripes) and largest (white) lesions made to the ADN.

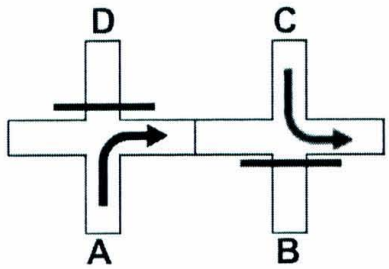
Figure 10: Mean (+SEM) trials correct in the first eight trials (left panel) and mean (+SEM) trials to criterion (right panel) for ADN lesioned rats and sham controls in phase 1 and phase 2 of the water maze task in Experiment 5.

Figure 11: Panel A shows the mean (+SEM) total errors for ADN lesioned rats and sham controls over blocks of 12 trials on the foraging task in Experiment 5. Panel B shows the mean (+SEM) number of Memory, Adjacent and Other errors for the ADN lesioned rats and sham controls. Panel C shows the mean (+SEM) trials to a criterion of 9/10 and the

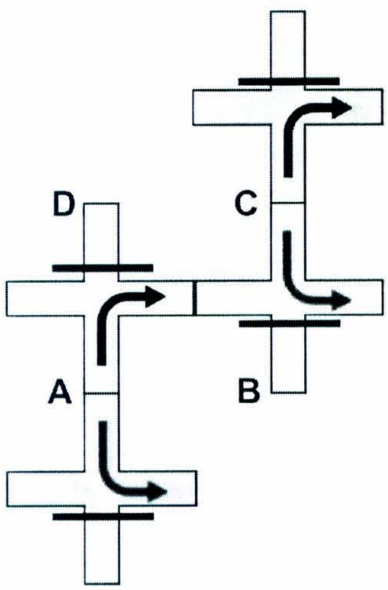
individual scores for sham controls and ADN lesioned rats on foraging task using a fixed start location.


Place Problems
Translation
Rotation
D
C
X
A
B
E
X
A

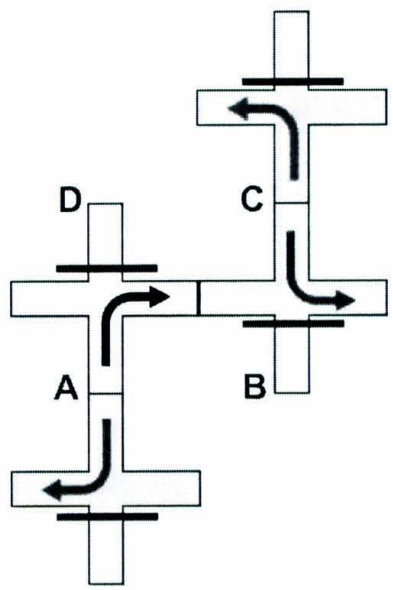
Direction

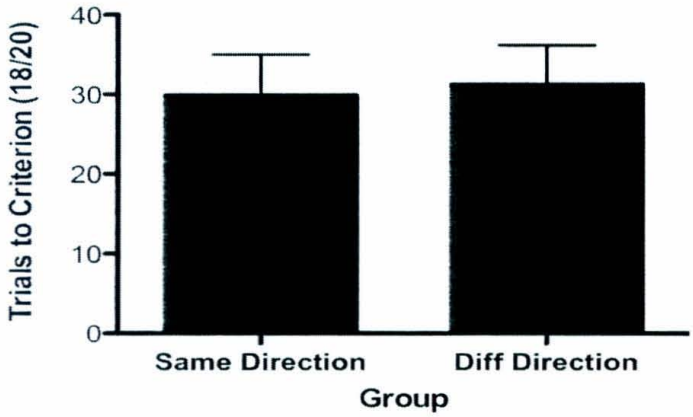


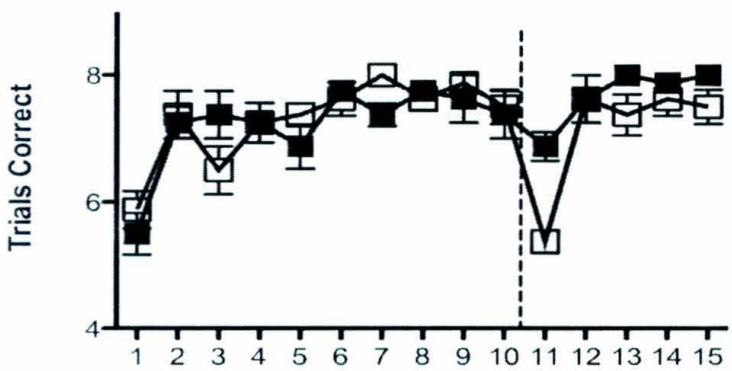
Same Direction – Exp 1
Orientation Cues – Exp 2

