THE BEHAVIOURAL EFFECTS OF LESIONS TO THE HEAD DIRECTION CELL CIRCUIT ON SPATIAL LEARNING IN RATS

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Abstract

The head direction (HD) cell circuit acts as a neurological compass, providing information on directional heading to the hippocampus. The HD signal originates in reciprocal connections between the lateral mammillary nuclei (LMN) and the dorsal tegmental nuclei (DTN); the signal is then relayed to the anterodorsal thalamic nuclei (ADN) and cortical areas. The functional role of these regions in spatial learning problems is not well understood. Rats underwent neurotoxic lesions of either the LMN or ADN and were tested using three tasks thought to depend on directional heading. Impairments were seen in both lesioned groups in the water T-maze tasks that required discrimination of headings that were 90° (rotation task) or 180° (direction task) apart. Lesioned rats were also impaired on a 12-arm maze if they were required to discriminate between adjacent arms that were 30° apart, but not when arms were separated by at least 90°. Performance on a response reversal task, where the maze was rotated by 90° each time the response-reinforcer contingency changed, was unaffected by lesions to the LMN or ADN, indicating that this task may not be critically dependent on the HD cell signal. Our results suggest that the difficulty of spatial problems relates to the angular specificity of direction discrimination required.

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The Behavioural Effects of Lesions to the Head Direction Cell Circuit on Spatial Learning in Rats

Spatial navigation is a skill so integral to daily life that it is rarely considered a skill at all, but even something as ubiquitous as remembering a daily commute relies heavily on a system that is not yet fully understood. It is not a system unique to humans either; evidence of spatial learning has been found across a staggering and ever-increasing variety of species, from insects (Collett, Chittka & Collett, 2013), to crayfish (Tierney, Baker, Forward, Slight & Yilma, 2018) to terrestrial toads (Daneri, Casanave & Muzio, 2015) to pigeons (Miyata & Fujita, 2010) to lemurs (Teichroeb & Vining, 2019). Moreover, spatial navigation is closely tied to memory, to the point where many common laboratory assessments of learning and memory in rodents are at least partially dependent on spatial performance. Given this overlap, it is absolutely crucial to have a thorough understanding of spatial learning in rodents, independent of memory systems, so as to avoid any unnecessary confounds in experimental design. This understanding will also serve as a foundation for further exploration of how and why this system malfunctions in both rodents and humans.

Regardless of species, successful navigation relies on knowledge of both location and direction; a “you are here” dot on a map is useless without knowledge of orientation. This information originates in the head direction (HD) cell signal, first described by Taube, Muller and Ranck (1990), who recorded cells in the postsubiculum (PoS) of rats which fired based on the direction the rat’s head was facing, regardless of the animal’s body movement or location (see Taube, 2007 for a review of HD cell properties). Subsequent research identified HD cells in a number of brain regions including the

anterodorsal thalamic nucleus (ADN), lateral mammillary nucleus (LMN), entorhinal cortex, retrosplenial cortex (RSP) and dorsal tegmental nucleus (DTN), among others (Blair, Cho & Sharp, 1998; Taube, 2007; Taube & Muller, 1998). In the three decades since their discovery, work in mapping out these various regions has lead to the defining of the HD cell circuit, which is thought to originate in reciprocal connections between the LMN and DTN, with input from the vestibular nuclei (Butler & Taube, 2017; Clark & Taube, 2012; Taube, 2007). Though the full circuit and its connections is incredibly complicated, involving nearly 20 brain regions and a web of reciprocal and cross connections, there is much evidence to support a central or primary pathway in the direction of DTN→LMN→ADN→ PoS/RSP (see Figure 1), which then projects to the hippocampus via the entorhinal cortex (Blair et al., 1998; Taube, 2007; Taube & Muller, 1998). It is through this connection that directional heading information integrates with the equally important knowledge of location.

Decades of research have determined that knowledge of location originates in the preferential firing patterns of two specific types of neurons known as place cells and grid cells. Discovered in rats in 1971 (O'Keefe & Dotrovsky), place cells located in CA1 and CA3 of the hippocampus fire in response to the head's location relative to the environment (see Muller, 1996 for review); generally speaking, each place cell fires for a unique global location, so long as it is distinguishable (Harland, Grieves, Bett, Stentiford, Wood, & Dudchenko, 2017). Grid cells, on the other hand, fire in response to a number of constant locations separated by clear areas of silence, such that their firing pattern can be mapped onto the environment as a grid made up of equilateral triangles (Moser, Rowland & Moser, 2015). Located primarily in the entorhinal cortex, grid cells are functionally

upstream of place cells and provide additional spatial information to the hippocampus (Moser et al., 2015).

Rather than separate systems relaying disparate pieces of information to the hippocampus, it seems that place cells, grid cells, and HD cells, along with other spatially oriented neurons such as angular head velocity cells and border cells (Butler & Taube, 2017; Harland et al., 2017; Taube 2007) communicate with each other, both within and upstream of the hippocampus, creating a complex and interconnected spatial neuron network (Taube, 2007). The entorhinal cortex, a major source of input into the hippocampus, contains place cells, grid cells and HD cells (Clark & Taube, 2012), indicating that it may be a key region in the integration of these signals (Giocomo, Stensola, Bonnevie, Van Cauter, Moser, & Moser, 2014; Moser, et al., 2015; Taube 2007). Though it is thought to be the origin of the HD cell signal, the DTN actually contains a higher proportion of angular head velocity cells (Taube & Bassett, 2003; Taube 2007). Indeed, a recent study by Harland et al. (2017) has confirmed that place cells are also directly informed by the HD cell circuit. Place cells will respond the same way to identical chambers within a room, so long as the chambers are oriented in the same direction, but will distinguish between the two locations if the chambers are rotationally distinct from one another. Capitalising on this, Harland et al. (2017) performed LMN lesions on rats and allowed them to explore a series of connected chambers, oriented either parallel or at 60° angles to one another, while recording place cell firing rates; they found that LMN lesions abolished the ability of place cells to distinguish between rotated identical chambers, implying that place cells rely on the input of HD cells to distinguish between similar environments.

