

The Effects of Lesions to the Postsubiculum or the Anterior Dorsal Nucleus of the Thalamus on Spatial Learning in Rats

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To investigate the role of the head direction (HD) cell circuit in spatial navigation, rats with bilateral, neurotoxic lesions to the postsubiculum (PoS; Experiment 1) or the anterior dorsal nucleus of the thalamus (ADN; Experiment 2) were compared to sham controls on 2 tasks that could be solved using directional heading. Rats were first trained on a direction problem in a water T maze where they learned to travel either east or west from 2 locations in the experimental room. ADN lesioned rats were impaired relative to sham controls on the first block of 8 trials, but not on the total trials taken to reach criterion. This transient deficit was not observed in rats with lesions to the PoS. In the food-foraging task, rats were trained to leave a home cage at the periphery of a circular table, find food in the center of the table, and return to the home cage. Both PoS and ADN lesioned rats showed impairments on this task relative to sham rats, making more errors on the return component of the foraging trip. The spatial deficits produced by lesions to the PoS and the ADN, downstream structures in the HD cell circuit, are not as severe as those observed in earlier studies in rats with lesions to the dorsal tegmental nucleus.

Keywords: direction learning, head direction cells, water maze, T maze, foraging task

Since their discovery, head direction (HD) cells have been thought to underlie an animal's sense of direction or orientation (Taube, Muller, & Ranck, 1990a, 1990b). HD cells fire when an animal's head is pointed in a particular direction independent of the animal's location (Taube, 2007; Taube et al., 1990a, 1990b). The preferred firing direction of HD cells can be controlled by both external (allothetic) cues (e.g., visual) and internal (idiothetic) cues (e.g., vestibular and proprioceptive; Taube, 2007; Taube & Burton, 1995; Taube et al., 1990b). Although HD cells have been identified in various brain regions (Chen, Lin, Green, Barnes, & McNaughton, 1994; Cho & Sharp, 2001; Mizumori, Ragozzino, & Cooper, 2000; Taube, 1995; Taube et al., 1990a; Wiener, 1993; see Wiener & Taube, 2005, for review), they appear to be most abundant, and best studied, in limbic areas that form part of the classical Papez circuit (see Taube, 2007, for review). The HD signal is thought to originate in the reciprocal connections between the dorsal tegmental nucleus (DTN) and the lateral mammillary nuclei (LMN) and the signal is then processed through mostly serial projections from DTN → LMN → anterior dorsal nucleus of the thalamus (ADN) → postsubiculum (PoS)/also parasubiculum (PaS) and retrosplenial cortex (RSP; see Clark & Taube, 2012, for review). The connections between the PoS and the entorhinal

cortex, the major input to the hippocampus, permit the directional signal to be integrated with location information from grid cells and place cells, the other major cell types thought to be involved in spatial learning and navigation (see Taube, 2007, for review).

The importance of direction or orientation has been revealed by its impact on place cell activity (Knierim, Kudrimoti, & McNaughton, 1995), its importance to place learning (Dudchenko, Goodridge, Seiterle, & Taube, 1997; Gibson, Shettleworth, & McDonald, 2001; Martin, Harley, Smith, Hoyles, & Hynes, 1997), and its representation in many neural structures involved in navigation (see Taube, 2007; Wiener & Taube, 2005, for reviews). The importance of directional information also is highlighted by the recent finding that HD cells fire in an adult-like fashion earlier than place and grid cells (Langston et al., 2010; Wills, Cacucci, Burgess, & O'Keefe, 2010). This has led to speculation that HD cells set up the entire "spatial network" in the mammalian brain. Behavioral studies in the past decade have strengthened the belief that direction learning underlies the solution to many spatial problems previously attributed to place learning (e.g., Hamilton, Akers, Weisend, & Sutherland, 2007; Skinner et al., 2003).

Some of the earliest studies examining spatial learning by rats on mazes used tasks with a strong directional component (e.g., Tolman, Ritchie, & Kalish, 1946). In the original "place" task used by Tolman and colleagues, rats were trained to run in a consistent direction from the choice point to the goal on a plus maze. Blodgett, McCutchan, and Mathews, 1949 argued that Tolman et al.'s demonstration of superior place learning compared to response learning was actually evidence of a directional disposition. Blodgett et al. demonstrated that going to a fixed place was difficult if rats had to travel in two different directions from the choice point to the goal. The strategy of moving mazes between adjacent locations to adequately separate place and direction learn-

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ing originally developed by Blodgett et al. has become more widely used in the past decade (Clark et al., 2013; Hamilton et al., 2007, 2008; Skinner et al., 2003; Stackman, Lora, & Williams, 2012; Whyte, Martin, & Skinner, 2009).

Skinner et al. (2003) replicated the findings of Blodgett et al. (1949) and suggested that the poor performance by rats on the place task may be attributed to indistinguishable start points at the two maze positions. In the place task, the start points were on the same side of the room and the view from the maze at the two positions contained many of the same cues. In the response and direction tasks, the start points were on the opposite side of the room and the view from the maze at the two points was different. When Skinner et al. (2003) designed a new place task, rotating the maze by 90° between trials to create more distinct start points, rats solved the task as well as the response and direction tasks. Based on this finding, Skinner et al. (2003) postulated that distinguishable start points were an essential part of the strategy to solve place problems. More recent evidence suggests that a conditional strategy based on orientation of the start arm probably underlies the solution to both place (Skinner, Horne, Murphy & Martin, 2010) and direction problems (Peckford, McRae, Thorpe, Martin, & Skinner, 2013). Orientation, or directional heading, appears to be important to the solution of other spatial tasks as well. Wright, Williams, Evans, Skinner, and Martin (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.

If HD cells are the neural representation of an animal's sense of direction, then disrupting the flow of information within the HD cell circuit should disrupt behavioral demonstrations of direction learning. Dwyer et al. (2013), recently demonstrated that lesions of the DTN, in which the HD signal is thought to be generated (Clark & Taube, 2012), produced a severe impairment on two tasks that could be solved using directional heading. Rats were first trained on a direction problem in a water T maze where they learned to travel either east or west from two locations in the experimental room. Rats with electrolytic or neurotoxic lesions to the DTN were impaired relative to sham controls. In the food-foraging task, rats were trained to leave a home cage at the periphery of a circular table, find food in the center of the table, and return to the home cage. Again, rats with electrolytic or neurotoxic lesions to the DTN were impaired relative to sham rats, made more errors on the return component of the foraging trip. Similar results have been reported with electrolytic lesions to the DTN on a foraging task (Frohardt, Bassett, & Taube, 2006) and in the water maze (Clark et al., 2013). These behavioral data are consistent with earlier cell-recording studies, which showed that lesions to the DTN disrupted the HD signal in downstream components of the circuit (Bassett, Tullman & Taube, 2007; see Taube, 2007, for review).