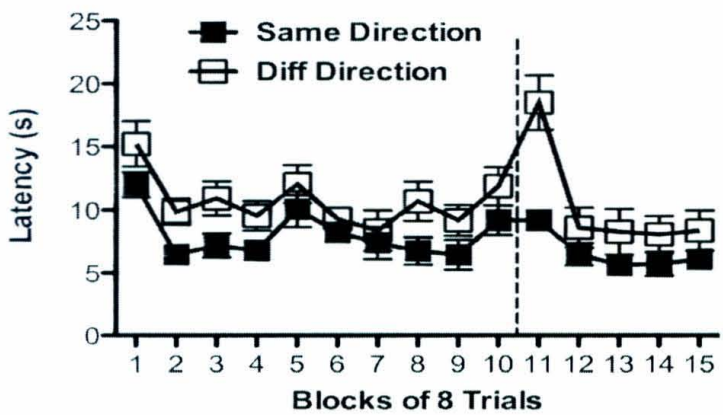


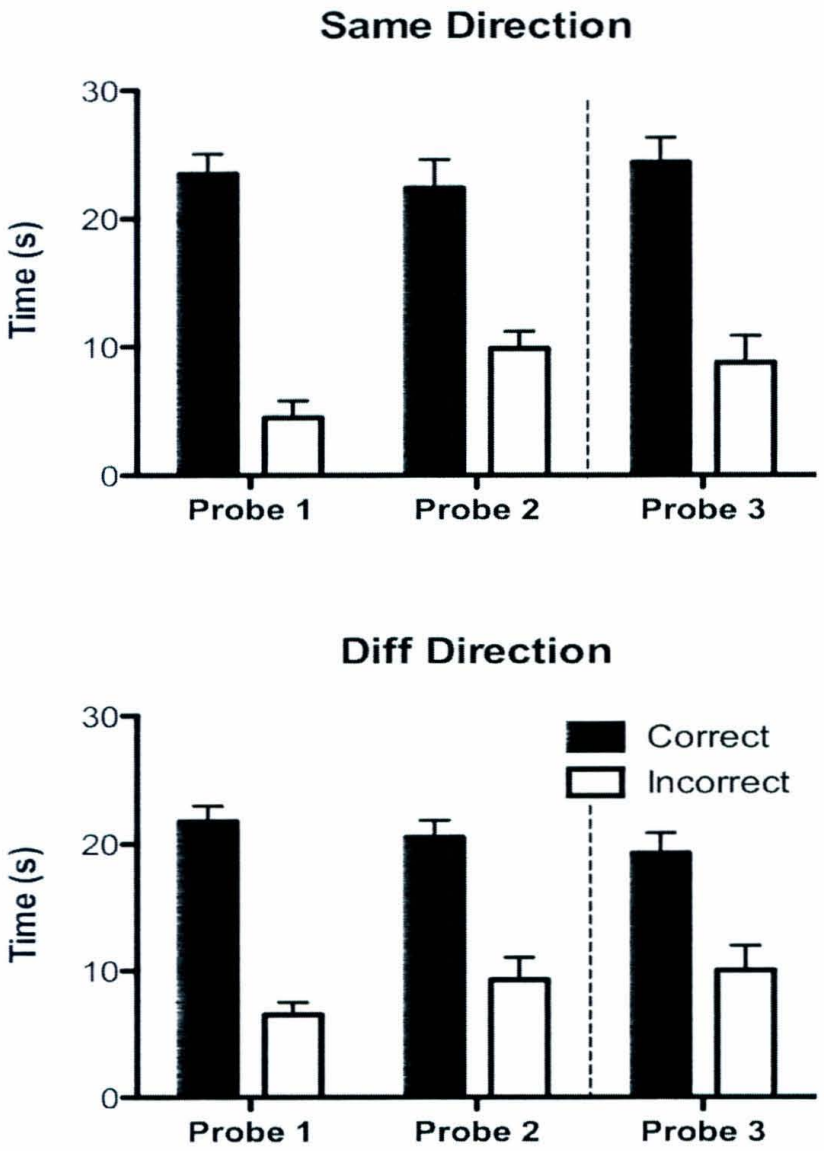
Different Direction – Exp 1
Location Cues – Exp 2