All of this research, however crucial to our understanding of spatial learning, is primarily neurological, and deals little with actual behaviour; the functional aspects of these neurological circuits. As insightful as early work on HD cells was, much of it failed to properly distinguish between types of spatial learning, as had behavioural research before it (Skinner, Etchegary, Ekert-Maret, Baker, Harley, Evans, & Martin, 2003). The hallmark studies by Tolman, Ritchie and Kalish (1946a; 1946b) trained rats to navigate a plus maze or a more complex starburst maze for a food reward in an attempt to distinguish response learning from place learning. In both experiments a significant portion of the rats headed towards the goal area during test conditions, and the authors argued that they were exhibiting a place disposition; however, in both sets of experiments the location of the food reward relative to room cues was confounded with the direction the rats had to run in order to approach the goal, making it impossible to separate place learning from direction learning. The interpretation of these findings was called into question a few years later when Blodgett, McCutchan and Mathews (1949) used a T-maze to design an experiment in which the place condition required travel in a different direction, as well as a different response, and found that rats in the place condition made the largest number of errors, while those in the direction condition made the fewest errors. These findings should have fast tracked the study of spatial learning, but instead were misinterpreted in the literature as support for rats using place solutions under good lighting conditions (Restle, 1957), leading to the false belief within the literature that place solutions are a primary strategy ahead of direction or response solutions (Skinner et al. 2003).

In order to clarify this issue, Skinner et al. (2003) replicated the findings of Blodgett et al. (1949) in a series of appetitive open field maze experiments in which the rats had full visual access to room cues. In the first experiment, rats were placed in a constant start location relative to the maze (which was moved between trials, dependent on the group) and trained to find a food reward by turning left (or right), traveling East (or West) or heading to the same location relative to room cues, named the response, direction and place groups, respectively; only the place group failed to learn the location of the food reward. Subsequent experiments found that place problems became soluble when start points were more distinct with suspected contributions from both distinct cues and path traveled from the holding cage to the maze (Skinner et al., 2003). This indicates that a crucial aspect of solving place problems is determining one's starting point and orientation, which may depend on the firing patterns of HD cells.

The issue was investigated further by Whyte, Martin and Skinner (2009) using an aversive water T-maze to assess the influence of changes to orientation in spatial problems; their findings again showed that place learning is more difficult than both response and direction learning, but that place learning is possible when the start points at the different maze positions are made more distinct by rotating the maze. The initial place problem, used by Blodgett et al. (1949) and Skinner et al. (2003), required the rats to discriminate between start points that were separated in space but oriented in the same direction, as the maze was simply translated (shifted to the left or right) between trials; in the response and direction tasks, the start points were oriented 180° apart (see Figure 2 for diagram of the translation (place) and direction problems). In the new (rotation) place task, the start points at the two maze positions were oriented 90° apart. Using both dry

land appetitive and aversive water T-mazes with a combination of translation and rotation techniques to distinguish start point location from orientation in both direction and place learning, orientation seems to be the more crucial aspect, as rats show more learning impairment when given distinct start locations with similar headings than in similar locations with distinct headings (Peckford, McRae, Thorpe, Martin & Skinner, 2013; Skinner, Horne, Murphy & Martin, 2010). These behavioral findings are consistent with recent evidence that directional information can cause remapping of hippocampal place cells between identical compartments. Place cells show repetition of place fields when multiple compartments are parallel but not when compartments are radially oriented (Grieves, Jenkins, Harland, Wood, & Dudchenko 2016).

In behavioural work, significant evidence has pointed at start point orientation as a crucial factor, and in neurological work the firing patterns and connectivity of the HD cell circuit has been relatively well mapped; however, the connection between cell activity and behaviour has remained comparatively patchy (Dwyer, Ingram, Snow, Thorpe, Martin, & Skinner, 2013; Muir and Taube 2002; 2004; Stackman, 2010; but see Valerio & Taube, 2012). Several studies have shown that lesions to the DTN produce significant impairments on a variety of navigation tasks, including a food-carrying task, a modified water maze task and others (Bassett, Tullman & Taube, 2007; Clark, Rice, Akers, Candelaria-Cook, Taube, & Hamilton, 2013; Frohardt, Bassett & Taube, 2006; Taube & Bassett, 2003). Dwyer et al. (2013) compared the behavioural effects of electrolytic vs. neurotoxic lesions to the DTN using a direction task in a water T-maze and a food foraging task. The food task consisted of a circular open field maze with eight evenly distributed access holes around the perimeter (45° apart) and three food cups in a