It is surprising that lesions to structures later in the circuit do not produce as severe behavioral impairments. For example, Frohardt et al. (2006) showed that the impairments produced by lesions to the ADN on a food-foraging task were not as severe as the impairments produced by lesions to the DTN. Lesions to the PoS also did not produce impairments on a similar foraging task (Bett, Wood, & Dudchenko, 2012). In both of these earlier studies, the rats were trained on the foraging task prior to surgery. It is possible that the rats had an established sense of direction or orientation in the familiar environment that no longer depended on the integrity

of the ADN or PoS. Given that we have developed spatial tasks where directional heading is critical to the solution (Peckford et al., 2013; Skinner et al., 2010), we assessed whether lesions to the PoS or ADN would impair performance on the direction task used previously with DTN lesioned rats (Dwyer et al., 2013). We also assessed performance on a food-foraging task similar to that used in earlier studies, but the rats were not trained prior to surgery.

Experiment 1

HD cells were first discovered in the PoS (Taube et al., 1990a, 1990b) and this is thought to be a site where internal representations of orientation and external representations of landmarks converge (Calton et al., 2003; Goodridge & Taube, 1997; see Yoder, Clark, & Taube, 2011, for review). Lesions to the PoS do not abolish the HD cell signal in the ADN but this signal is no longer controlled by visual cues (Goodridge & Taube, 1997). Early studies revealed that lesions to the PoS produced impairments on traditional spatial tasks (such as the Morris water task and the radial arm maze), but animals often showed improvement with training (P. Liu, Jarrard, & Bilkey, 2001; Taube, Kesslak, & Cotman, 1992). These transient impairments suggest that the PoS might play a bigger role early in training, perhaps when a sense of direction is being established in an environment.

Recent work using a food-foraging task has shown that the PoS is not critical for path integration (Bett et al., 2012), despite earlier findings of correlations between HD cell activity and performance on the same task (van der Meer, Richmond, Braga, Wood, & Dudchenko, 2010). Rats trained preoperatively showed accurate navigation after lesions to the PoS (Bett et al., 2012); however, on some trials the distribution of choices around the home base location (0°) was less sharply tuned in the lesioned rats compared to sham controls. The lesioned rats were, however, impaired on a T maze alternation task that was not acquired prior to surgery. In Experiment 1, we assessed the effects of lesions to the PoS on acquisition of a direction task in a water T maze that has been demonstrated depends critically on orientation in the start arm (Peckford et al., 2013) and on the integrity of the DTN (Dwyer et al., 2013). We also assessed rats' performance on a food-foraging task, similar to the one used by Frohardt et al. (2006) and Bett et al. (2012), that has been shown to be sensitive to damage to the DTN (Dwyer et al., 2013).

Method

Subjects. Nineteen naïve, male, Long-Evans rats were obtained from Charles River Company (St. Constant, Quebec, Canada) and weighed 300 to 350g prior to surgery. The rats were singly housed in clear plastic cages (45 × 25 × 21 cm) with metal lids in a temperature controlled room (approximately 20 °C) and maintained on a 12-hr light–dark cycle with lights on at 0800. All rats had continuous access to food and water in their home cages during training in the water maze. Prior to the foraging task, the rats were placed on a food deprivation schedule to maintain them at 85% of their free feeding weight. All procedures used in this experiment were approved by Memorial University's Institutional Committee on Animal Care.

Apparatus and materials. The water maze 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 to a T maze using a section of clear Plexiglas that 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 nontoxic 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 room used for water maze training (528 × 464 × 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.

The room used for the foraging task (528 × 464 × 267 cm) had a door on the east, west, and south walls and a large window, covered in black curtains, on the north wall. There were cupboards, a counter, and a sink in the southeast corner and a desk in the northwest corner. There was a metal rack (150 × 50 × 165 cm high) along the north wall, where rats were held in wire mesh cages (20 × 25 × 19 cm) between trials.

The apparatus used for the foraging task consisted of a large, black, circular wooden 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 small wooden food cups (6 × 6 × 4 cm high) were placed on the surface (see Figure 1). A 1g pellet (BioServ, Frenchtown, NJ) was placed in one of the three food cups. Wire mesh cages (20 × 25 × 19 cm) were attached on runners beneath a hole at the periphery of the table. Wooden blocks were placed in the cage at the beginning of a trial to allow for easy access to the table. Once the 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.

Surgery. All rats were anesthetized with an intraperitoneal injection of 0.24M chloral hydrate (1 mL/100g) and placed in a stereotaxic instrument (Model 900, Kopf, Tujunga, CA) in the skull-flat position. Additional supplements of 0.5 ml of chloral hydrate were administered as necessary. After a scalp incision was made, holes were drilled into the skull above the target site. Eleven of the rats were given lesions to the postsubiculum. Lesions were produced by injecting 0.3 µl of NMDA (Sigma Chemical, St. Louis, MO) at each of two sites per hemisphere using bregma as a reference point (AP: -6.8, ML: ± 2.6, DV: -2.8 from the surface of the brain; AP: -7.8, ML: ± 3.3, DV: -3.0 from the surface of the brain). The injections were delivered at a rate of 0.05 µl/min using a 1 µl Hamilton syringe. At each injection site the needle was left in place for 30 s before the injections were made. After the injections were made the needle was left in place for an additional 5 min. The incision was then sutured. The remaining

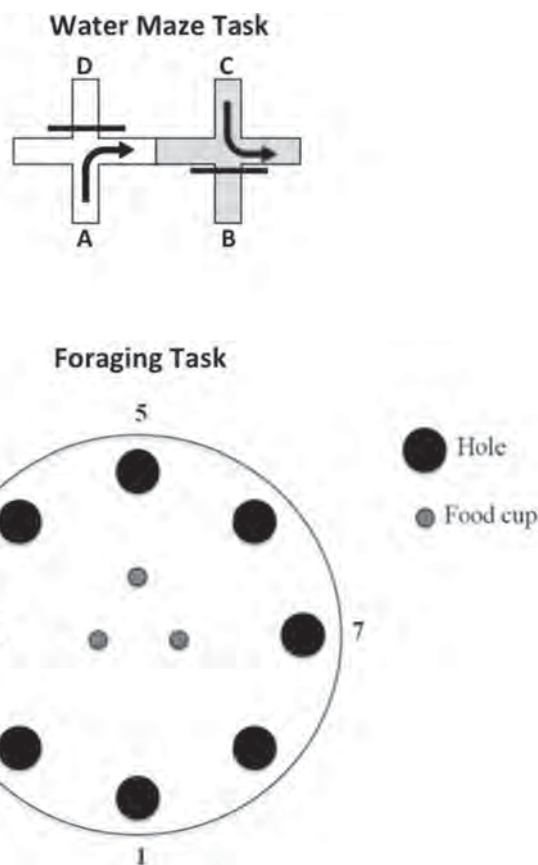


Figure 1. The top panel shows a schematic diagram of the water T maze task. Half of the rats were trained to go east from maze positions A and C as indicated whereas the other half were trained to go west from maze positions B and D (not shown). Arrows indicate the correct path and solid black lines indicate a Plexiglas barrier. The bottom panel shows a schematic diagram of the food-foraging apparatus. The black circles represent holes in the wooden table under which a wire mesh cage was positioned. Only holes 1, 3, and 5 were used as variable start points. Rats were trained to locate a large food pellet placed in one of three food cups (indicated in gray) and to return to the variable home location.

eight rats were given sham surgeries; holes were drilled in the skull but no NMDA was administered. All rats were given a 1-week recovery period before behavioral testing.