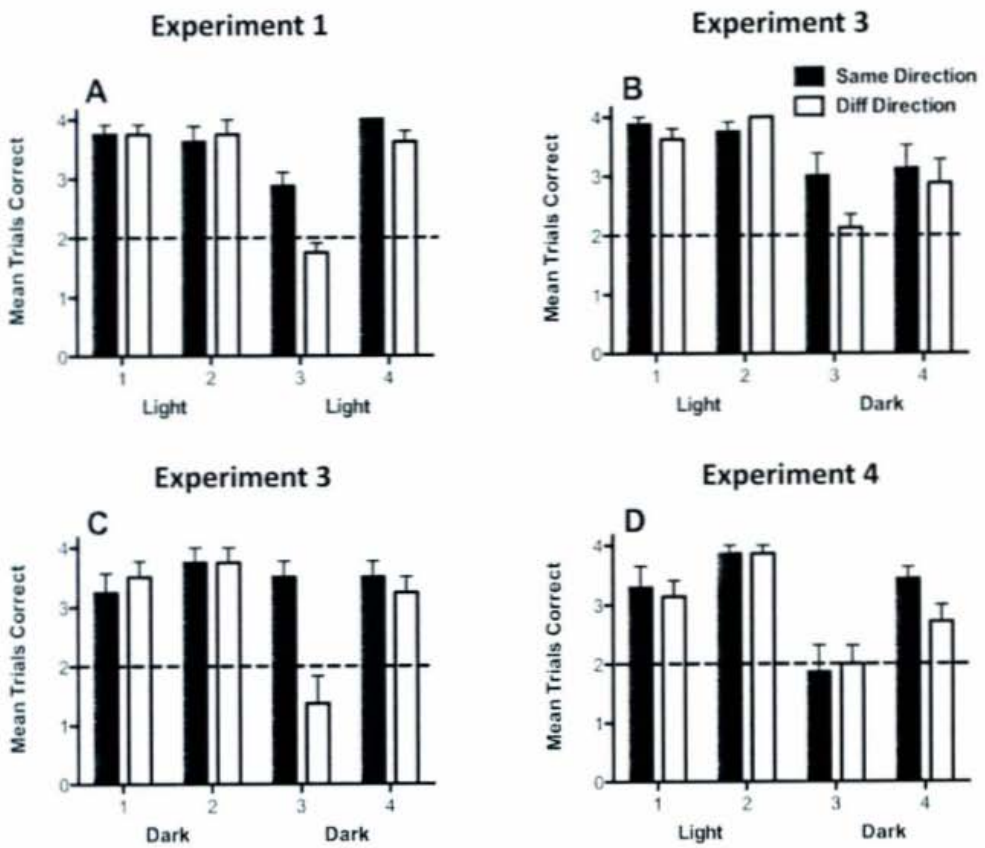


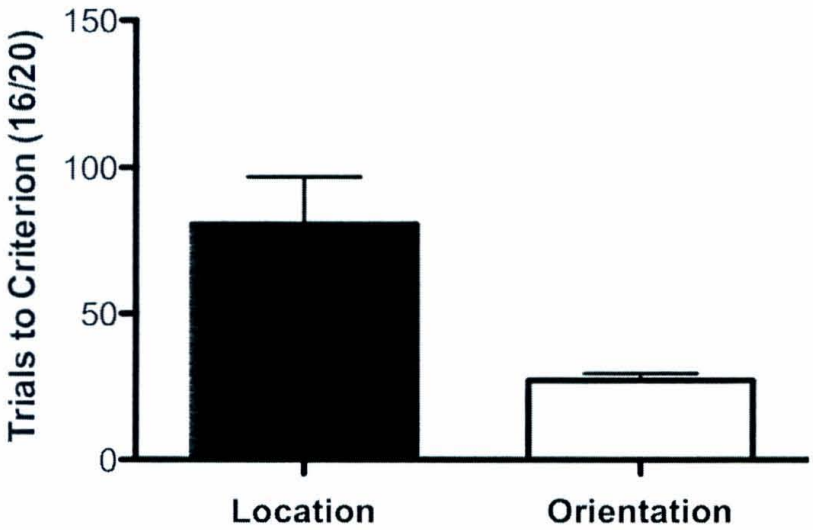

Trials to Criterion (18/20)
40
30
20
10
0
Same Direction