triangular formation in the center of the maze. During training the rats cage was placed under one of the access holes so they could enter the maze, retrieve a food pellet from the center, and return to their home cage via the same hole. Rats were trained to forage from three different holes, and performance measure by number of errors, i.e. returning to the wrong hole. As the only contextual cues available were distal cues from the room, discriminating between the access holes would theoretically rely on the HD signal (Dwyer et al., 2013). They found that both electrolytic and neurotoxic lesions produced similar impairments on both tasks, indicating that the theorized conditional strategy based on start orientation is, in fact, dependant on the DTN. Further neurotoxic lesion work by Peckford, Dwyer, Snow, Thorpe, Martin, and Skinner (2014) showed that lesions to the PoS did not impair performance on a water T-maze task (but see Taube, Kesslak & Cotman, 1992), though the same rats did show impairment in a food foraging task. Rats with lesions to the ADN were also impaired on the food foraging task and showed early impairment on the water T-maze task, though this impairment faded with additional training (Peckford et al., 2014). These results were notably less severe than the impairments seen following DTN lesions on the same tasks (Dwyer et al., 2013). Meanwhile, lesions to the LMN, which is the other half of the reciprocal connections to the DTN thought to give rise to the HD cell signal (Butler & Taube, 2017; Clark & Taube, 2012; Taube 2007; see Figure 1), also produce only small impairments on spatial learning tasks (Bassett et al., 2007; Vann, 2005; 2011). These impairments increase if the mammillary bodies or mammillothalamic tract are included in the lesions (Sziklas & Petrides, 2000; Vann & Aggleton, 2003); however, as these structures do not contain HD cells, it can be surmised that these deficits are not connected to the HD cell circuit.

Despite these inconsistencies, it has been clearly shown that lesions to the LMN disrupt downstream firing patterns in the ADN (Blair et al., 1998).

There is a gap between behavioural research on spatial learning and electrophysiological research on the activity and connections of the HD cell circuit, limiting our understanding of the functional significance of the components of this circuit. While lesions to the DTN result in robust impairments on several spatial learning tasks, the impairments resulting from lesions to downstream structures in the HD cell circuit, including the LMN, ADN and PoS, have been both smaller in magnitude and less consistent; the variety of tasks used to assess navigation further contributes to this lack of clarity. In the current study, we assessed the outcome of neurotoxic lesions to the LMN and ADN on a water T-maze task, response reversal task and radial arm maze task, three tasks where directional heading might play a role in problem solution. Evidence from previous studies indicate that distinct start orientation plays a key role in the solubility of these tasks, implying the involvement of the HD cell circuit.

Each HD cell has a preferred firing range, and the range of each cell overlaps with the range of many other cells providing a comprehensive coverage of all directions (Preston-Ferrer, Coletta, Frey & Burgalossi, 2016; Taube, 2007). In a spatial problem requiring discrimination between two orientations, the closer the angle of those orientations, the more overlap there will be in the firing ranges of the HD cells activated; it stands to reason that the more overlap there is, the more difficult the discrimination will be, and this is supported by behavioural data that less distinct start points increase the difficulty of the spatial problem (Cahill, Fifield, Thorpe, Martin & Skinner, 2015; Dwyer et al. 2013; Peckford et al. 2013; Skinner et al., 2010). Further evidence is that mice,

which have broader HD firing ranges than rats, perform worse than rats on spatial problems requiring discrimination of start points separated by 90° (Cahill et al., 2015; Yoder and Taube, 2009).

The water T-maze is a commonly used spatial problem, and a significant body of research indicates that, when different maze positions are used, differences in the start point orientation between positions are critical for the solubility of both place and direction problems (Cahill et al., 2015; Peckford et al., 2013; Peckford et al., 2014; Skinner et al., 2010; Whyte et al., 2009). Specifically, rats will readily learn direction problems, in which the start arms are separated by 180,° as well as rotation problems where start arms are 90° apart, but have difficulty learning translation problems where the start arm, though moved in space, is oriented in the same direction. Interestingly, mice, unlike rats, acquire the direction problem more rapidly than the rotation problem, potentially due to the broader HD cell firing ranges of mice compared to rats (Cahill et al., 2015; Yoder & Taube, 2009). It has also been shown that the direction problem, with 180° distinct start arms, is easily soluble for control rats but very difficult for rats with lesions to the DTN (Dwyer et al., 2013), suggesting a dependence on the HD cell signal. The experiments discussed in this paper used both the direction and rotation problem versions of this task.

The current study also utilised response reversal learning, a well documented measure of acquiring competing behaviours in similar environments that is known to be facilitated by changes in context between reversals. What is interesting is which type of contextual changes facilitate learning, and which do not; changes in room result in rapid acquisition of the new response, while changes to visual cues in the same room have no

effect on learning (Skinner et al., 2014; Wright, Williams, Evans, Skinner & Martin, 2009). Rotating the start arm, even in the same room and with no other cue changes, is sufficient to facilitate rapid acquisition of response reversal learning (Wright et al. 2009), indicating dependence on start point orientation and the HD cell circuit.

The final task used in this paper is the radial arm maze, a spatial problem based on location discrimination and which was shown in 1997 (Dudchenko & Taube; McDonald & White, 1993) to be directly correlated with HD cell firing. In this task, start location is consistent, as are extramaze cues, and the rat must learn to distinguish between one consistent baited arm and two variable non-baited arms using directional heading and/or distal cues. Previous work using an eight arm version of the maze found impairments following lesions to the PoS, retrosplenial cortex and anterior thalamic nuclei (Harvey et al., 2017; Pothuizen et al., 2008; Taube, Kesslak & Cotman, 1992). Oddly, an LMN lesion study found no impairment on this task (Vann, 2018), but it is possible that this was due to the procedure used, which required rats to visit all eight arms sequentially; discrimination between arms was not required. The current study used a twelve-arm apparatus, which allows for control of the distance between arms down to 30° apart, and a task which should directly tax the HD system; rats were trained to discriminate a single baited arm location from two non-baited arms using only directional heading based on distal cues.