Water maze training.

Phase 1. The 19 rats (11 lesions and eight shams) were trained to go in a consistent direction (half went west; half went east) to locate a hidden platform from two maze positions. Between trials, the maze was translated and the start point was rotated 180° as illustrated in Figure 1. The rats were brought into the training room in squads of six or seven 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 in which 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. On 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 intertrial interval was approximately 5 to 10 min. The rats were given eight trials per day until a criterion of 18 to 20 correct trials was reached. No more than two trials in a row were given from the same maze position. Because the lesioned rats did not appear to be impaired on this task, we trained them on a second version of the direction task, which was essentially a direction reversal.

Phase 2. On completion of the first direction task, the maze was then moved to two new positions and the direction of the hidden platform was reversed (i.e., rats trained to travel east from maze positions A and C (see Figure 1) were now trained to travel west from maze positions B and D). The rats were given eight trials per day on this new problem until they reached a criterion of 18 to 20 correct trials.

Foraging task. During this task, the rats were transferred to the hanging wire mesh cages in the colony room. All rats were initially pretrained in the colony room to leave the home cage and retrieve a food pellet. During pretraining, the rats were placed beneath a hole that allowed access to a modified test apparatus, which was the same as described above except folded in half. Initially, a 1g pellet was placed near the opening of the home location. 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.

Once pretraining was completed, the apparatus was moved to a new room and all rats were trained to leave holes 1, 3, and 5 (three trials per day; one from each starting point) and find a pellet that was located in one of the three centrally positioned food cups (see Figure 1). For each trial, the rats were carried in their holding cages around the circular maze in a counterclockwise direction and placed below the designated start hole. A trial began when the rat emerged (all four limbs) from the wire mesh cage, and ended when the rat returned to the cage. The wire mesh cage was then removed from the table and the rat was returned to the metal transport rack. The rats were run sequentially with an intertrial interval of approximately 20 min. The circular table was rotated 135° every third trial and washed with soap and water to minimize scent trails. A trial was considered to be correct when the rat retrieved the pellet and immediately returned to the hole from which it started without visiting any other holes. First choice errors were divided into three types. Adjacent-hole errors occurred when a rat nose-poked the hole 45° to the left or right of its start hole; memory errors consisted of exploration of start holes (1, 3, or 5) from a previous trial; and other errors included visits to any other hole (Martin, Evans, Harley, & Skinner, 2005; Martin et al., 2011). Because the rats often made more than one error per trial, we also analyzed total errors by the two groups. The rats were run until

they obtained a pellet from the centrally located food cups 60 times. Trials in which the rat did not leave its cage or did not find food were not included in the analyses.

Histology. On completion of the experiment, the rats were euthanized using carbon dioxide and decapitated. The brains were removed, submerged in cooled 2-methylbutane, and stored in a -70 °C freezer. The brains were sectioned at 30-μm and nissl stained (cresyl violet) for verification of lesion sites. To quantify the extent of tissue damage created by the lesions, digital pictures were taken at the level of the PoS (approximately 6.8 mm posterior to bregma). Undamaged PoS with intact neurons and few glial cells was outlined and measured using Image-J software (Rasband, 1997–2014) blind to the animal's individual performance in the behavioral testing. The total amount of tissue damage was calculated using the following equation as per Clark et al. (2013):

$$\text{tissue damage} = \left[\frac{\text{average area of PoS in control rats (pixels}^2\text{)} - \text{total area of spared PoS tissue in lesioned rats (pixels}^2\text{)}}{\text{average area of PoS in control rats (pixels}^2\text{)}} \right] \times 100\%.$$

Results

Histology. Based on histological analysis performed blind to the behavioral performance of the subjects, nine of the 11 lesioned animals sustained substantial damage to the PoS (>70%). The remaining two rats were excluded from the behavioral analysis due to insufficient damage to the PoS. Figure 2 shows representative brain sections from a sham control and a PoS lesioned rat, and a schematic diagram indicating the smallest and largest lesions. In addition to damage to the PoS, the majority of rats had some damage that extended beyond the PoS, including damage to the overlying (visual) cortex, the forceps major of the corpus callosum and the subiculum. However, as there was no correlation between lesion size and trials to criterion on the water maze ($r = -.33, p = .38$) or total errors on the foraging task ($r = .31, p = .46$) we included these rats in the behavioral analyses.

Water maze training. We compared performance of the two groups on the first eight trials, on total trials to criterion, and on total errors made during acquisition of both the initial direction task and the direction reversal. For all statistical tests alpha was set at .05. Rats with lesions to the PoS were comparable to sham controls in all aspects of performance on the water maze task. A two-way analysis of variance (ANOVA), Group (sham or PoS) $\times$ Phase (1 or 2) over performance on the first eight trials revealed no significant effects ($F_s < 1$; see Figure 3A). A similar two-way ANOVA on total trials to criterion also revealed no significant effects, largest $F(1, 15) = 1.42$ (Figure 3B). Although the rats with PoS lesions made more errors than sham rats during Phase 1, a two-way ANOVA revealed no significant effects, largest $F(1, 15) = 1.65$ (see Figure 3C).