Diff Direction
Group

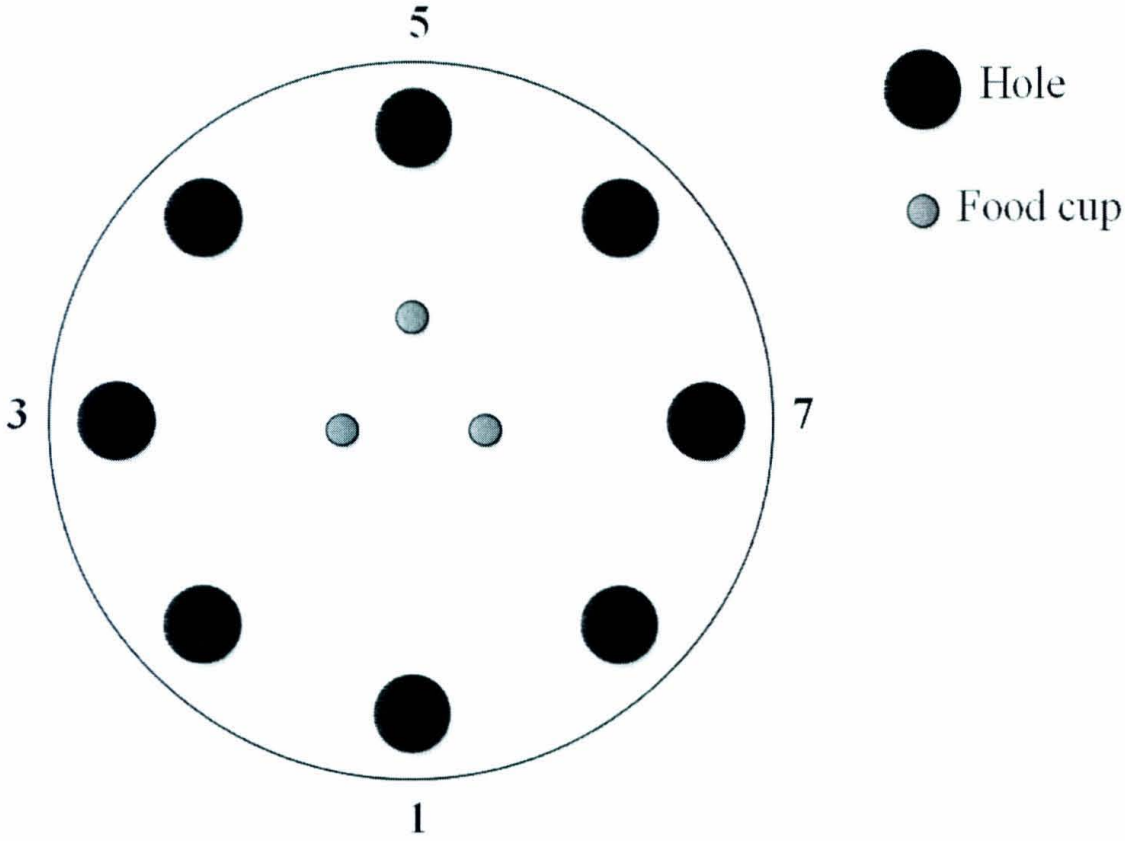

Trials Correct
8
6
4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