Experiment 1

Rats with neurotoxic lesions to the LMN were compared to sham controls on the rotation and direction problems in the water T-maze, response reversal learning and the

adjacent and separate conditions of the radial arm maze. Previous evidence from the literature would suggest that the abatement of the HD signal caused by LMN lesions would result in impairments on all three of these tasks.

Method

Subjects. Thirty-two experimentally naïve, male Long-Evans rats, weighing 250g-300g at the beginning of experimentation, were obtained from the Charles River Laboratory (Montreal, Quebec, Canada). Rats were individually housed in transparent plastic cages (45 x 25 x 21 cm) with sealing plastic lids which connected to a SmartFlow oxygen ventilation system. The colony room was temperature controlled to 20° Celsius and maintained on a 12 hour light-dark cycle, with lights on at 0700. All rats had ad libitum access to food and water during surgery, recovery phase and water T-maze training. One week prior to beginning the response reversal task, rats were placed on food restriction, with one meal per day. Rats were weighed twice a week and food amounts adjusted once a week, as necessary, to maintain 85% of their free-feed weight. This feeding schedule was kept in place until the end of experimentation. All procedures were conducted in accordance with the Canadian Council on Animal Care guidelines and were approved by Memorial University of Newfoundland's Committee on Animal Care.

Surgery. Rats were anesthetized using isoflurane and placed in a stereotaxic device (Model 900, Kopf, Tujunga, CA) in the skull-flat position. Each rat was administered 2ml of saline and 1mg/kg of Meloxicam subcutaneously during surgery. Following a scalp incision, two holes were drilled in the skull above the target injection sites, using bregma as the reference point for coordinates (in mm, AP: -4.5, ML: $\pm$1.0). Sixteen rats were given neurotoxic lesions to the lateral mammillary nucleus, for which a

1µl straight Hamilton syringe was used to inject 0.15µl of N-methyl-D-aspartate (NMDA; 10mg/ml) in one site per hemisphere (DV: -9.2 from the surface of the brain). The needle was left in place for 3 min before and 5 min after each injection in order to counteract any tissue compression cause by insertion of the needle. The needle was then removed and the incision sutured. An intraperitoneal injection of diazepam (1mg/kg) was administered as soon as the anaesthesia began to wear off in order to minimize seizures. The remaining fourteen rats received sham surgeries in which holes were drilled in the skull but no intracranial injections were administered. All rats were given at least one week of recovery before beginning behavioural testing.

Apparatus and Materials. The water T-maze apparatus consisted of a circular metal tank (120cm in diameter, 31cm high) with plexiglass walls extending 31cm further above the metal tank. A plexiglass plus maze, also 31cm taller than the metal tank, was inserted into the tank and converted into a T-maze by using a section of clear plexiglass snapped onto the arm opposite the start arm using butterfly clips, such that access to that arm was blocked without obstructing visual access. The arms of the maze were 11.5 cm wide and 52.5cm long, and the water level was kept approximately 2.5cm below the rim of the metal tank. The whole apparatus was placed on a metal frame with wheels. The water was rendered opaque by adding approximately 250ml of nontoxic white Tempera paint (Rich Art Color Company, Northvale, NJ), and was left overnight to equilibrate to the temperature of the room (approximately 20°C). 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 plexiglass base for stability; the platform rested 1-2 cm below the surface of the water, thus obscuring it from the view of the rat.

The response reversal apparatus consisted of a wooden plus maze with four arms (38.5cm×15.5cm) radiating at 90° from a square center (15.5cm×15.5cm). The maze was elevated 61.3cm off the floor, and a circular 2.5cm depression at the end of each arm served as a food cup. The plus maze was converted to a T-maze by the use of two clear plastic mouse cages, inverted and stacked on the arm opposite the start arm to create an impassable barrier.

The radial arm maze consisted of twelve painted metal arms (7.5×90cm) radiating from a circular center platform (37cm in diameter) elevated 88.5cm off the ground on a metal base. At the end of each arm a small hole held a 3cm circular metal food cup. The entire maze was rotated between trials. As only two arms were open for any given trial, the remaining ten arms were each blocked with two stacked and inverted clear plastic mouse cages.

Procedure

Water T-Maze. All rats were trained to locate a hidden platform from two maze positions; lesion and sham rats were divided evenly between the Direction and Rotation conditions. In the Direction group, the maze was translated and the start point rotated 180° such that only the direction travelled (East/West), and not the location of the platform or the response required (left/right), was held constant. In the Rotation condition, the maze was rotated between trials, shifting the start point by 90°, such that the location of the platform was held constant while the response required and direction travelled varied. In both conditions the maze positions used were counterbalanced so that half the Direction rats went East, using positions A and C, while half went West using

positions B and D (see Figure 3). In the Rotation group, half were trained to positions A and E, while half were trained to positions B and F.

The rats were brought into the training room in groups of seven or eight and transferred to plastic holding cages similar to their home cages; while not being trained rats remained in their home cages in the colony room. On each trial, a rat in its holding cage was carried in a clockwise direction to a chair positioned at the start arm. The rat was placed in the start arm facing the wall of the maze, and 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 whole body, minus the tail, was inside the arm. A correct trial was one in which the rat located the hidden platform in under 60s without going down any incorrect arms. Once the platform was located, the rat was allowed to sit on it for 5s before being removed and returned to the holding cage. If the rat did not locate the platform within 60s, it was placed on the platform by the experimenter. For the duration of the trial, until the platform was located or assistance was required, the experimenter remained at the end of the start arm. Upon completion of the trial, the rat, in its holding cage, was carried counterclockwise back to the holding table before beginning the next rat's trial. The intertrial interval was approximately 5-10 minutes, and each rat received eight trials per day until a criterion of 18/20 trials correct was reached. No more than two trials in a row were given from the same maze position.