Foraging task. One lesioned rat was not included in the foraging task as it refused to exit the hole and search for food on the table. The data from the 60 trials in which rats found food were divided into five blocks of 12 trials for analyses. Rats with lesions to the PoS did show deficits relative to sham controls on the foraging task. A two-way ANOVA (Group $\times$ Trial Block) on trials

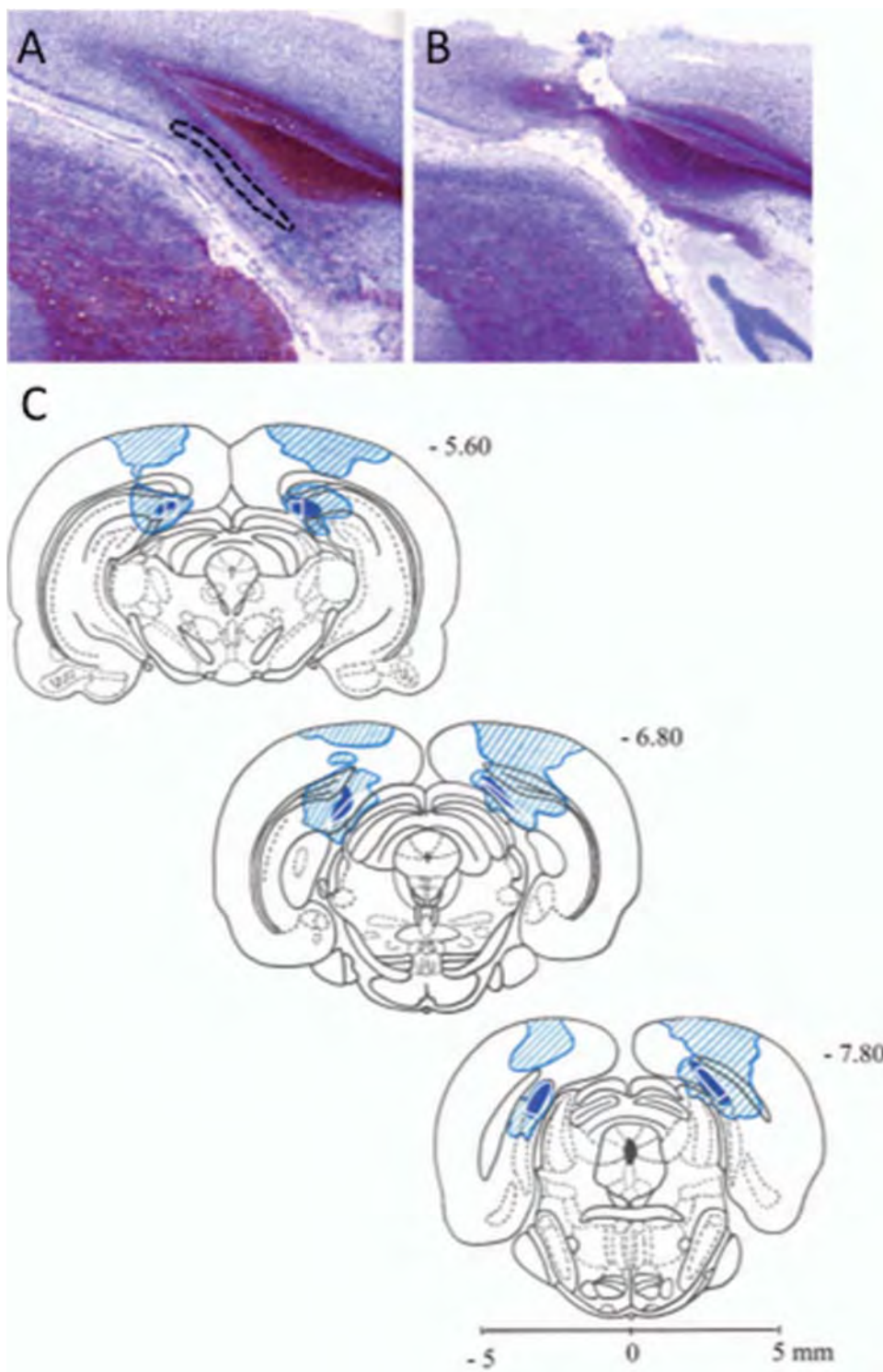


Figure 2. Panel A shows a section of a sham brain with the area of the postsubiculum (PoS) outlined in black and Panel B shows the corresponding section from a typical lesioned brain. Panel C shows the anterior and posterior extent of the lesions with hatch marks indicating the largest lesion and solid shading showing the smallest lesion. The area of the PoS is outlined in white. From *The Rat Brain in Stereotaxic Coordinates*, by G. Paxinos and C. Watson, 1998 (4th ed.), San Diego, CA: Academic Press. Copyright 1998 by Elsevier Academic Press. Adapted with permission. See the online article for the color version of this figure.

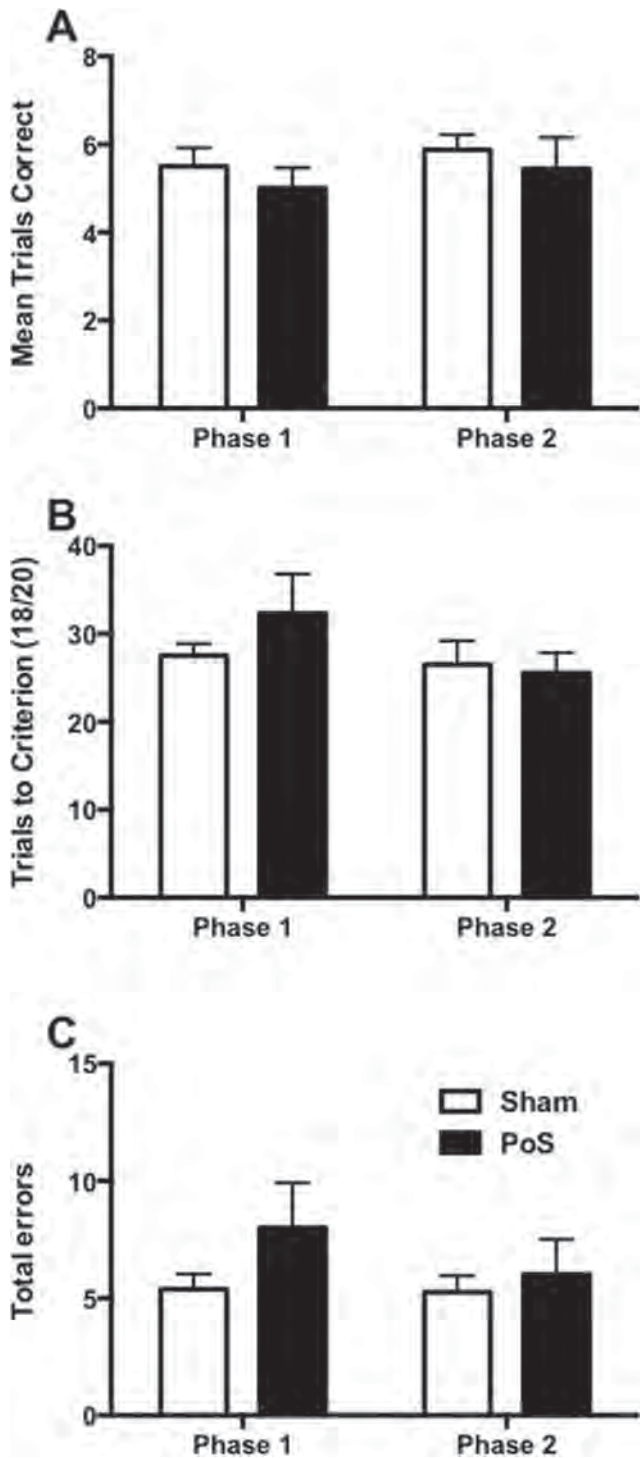


Figure 3. (A) Mean ($\pm$ SEM) trials correct for the first eight trials for postsubiculum (PoS) lesioned rats and sham controls on the direction task in the water T maze (B) Mean ($\pm$ SEM) trials to criterion of PoS lesioned rats and sham controls on the direction task (C). Mean ($\pm$ SEM) Total errors made by the sham and PoS lesioned rats on the direction task. ADN = anterior dorsal nucleus of the thalamus; SEM = standard error of the mean.