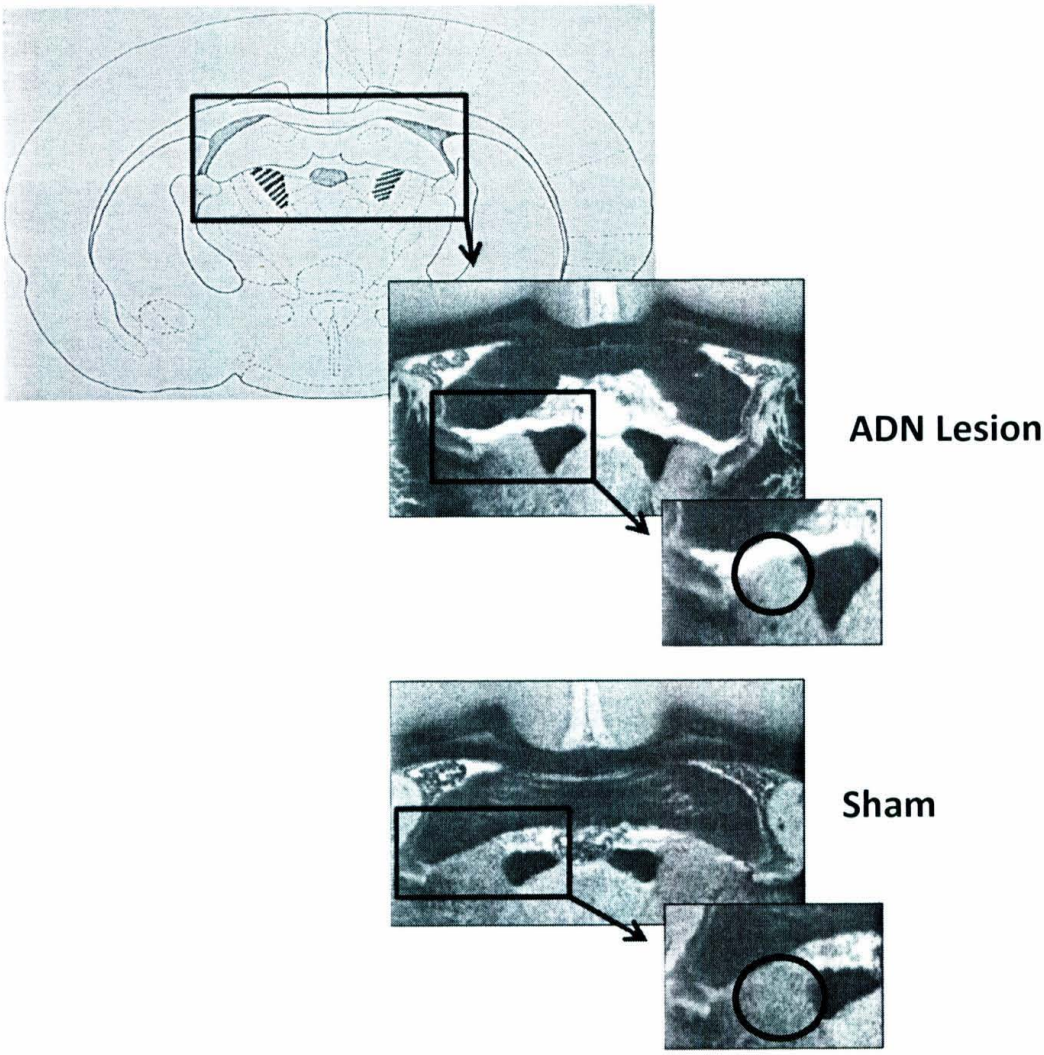

Same Direction
Diff Direction
Latency (s)
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Blocks of 8 Trials