Response Reversal. For at least three days before testing, rats were given three Kellogg's Froot Loops™ in their home cage each day. Pretraining took place in the same room as water T-maze testing, though the water T-maze itself had been removed and the response reversal apparatus put in its place. Rats were placed on the start arm of the maze,

with multiple Froot Loops™ scattered around the maze, for 5 min or until they ate all of the Froot Loops™. With each successful trial, the number of Froot Loops™ on the maze was reduced until there were only two, one in each food cup. Rats were given one to two trials a day until they ate from the food cups within 60s of being placed on the maze. Once all rats had completed pretraining, they were matched based on performance and divided into Control and Direction groups.

Testing took place in a different room, which the rats had not been habituated to, using the same apparatus from pretraining. Rats were brought into the room in groups of four and placed on a holding table in the south-west corner of the room, where they remained until the whole group had completed testing. For each trial the rat was removed from its home cage and placed on the start arm facing the experimenter for 60s or until a choice was made. A rat was considered to have made a choice when it's full body, minus the tail, crossed into either the right or left choice arm. If an incorrect choice was made, the rat was removed from the maze without being allowed to explore the other arm. The correct arm had half a Froot Loop™ hidden in the food cup at the end of the arm.

All rats were trained to make a consistent response - half were trained to go right, half to go left - until they reached a criterion of 9/10 trials correct. A total of five trials were given each day with an intertrial interval of approximately 2-4 minutes, regardless of which trial they reached criterion on. The day after criterion was reached, rats began the first reversal, and continued following the same protocol until they achieved criterion. In the Control group the response requirement was reversed (from right to left or vice versa) but the maze position remained constant. In the Direction group, the orientation of the maze was shifted by 90° when the response requirement was changed (see Figure 4);

the mouse cages used to block off the fourth maze arm were also moved to be opposite the new start arm for these trials. This procedure was repeated for four reversals following acquisition. The apparatus was wiped down with Peroxigard™ disinfectant between trials.

Radial Arm Maze. Radial arm maze (RAM) training took place in the same room as water T-maze testing, though the water T-maze had been removed and the RAM apparatus put in its place. Rats were brought into the room in squads of six to eight and placed on a holding table at the south end of the room in their home cages. The pretraining phase lasted two days; each rat was placed in the center of the apparatus facing away from the experimenter and allowed to freely explore for 5 min. On the first day the assigned food arm and the left arm position were open; on the second day the assigned food arm and the right arm position were open. No food was placed on the maze for pretraining, but each rat received three Froot Loops™ in their cage at the end of each day of pretraining.

In the testing phase, each rat was trained to a single arm position which consistently had half a Froot Loop™ in the food cup. On each trial one other arm was open, either to the left or the right of the assigned food arm (the same positions they were exposed to in pretraining). The order of which incorrect arm was open was pseudorandomized in order to avoid pattern recognition; the same arm positions were never presented more than twice in a row. The rats were evenly divided into Adjacent and Separate groups. For the Adjacent group, the incorrect arms presented were directly next to the food arm, separated by 30°. In the Separate group, the presented arms were at least 90° away from the food arm, separated by at least two other blocked arms (see Figure 5).

For all trials the rat was carried clockwise from its home cage around the maze and placed in the center facing away from the experimenter, who stood equidistant between the two open arms and remained there for the duration of the trial. The trial ended when the rat ate the Froot Loop™, made an incorrect choice, or reached 60s without having made a choice. A rat was considered to have made a choice if its body, not including the tail, passed the barrier on the adjacent arm, approximately one third down the length of the arm. In the event of an incorrect choice, the timer continued until the rat either reached the end of the arm with the empty food cup or reversed back to the center of the maze, at which point it was removed from the apparatus without the opportunity to explore further. At the end of each trial the rat was carried back to its home cage in a counterclockwise direction, following the same path the experimenter walked to the maze. The intertrial interval was 60s, which began as soon as the rat was picked up from the maze and ended as the rat was placed on the maze to begin the next trial. Ten trials were given each day until a criterion of 18/20 trials correct was reached, or for a total of 140 trials for those rats that did not reach criterion. The maze was rotated between trials so that each open arm had not been used in the previous trial, and was cleaned with Peroxigard™ disinfectant between rats.

Histology. Following completion of all behavioural tasks, rats were euthanized using carbon dioxide and the brains were immediately extracted. A solution of 2-methylbutane kept at -76° was used to freeze the brains, which were subsequently sliced coronally to a thickness of 30μm using a Lecia CM3050 S cryostat machine. Slices were then stained with a solution of 10% Cresyl Violet and digitally imaged. Three slices of the LMN were chosen for analysis from each brain, based on comparison to the rat brain atlas

(Paxinos & Watson, 1998) at approximately -4.5mm to -4.8mm rostral-caudal relative to Bregma. The selected sites were analysed using Image-J software to calculate percentage of damage by area; the mean area spared was determined for each subject by averaging the sum of spared tissue in all three slices, bilaterally. This was then compared to the total mean area from sham brains, determined in the same fashion, to estimate the percent damage using the formula: LMN Damage = [(Total Area - Spared Area) / Total Area] X 100. Subjects with less than 50% damage were determined to have incomplete lesions and were omitted from statistical analysis. Nine rats were omitted based on this criterion, leaving seven successful lesions.