correct over blocks of 12 trials revealed significant effects of group, $F(1, 14) = 5.06$, confirming that lesioned rats performed worse than sham rats ($\omega^2 = .20$; see Keppel, 1991 for use of omega-squared for effect sizes), and trial block, $F(4, 56) = 6.91$, reflecting a deterioration in performance over blocks (see Figure 4A). A similar ANOVA on errors per block of 12 trials revealed only a significant effect of group, $F(1, 14) = 9.99$, again reflecting

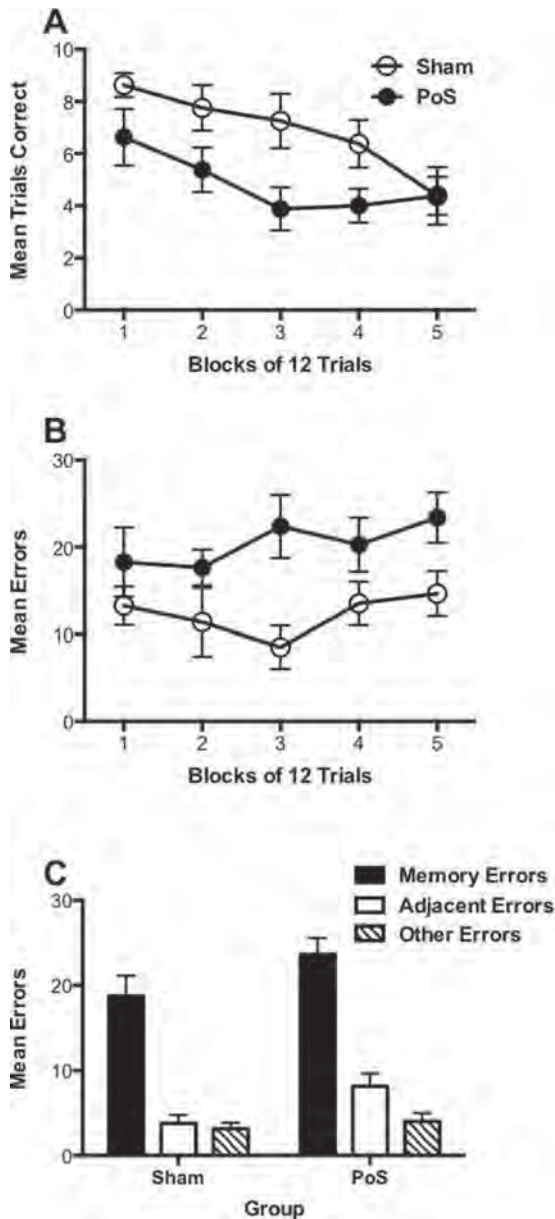


Figure 4. (A) Mean ($\pm$ SEM) trials correct over five blocks of 12 trials for postsubiculum (PoS) lesioned rats and sham controls on the food-foraging task. (B) Mean ($\pm$ SEM) total number of errors made over five blocks of 12 trials for PoS lesioned rats and sham controls on the food-foraging task (C) Mean ($\pm$ SEM) Number of first choice memory, adjacent, and other errors made over 60 trials for PoS lesioned rats and sham controls on the food-foraging task. ADN = anterior dorsal nucleus of the thalamus; SEM = standard error of the mean.

the poorer performance of the lesioned rats (see Figure 4B). In a separate two-way ANOVA (Group $\times$ Error type) we examined the different types of errors made by rats in the two groups on their first choice. In addition to the main effect of group, $F(1, 14) = 5.06$, this ANOVA revealed a significant effect of error type, $F(2, 28) = 98.47$. The most common type of error was memory errors (see Figure 4C). Rats in the PoS lesioned group made more adjacent errors than sham controls, $t(14) = 2.40$, but the groups did not differ on memory or other errors ($p > .05$).

Experiment 2

To the extent that information from HD cells is important for the acquisition of direction tasks, the key structures appear to be upstream of the PoS. Experiment 1 revealed no impairment on the direction task in the water maze and small impairments in heading on the foraging task in rats with lesions to the PoS. The PoS receives projections from the ADN, which contains the most abundant proportion of HD cells in the circuit. Sixty percent of the cells in the ADN fire in response to heading (Taube, 1995), and both electrolytic and neurotoxic lesions to the ADN disrupt HD cell activity in the PoS (Goodridge & Taube, 1997). Behavioral impairments are relatively small (e.g., Frohardt et al., 2006), unless damage extends to other thalamic nuclei (e.g., Aggleton, Poirier, Aggleton, Vann, & Pearce, 2009; Wilton, Baird, Muir, Honey, & Aggleton, 2001). It is interesting that Stackman et al. (2012) showed that inactivation of the anterior thalamic nuclei in mice produced deficits in directional heading in a water maze task, similar to the one used in Experiment 1, that involved moving the maze to two locations. In Experiment 2, we assessed the effects of lesions to the ADN on acquisition of the two tasks used in Experiment 1: the direction task in a water T maze and the food-foraging task.

Method

Subjects. Twenty naïve, male, Long-Evans rats, obtained from Charles River Company (St. Constant, Quebec, Canada) and weighing 303 to 357g prior to surgery, were used. The rats were maintained as in the previous experiment.

Apparatus and materials. The water maze and foraging apparatus, as well as the training rooms, were identical to those used in Experiment 1.

Surgery. Thirteen rats were given neurotoxic lesions to the ADN and seven rats served as sham controls. All rats were anesthetized with an intraperitoneal injection of 0.24M chloral hydrate (1 mL/100g) and placed in a stereotaxic instrument (Model 900, Kopf) in the skull-flat position. Additional supplements of 0.5 ml of chloral hydrate were administered as necessary. After a scalp incision was made, holes were drilled into the skull above the target site. Lesions to the ADN were produced by injecting two 0.15 μ l injections of 100 mM NMDA (Sigma Chemical, St. Louis, MO) into each hemisphere using a 1- μ l syringe (Hamilton Company, Reno, NV). 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: $\pm$ 1.2; DV coordinates: 5.0 and 4.6 mm below brain surface). The 0.15 μ l of NMDA was injected over a 2-min period. At each injection site the needle was left in place for 30 s before the injections were made. After the injections were

made the needle was left in place for an additional 5 min. The incision was then sutured. Sham control rats had holes drilled in the skull but did not receive injections. All rats were given a minimum of 1 week to recover before any behavioral testing.