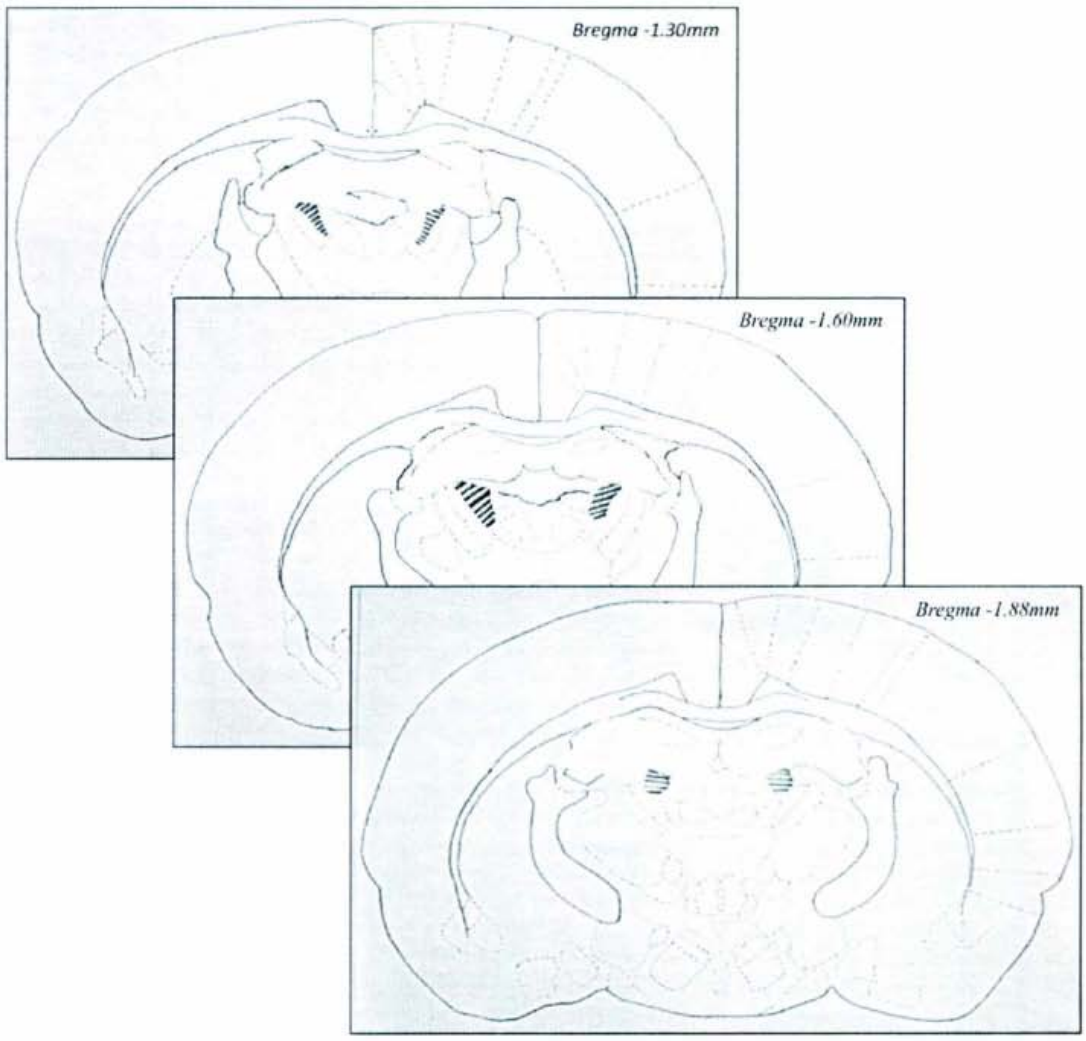

Same Direction
Time (s)
30
20
10
0
Probe 1
Probe 2
Probe 3
Diff Direction
Correct
Incorrect
Time (s)
30
20
10
0
Probe 1
Probe 2
Probe 3

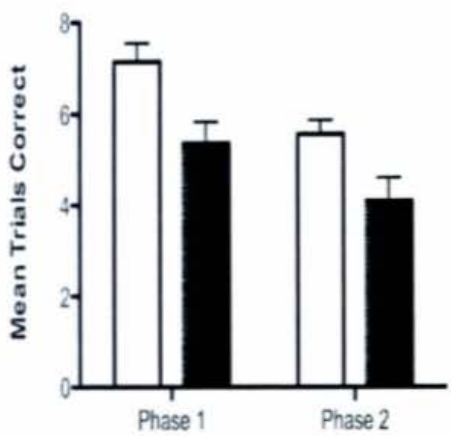

Experiment 1
A
Mean Trials Correct
0
1
2
3
4
1
2
3
4
Light
Light
Experiment 3
B
Same Direction
Diff Direction
Mean Trials Correct
Light
Dark
Experiment 3
C
Mean Trials Correct
Dark
Dark
Experiment 4
D
Mean Trials Correct
Light
Dark