Results

Of the 16 lesioned animals, nine were excluded from behavioural analysis due to insufficient damage to the LMN (<50%). Figure 6 shows representative sections from an LMN-lesioned brain as well as a sham brain for comparison; damage to the LMN is evidenced by the holes visible in the ventral aspect of the slice, just lateral to the midline of the brain.

Water T-Maze. Sham rats performed better than lesioned rats on both the direction and rotation problems, requiring fewer trials to reach criterion (see Figure 7). A two-way (Group (LMN vs. Sham) x Task (Direction vs. Rotation) analysis of variance (ANOVA) showed a significant main effect of Group, $F(1, 17) = 5.622$, $p < .05$, $\eta_{p^2} = 0.248$, as well as a significant main effect of Task, $F(1, 17) = 5.496$, $p < .05$, $\eta_{p^2} = .244$, showing that rats required more trials to reach criterion on the rotation task than on the direction task. These effects were large, each accounting for nearly 25% of the variance.

Response Reversal. Trials in which the rat did not make a choice were excluded from the analysis. Both the LMN lesioned and sham rats in the Direction condition showed a facilitation of response reversal learning, reaching criterion in fewer trials on the later reversals. This pattern was not evident in the Control condition (Figures 8 & 9). A three-way (Group (LMN vs. Sham) x Condition (Direction vs. Control) x Task (ACQ-R4)) ANOVA showed a significant Condition x Task interaction, $F(4, 68) = 4.976$, $p = .001$, $\eta_{p^2} = 0.226$, confirming that the improvement in task acquisition across reversals was more evident in the Direction condition than in the Control condition. Lesions to the LMN did not impair this facilitation of reversal learning.

Radial Arm Maze. Both LMN-lesioned and sham rats performed better in the Separate condition than the Adjacent condition, requiring fewer trials to reach criterion (see Figure 10). This was confirmed by a two-way (Group (LMN vs. Sham) x Task (Separate vs. Adjacent)) ANOVA which found a significant main effect of Task, $F(1, 12) = 7.863$, $p < .05$, $\eta_{p^2} = .396$, but no significant interaction or main effect of Group. All sham rats reached criterion within 150 trials, as did all LMN lesioned rats in the Separate condition, but two out of three LMN lesioned rats in the Adjacent condition did not meet criterion within 150 trials. This indicates the possibility that an interaction may exist, and perhaps could have been seen if the power of the experiment, which was limited by number of subjects (LMN Adjacent n = 3, LMN Separate n = 4), had been greater. Sidak's multiple comparisons test revealed that the difference in trials to criterion in the Adjacent condition vs. the Separate condition was significant for the LMN lesioned rats ($p < .05$), but not for the sham rats.

Experiment 2

In Experiment 2, rats with lesions to the ADN were compared to sham controls on the same tasks used in Experiment 1, with the exception of the separate condition of the radial arm maze, which was not used as it did not yield any significant results in Experiment 1. It was anticipated that ADN lesions would yield some impairments on all three tasks.

Method

Subjects. Forty experimentally naïve, male Long Evans rats were obtained from Charles River Laboratory (Montreal, Quebec, Canada) and weighed 250-300g at the beginning of experimentation. The rats were maintained as in Experiment 1. All procedures were conducted in accordance with the Canadian Council on Animal Care guidelines and were approved by Memorial University of Newfoundland's Committee on Animal Care.

Surgery. Seventeen rats received neurotoxic lesions of the ADN using 10mg/kg NMDA. One large hole per hemisphere was drilled in the skull, using Bregma as a reference point, and two injections were made per site (in mm, AP: -1.5, -1.8, ML: $\pm$1.5, DV: -5.4 from the surface of the brain) of 0.1µl of NMDA. Surgical procedures were otherwise identical to those outlined in Experiment 1. Sixteen rats received sham operations in which the holes were drilled but no NMDA was infused.

Apparatus and Materials. The water T-maze and radial arm maze apparatuses used were the same as those described in the previous experiment, and took place in the same room. Response reversal testing used the same apparatus and room as described in

Experiment 1; however, the pretraining phase of response reversal took place in the same room as the testing phase, using a different apparatus consisting of a single wooden alley (92.5cm×15.5cm) elevated 61.3cm off the ground.

Procedure. The water T-maze and response reversal tasks were identical to those described in Experiment 1. Except for the noted change in apparatus and room, the pretraining protocol for the response reversal task remained the same. The radial arm maze was identical to Experiment 1 except for the omission of the Separate condition; as there were no differences between sham and LMN lesioned rats in the separate condition, all rats in Experiment 2 were placed in the Adjacent condition.

Histology. Brain extraction and lesion analysis procedures were identical to those outlined in Experiment 1, except that the rostral-caudal sites used were at -1.8, -1.88, and -2.12 in mm relative to Bregma, referenced from the rat brain atlas (Paxinos & Watson, 1998). The same equation was used (ADN Damage = [(Total Area -Spared Area) /Total Area] X 100) and the 50% damage cut-off was still used as the basis for inclusion. Based on this criterion, nine rats were omitted from analysis, leaving eight successful lesions. Additionally, two control rats were removed from analysis as one died during testing (suspected stroke/brain hemorrhage) and another became sick near the end of the experiment; both were later found to have extensive cortical damage from an unknown cause. As the cortical damage was in the same location as the holes drilled in the skull, we can surmise that the cortical loss was related to the surgery.