Water maze training. The 20 rats (13 lesions and seven shams) were trained to go in a consistent direction (half went west; half went east) to locate the hidden platform from two maze positions (see Figure 1). As in Experiment 1, rats were given eight trials per day until they reached a criterion of 18 to 20 correct trials. The maze was then moved to two new positions and the direction of the hidden platform was 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 per day on this new problem until they reached a criterion of 18 to 20 correct trials.

Foraging task. The pretraining and training phases were identical to Experiment 1.

Histology. Once the behavioral testing was completed, the rats were euthanized using carbon dioxide and decapitated and the brains were processed as in Experiment 1. Digital pictures were taken at the level of the ADN (approximately 1.8 mm posterior to bregma) and the extent of tissue damage created by the lesions was quantified as in Experiment 1.

Results

Histology. Based on histological analysis performed blind to the behavioral performance of the subjects, seven of the 13 lesioned animals sustained substantial damage to the ADN ($> 70\%$). The remaining six rats were excluded from the behavioral analysis due to insufficient damage to the ADN. Figure 5 shows representative brain sections from a sham control and an ADN lesioned rat, and a schematic diagram indicating the smallest and largest lesions. In addition to damage to the ADN, the majority of rats had some damage that extended beyond the ADN, including damage to the overlying cortex, the fimbria of the hippocampus, and adjacent thalamic nuclei. However, as there was no correlation between lesion size and trials to criterion on the water maze ($r = -.19$, $p = .68$) or total errors on the foraging task ($r = -.10$, $p = .83$) we included these rats in the behavioral analyses.

Water maze training. ADN lesioned rats were impaired early in training on the direction task. A two-way ANOVA (Group $\times$ Phase) on the first block of eight trials (the first day of training) revealed a significant effect of group, $F(1, 12) = 13.50$, confirming that sham controls performed better than ADN lesioned rats early in training (see Figure 6A). There was also a significant effect of phase, $F(1, 12) = 12.21$, 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 $\times$ Phase) on trials to criterion showed only a significant effect of phase, $F(1, 12) = 5.04$ (see Figure 6B). However, the lesioned rats did make more errors than the sham controls, $F(1, 12) = 6.25$, and both groups made more errors in Phase 2 than in Phase 1, $F(1, 12) = 18.97$ (see Figure 6C). The difference in the pattern of results for the trials to criterion measure and the errors measure is due to rats making multiple errors on a single trial.

Foraging task. The data from the 60 trials in which rats found food were divided into five blocks of 12 trials for analyses. The

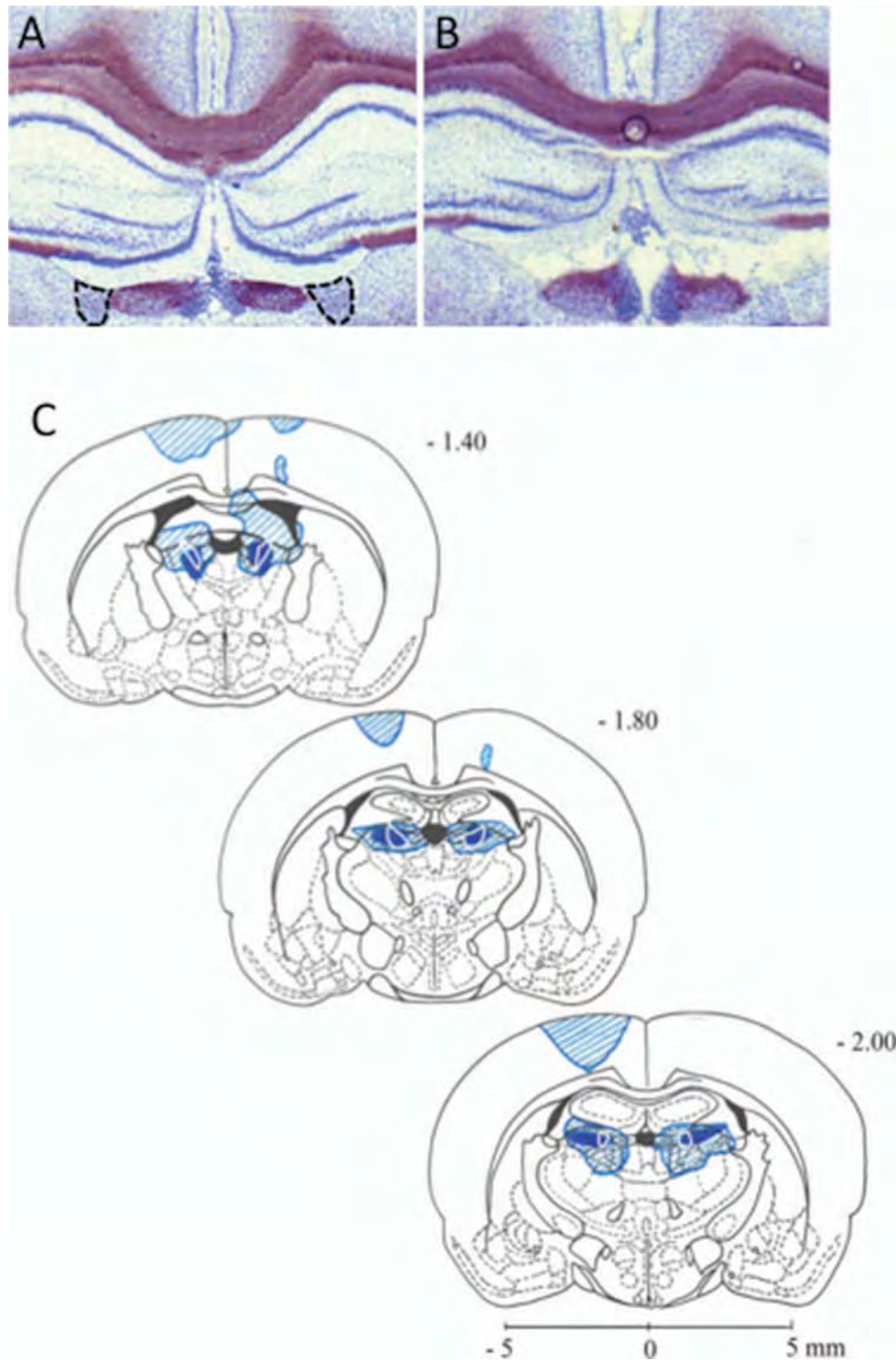


Figure 5. Panel A shows a section of a sham brain with the area of the anterior dorsal nucleus of the thalamus (ADN) outlined in black and Panel B shows the corresponding section from a typical lesioned brain. Panel C shows the anterior and posterior extent of the lesions with hatch marks indicating the largest lesion and solid shading showing the smallest lesion. The area of the ADN is outlined in white. From *The Rat Brain in Stereotaxic Coordinates*, by G. Paxinos and C. Watson, 1998 (4th ed.), San Diego, CA: Academic Press. Copyright 1998 by Elsevier Academic Press. Adapted with permission. See the online article for the color version of this figure.