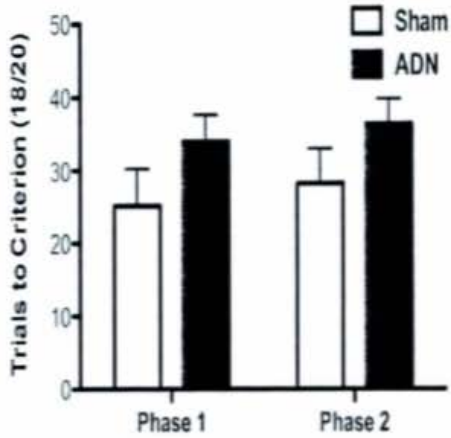

Trials to Criterion (16/20)
150
100
50
0
Location
Orientation

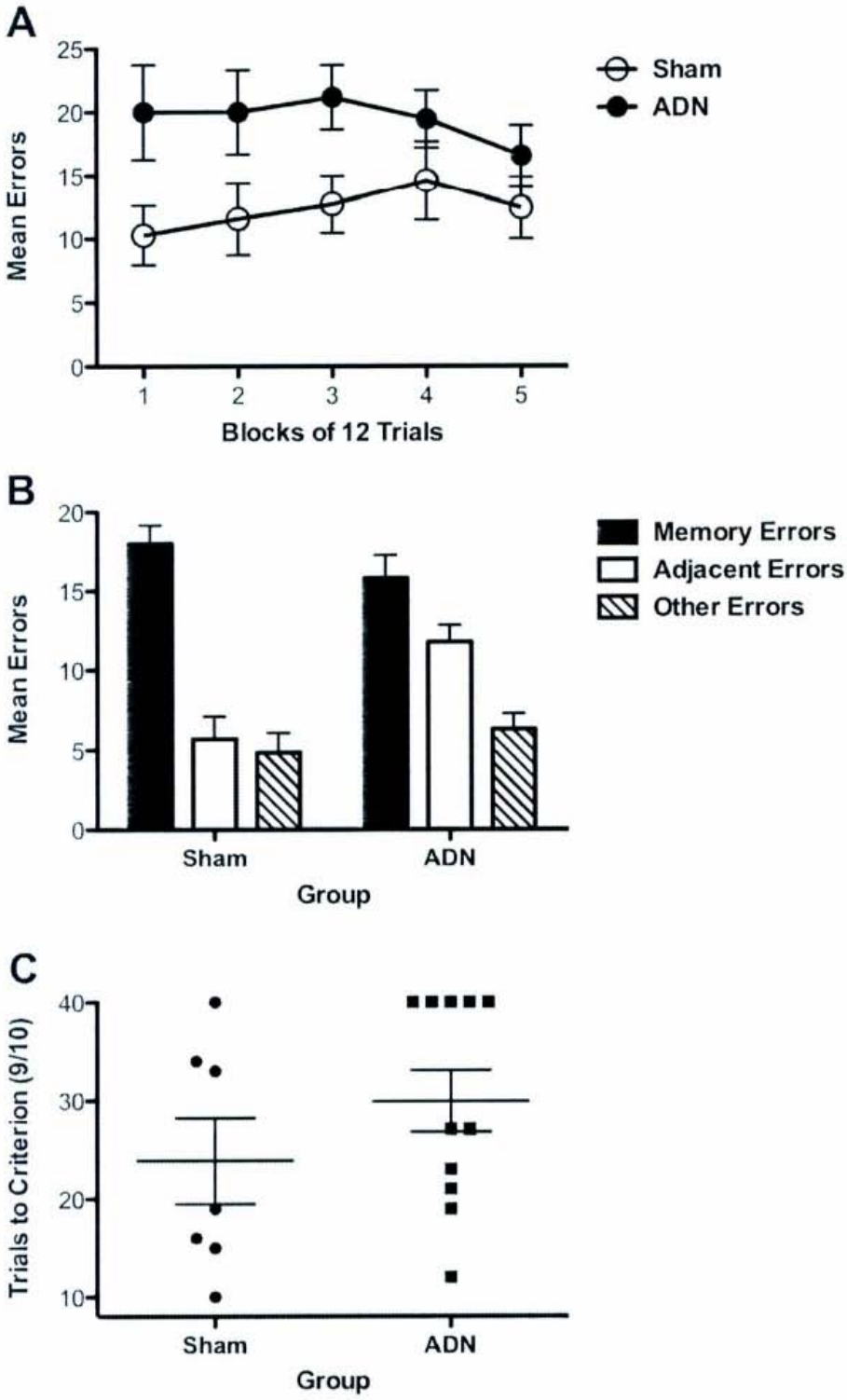

5
3
7
1
Hole
Food cup


ADN Lesion
Sham


Bregma -1.30mm
Bregma -1.60mm
Bregma -1.88mm


Mean Trials Correct
0
2
4
6
8
Phase 1
Phase 2


Trials to Criterion (18/20)
0
10
20
30
40
50
Sham
ADN
Phase 1
Phase 2


A
Mean Errors
25
20
15
10
5
0
1
2
3
4
5
Blocks of 12 Trials
Sham
ADN
B
Mean Errors
20
15
10
5
0
Sham
ADN
Group
Memory Errors
Adjacent Errors
Other Errors
C
Trials to Criterion (9/10)
40
30
20
10
Sham
ADN
Group