Results

Of the 17 lesioned animals, nine were excluded from behavioural analysis due to insufficient damage to the ADN (<50%). Figure 11 shows a representative ADN lesioned brain. One sham rat died during the response reversal task of a suspected stroke, and another sham rat became sick shortly after the completion of testing; histological analysis later revealed extensive cortical damage to both brains, and the animals were excluded from behavioural analysis.

Water T-Maze. Similar to the results seen in Experiment 1, sham rats performed better than ADN lesioned rats in both the Direction and Rotation conditions. A two way (Group (ADN vs. Sham) x Task (Direction vs. Rotation)) ANOVA showed a significant main effect of Group, $F(1, 18) = 11.89$, $p < .05$, $\eta_p^2 = .398$. The effect of Task approached significance, $F(1, 18) = 4.40$, $p = .05$, $\eta_p^2 = .196$. No significant interaction was found, though a visual trend in the data suggests that the disparity in trials to criterion was larger in the ADN lesioned rats than in the control rats (see Figure 12). As in Experiment 1, power was limited due to the number of successful ADN lesions (ADN Direction n = 4, ADN Rotation n = 4); it is therefore possible that an interaction exists but could not be detected due to insufficient power.

Response Reversal. One lesioned rat and one sham rat were excluded from the analysis due to anomalous performance, i.e. trials to criterion exceeding two standard deviations from the mean on at least one reversal. An initial three way Group (ADN vs. Sham) x Condition (Direction vs. Control) x Task (ACQ-R4) ANOVA using the 9/10 trials to criterion found a significant main effect of Condition, $F(1, 13) = 18.839$, $p <$

.001, η_{p^2} = .592; as expected, rats in the Direction condition performed better than rats in the Control condition. A significant main effect of Task, $F(1, 52) = 4.172$, $p < .005$, was also found, indicating that performance improved with additional reversals (see Figure 13). There was no significant Task x Condition interaction; however, trends in the data indicated that the improvement seen across reversals was primarily due to facilitation in reversal learning in the Direction condition only (see Figure 14). To confirm this, we performed separate two-way Group (ADN vs. Sham) x Task (ACQ-R4) ANOVAs, which showed a significant effect of Task in the Direction condition, $F(4, 20) = 9.650$, $p <$ .001, $\eta_{p^2} = .659$, but no significant effect of Task in the Control condition. Further analysis of the Direction condition with Tukey's multiple comparisons tests showed that the number of trials needed to reach criterion was significantly lower in reversals one, three and four than in acquisition ($p < .05$), indicating that the rats improved over the four reversals. As a significant improvement was only seen in the Direction condition, this suggests the presence of facilitation in response reversal learning. As no such improvement was seen in the Control condition, this indicates that facilitation did not occur.

Radial Arm Maze. Five sham rats were removed from analysis for failure to move or make a choice on the maze. As only the Adjacent condition was included in this task, an independent samples t-test was used to analyze the results. In line with our expectations, ADN rats required more trials to reach criterion (150.0 ± 0.0) than sham rats (119.9 ± 8.592), $t(15)=3.292$, $p < .01$, $R^2 = .419$, indicating poorer performance on this spatial discrimination task (see Figure 15). None of the eight ADN lesioned rats reached criterion within 150 trials, indicating that this task was very difficult, and potentially

insoluble, for animals with lesions to the ADN, while seven of the nine sham rats reached criterion before reaching the 150 trial cut-off point.

Discussion

The HD cell signal has long been thought of as a neurological "compass" of sorts (Butler & Taube, 2017; Taube, 2007; Valerio & Taube, 2007), providing information on directional heading to the hippocampus. Likewise, directional heading appears to be crucial for the acquisition of several spatial learning problems (Peckford et al., 2013; Skinner et al., 2003; Skinner et al., 2010; Stringer, Martin & Skinner, 2005). Despite these parallels, the link between the neurological and behavioural aspects of the HD cell circuit is not as well understood as either component individually. In an effort to further elucidate this link, we investigated the functional role of two major components of the HD circuit, the LMN and the ADN, by performing neurotoxic lesions to each region and assessing subsequent performance on three tasks which are thought to depend on directional heading.

The water T-maze results from Experiment 1 showed that rats with lesions to the LMN were impaired, relative to sham controls, on both the direction and rotation problems. Similarly, the results of Experiment 2 showed that ADN lesioned rats were also impaired relative to sham rats, on the same problems. The consistent deficits in performance of lesioned rats across conditions are in agreement with a large body of research indicating that the direction problem in the water T-maze task is dependent on the HD cell circuit, and that lesions to the LMN or the ADN disrupt the HD signal sufficiently to cause impairment in this spatial problem (Dwyer et al., 2013; Peckford et

al., 2014). The present results also confirm a deficit on the rotation problem, which was not assessed in the earlier studies (Dwyer et al., 2014; Peckford et al., 2014), and suggest that the rotation problem may be more difficult, as both the lesioned and sham rats required more trials to reach criterion on the rotation problem.

This apparent disparity in difficulty is likely due to the difference in start point orientations between conditions; the direction problem has the start arms angled 180° apart between maze positions, while in the rotation problem the start arms only differ by 90°. It is known that translation problems, where the start orientations between maze positions do not differ in angle, are very difficult for rats to solve compared to rotation or direction problems (Peckford et al., 2014; Skinner et al., 2003; 2010; Whyte et al., 2009). The current results suggest that the 90° rotation problem is more difficult than the 180° direction problem, which could be explained by the increased overlap of HD cell firing ranges in locations with a smaller difference in heading (Cahill et al., 2015; Preston-Ferrer et al., 2016; Taube, 2007) and is consistent with both problems being easier to solve than the translation problem.