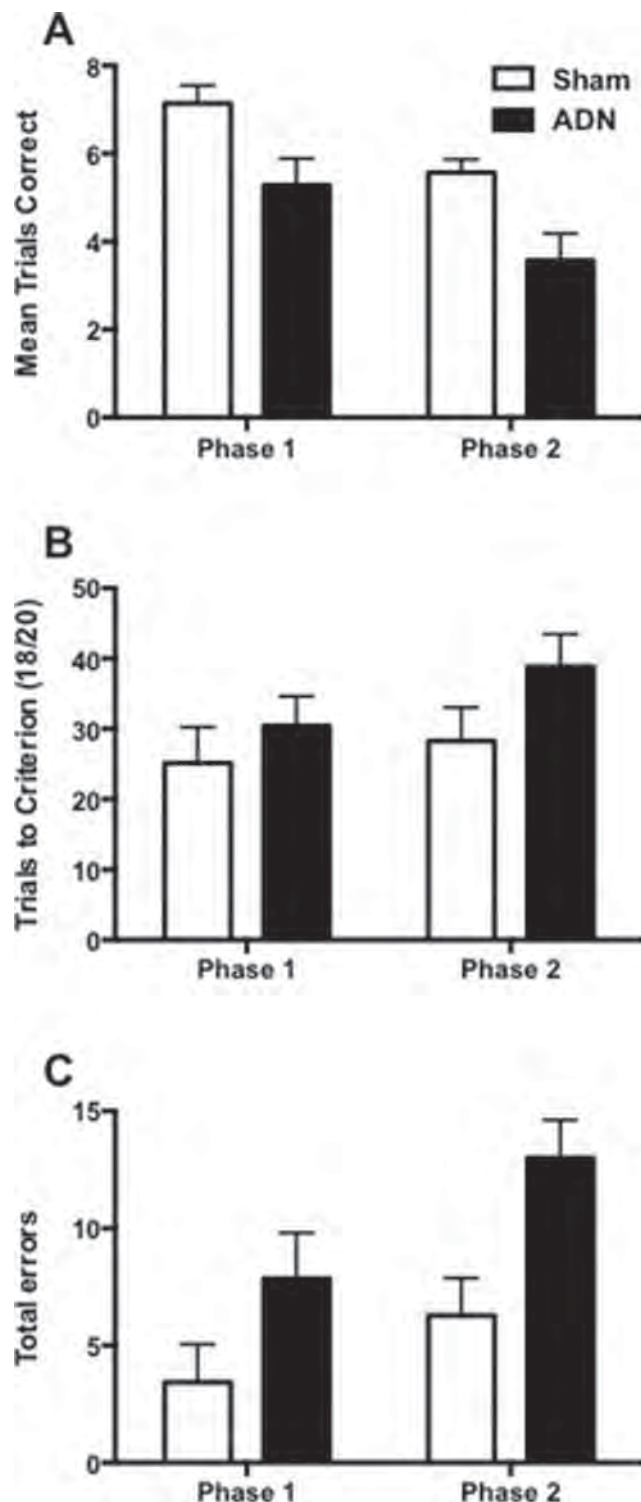


Figure 6. (A) Mean (+ SEM) trials correct for the first eight trials for ADN lesioned rats and sham controls on the direction task in the water T maze (B) Mean (+ SEM), trials to criterion of ADN lesioned rats and sham controls on the direction task (C). Mean (+ SEM). Total errors made by the sham and ADN lesioned rats on the direction task. ADN = anterior dorsal nucleus of the thalamus; SEM = standard error of the mean.

ADN lesioned rats showed impairments on the foraging task. A two-way ANOVA (Group $\times$ Trial Block) on trials correct over blocks of 12 trials revealed only a significant effect of group, $F(1, 12) = 6.19$; $\omega^2 = .27$, confirming that lesioned rats performed worse than sham rats (Figure 7A). A similar two-way ANOVA on errors per block of 12 trials revealed only a significant effect of group, $F(1, 12) = 9.44$, again reflecting the poorer performance of the lesioned rats (Figure 7B). A separate two-way ANOVA (Group $\times$ Error type) examining the different types of errors made by rats in the two groups on their first choice revealed a significant effect of group, $F(1, 12) = 6.19$, and a significant effect of error type, $F(2, 24) = 64.86$. Bonferroni multiple comparisons tests revealed that rats made more memory errors than adjacent ($p < .05$) or other ($p < .05$) errors, and more adjacent errors than other errors ($p < .05$). The ANOVA also revealed a significant Group $\times$ Error type interaction, $F(2, 24) = 5.14$, reflecting the fact that lesioned rats made more adjacent errors than sham controls ($p < .05$). The two groups did not differ in memory ($p > .05$) or other ($p > .05$) errors (see Figure 7C).

General Discussion

As HD cells are thought to underlie an animal's sense of direction, we lesioned two components of the HD cell pathway and assessed acquisition on two tasks in which directional heading would be important for successful performance. It has been previously shown that both electrolytic and neurotoxic lesions to the DTN, an early structure in the HD cell pathway, produced severe impairments on both tasks used in the present study (Dwyer et al., 2013). Here we lesioned the PoS and the ADN, two downstream structures in the HD cell circuit.

In Experiment 1, rats with lesions to the PoS did not differ from sham controls on the direction task in a water T maze. This was surprising given the large impairment observed on this task with lesions to the DTN (Dwyer et al., 2013) and suggests that some compensatory mechanism, or alternate structure, is available after lesions to the PoS but not after DTN lesions. On the foraging task, PoS lesioned rats made more errors overall than sham control rats and were more likely than sham rats to return to holes that were adjacent to the start location as the first choice. These findings suggest there are impairments in heading after PoS lesions, but only on tasks in which rats have to head in multiple directions across trials.

In Experiment 2, 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. The deficit reappeared when the direction was reversed in Phase 2, but the rats quickly reestablished their heading and reached criterion in the same number of trials as sham controls. The initial impairment in the water task is consistent with the disruption in directional responding observed in mice after temporary inactivation of the anterior thalamic nuclei (Stackman et al., 2012). Whether the mice would have shown directional responding with continued inactivation of the anterior thalamus remains to be determined. Although multiple injections are difficult and risk tissue damage, future studies using optogenetic techniques (X. Liu et al., 2012) could answer this question. On the foraging task, ADN lesioned rats were more likely than sham

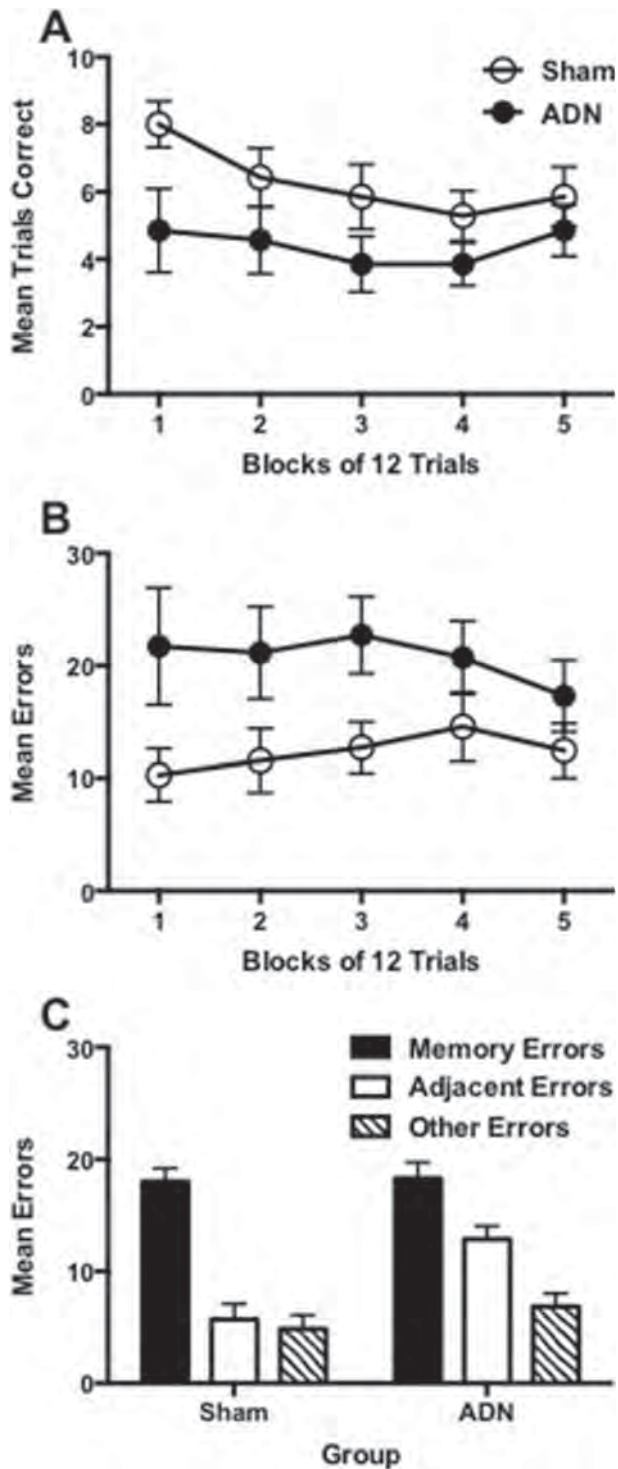


Figure 7. (A) Mean ($\pm$ SEM) trials correct over five blocks of 12 trials for ADN lesioned rats and sham controls on the food-foraging task. (B) Mean ($\pm$ SEM) total number of errors made over five blocks of 12 trials for ADN lesioned rats and sham controls on the food-foraging task. (C) Mean ($\pm$ SEM). Number of first choice memory, adjacent, and other errors made over 60 trials for ADN lesioned rats and sham controls on the food-foraging task. ADN = anterior dorsal nucleus of the thalamus; SEM = standard error of the mean.

controls to return to holes that were adjacent to the start location. This finding is consistent with other research showing impairment on path integration tasks in ADN lesioned rats (Frohardt et al., 2006) and suggests that the ADN is involved in heading.

In both experiments, proactive interference was evident in lesioned and sham controls 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 proactive interference errors when variable start locations are used in combination with variable reward locations (Martin et al., 2011). Although accurate performance on this task could reflect the use of allothetic (e.g., visual) or idiothetic cues, the fact that all rats made a high number of memory errors suggests that they were using allothetic cues to orient.

The behavioral deficits shown here after lesions to the PoS and ADN were smaller than those produced by lesions to the DTN. In the foraging task, for example, lesions to each of the three structures produce deficits in the number of correct trials relative to sham controls. However, the effect size with DTN lesions ($\omega^2 = .91$; Dwyer et al., 2013) was much bigger than with lesions to the ADN ($\omega^2 = .27$) or PoS ($\omega^2 = .20$). This pattern of findings is consistent with some earlier reports. Bett et al. (2012) reported no impairment on a foraging task similar to that used here after lesions to the PoS. In that study the animals were trained on the task prior to surgery. We showed some impairment on the foraging task in Experiment 1, when all components of the task were acquired after surgery. Frohardt et al. (2006) showed mild impairments on a foraging task after ADN lesions relative to lesions of the DTN, consistent with the small errors in heading observed in the present study (as seen by more adjacent errors in lesioned rats compared to sham controls). Thus, whether the task is acquired pre- or postlesion, the deficits with PoS lesions are very small and the deficit produced by lesions to the ADN, while somewhat larger, are not as severe as lesions to the DTN. The deficits produced by both lesions on the foraging task, manifest as increase choice of adjacent holes, might be attributed to a broadening of the firing range of HD cells.

In both experiments in this study, trials were conducted in well-lit rooms with an abundance of cues. With cortical pathways intact, the rats could have used visual cues to update their orientation. In fact, the high number of memory errors on the foraging task suggests that both sham and lesioned rats probably used this strategy. Rats with DTN lesions, however, were severely impaired even when visual cues were present (Dwyer et al., 2013). This finding suggests that the DTN lesions produced disorientation that prevented the rats from using visual cues or perceiving them as stable. This level of disorientation is not evident with damage to downstream structures in the HD circuit, such as the ADN and the PoS. Although DTN lesions presumably disrupt HD cell activity in the LMN, ADN, and PoS, rats with ADN and PoS lesions should have an intact HD signal in the LMN and intact angular head velocity (AHV) information in the DTN and LMN (see Clark & Taube, 2012; Taube, 2007, for reviews). The present findings, combined with earlier reports of more severe impairments after DTN lesions (Dwyer et al., 2013; Frohardt et al., 2006), suggest that HD and AHV information in the DTN and LMN might have behavioral significance without the ADN and PoS. As an alternative, information from the DTN and LMN may reach cortical areas

via pathways that do not include the ADN and PoS. What these pathways may be remains to be determined but tracing studies have revealed widespread projections from the DTN to mesencephalic, diencephalic, and telencephalic structures (Groenewegen & Van Dijk, 1984). Future work should examine the behavioral significance of these projections.

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