The results of the radial arm maze task further support the notion that decreasing the angle between start point headings increases the difficulty of the task. In Experiment 1, rats required more trials to reach criterion in the Adjacent condition than in the Separate condition, and further analysis revealed that this difference was only significant for the LMN lesioned rats, and not the sham rats. In Experiment 2, using only the Adjacent condition, our results show significant impairment in the ADN lesioned rats compared to sham control rats. In other words, lesions to the LMN and ADN produce impairment on the radial arm task when arms are angled 30° apart, while LMN lesions

have no effect on discrimination of arms that are at least 90° apart. At face value, this is inconsistent with the water T-maze results, which suggest that a 90° difference in heading is both difficult and reliant on the HD cell signal, while the radial arm maze results suggest that a 90° difference in heading is an easier problem which does not necessarily rely on the HD signal, as it was not disrupted by lesions to the LMN. One possible explanation for this is that the radial arm maze apparatus, which is elevated higher and lacks the 31cm walls of the water T-maze, provides more visual access to extra-maze cues, allowing for a cue-based strategy if the open arms are distinct enough, as in the Separate condition. The rats may be learning to move towards a certain distal cue or landmark, rather than along a certain directional heading, and a 90° difference in open arms is sufficient to allow a correct choice without necessarily relying on heading information. Conversely, when the open arms are only 30° apart, this strategy becomes unavailable, as any extra-maze cue would be visible when looking down either open arm, thereby forcing reliance on orientation-based learning and the HD cell system. Even if no other strategy is being used in the Separate condition, it is highly plausible that it is simply an easier problem for the same reason that the direction problem is easier than the rotation problem in the water T-maze task; the smaller angular difference in the open arms results in increased overlap of HD cell firing ranges, making it more difficult to discriminate between the two (Preston-Ferrer et al., 2016; Taube, 2007). Ergo, the adjacent problem is a more difficult spatial problem which more heavily taxes the HD cell system than the separate problem.

The results from the response reversal task in both Experiments 1 and 2 showed that rats improve over the four reversals in the Direction condition, but not in the Control

condition. Previous research with this task has shown that response reversal learning can be facilitated by rotating the start arm, or moving to a new room, between reversals, and that no improvement is seen if there is no change in start point orientation or contextual cues between reversals. (Chiszar & Spear, 1969; Skinner et al., 2014; Wright et al., 2009). The current results are consistent with these findings, and further show that the facilitation of response reversal learning seen in the Direction condition is not impaired by lesions to the LMN or the ADN.

One possible explanation for this lack of impairment is that response reversal learning does not depend on the HD signal, and therefore is unaffected by lesions to the HD cell circuit. It is possible that rats in the Direction condition of the response reversal task are able to use a cue-based learning strategy based on the extra-maze cues opposite the start arm. This strategy would not be available to the Control rats, as they face the same extra-maze cues across all reversals. This explanation contrasts with previous research which supports the idea that response reversal learning is dependent on start point orientation (Skinner et al., 2014; Wright et al., 2009), but is in line with a recent study by Wright, Martin, Thorpe, Haley & Skinner (2018) which found that the length of the start arm can facilitate response reversal learning in much the same way that start arm rotation can. These results clearly show that response reversal learning can be facilitated by metric cues other than directional heading, suggesting that it is not dependent on the HD circuit. The current results agree with this conclusion, as lesions to the central HD signaling pathway did not impair performance, though it does not answer the question of how, if not by start orientation, are the rats in the direction problem learning? The answer may lie in further exploration of a cue-based learning strategy.

Interestingly, Wright et al. (2018) found that while distance cues can facilitate response reversal learning, light cues do not, indicating that cue modality may be critical to their usefulness in facilitating task acquisition; they further supported this notion by showing that the same light cues can facilitate learning in a non-spatial go-no go discrimination task. The idea that cues will better support learning if they match the modality of the task is not a new one; a water-escape delayed matching-to-sample task is more readily acquired by rats given a spatial cue than a brightness cue (Means, Long, Jones & Curtis, 1996), in much the same way that verbal cues do not improve the solve rate of a visual insight problem in humans (Chronicle, Ormerod & MacGregor, 2001). As response reversal learning is a spatial task, it therefore follows that it can only be facilitated by spatial cues.

Skinner et al. (2014) dismissed the possibility of S-R learning in the response reversal task because switching from a square black curtain around the maze to a circular white one did not facilitate learning, despite stimulating hippocampal remapping. It is clear that place remapping is not enough to facilitate response reversal learning; however, the curtains used did not provide any distal cues which could support a new heading direction (Skinner et al., 2014). This means there were also no distal cues which could serve as landmarks, preventing both orientation and landmarks from being used to navigate. In rotating the start arm of a T-maze, the distal cues opposite the rats' start point change, though the hippocampal map of the room stays intact. The potential influence of cue modality suggests that individual distal cues could facilitate response reversal learning if used as landmarks, making them relevant to spatial mapping and mode-appropriate. As it has been determined that place mapping is entirely separate from

directional heading (Skinner et al., 2014; Whitlock & Derdikmen, 2012), an intact HD cell circuit is not necessary to map the location of landmarks. Subsequently, knowledge of directional heading within the larger environment is not necessarily needed for S-R learning based on landmark associations: e.g. at the blue door, turn left; at the black and white poster, turn right. This strategy would not be available in the Adjacent condition of the radial arm maze, as the available cues, or landmarks, beyond two arms separated by only 30° share a great deal of overlap, as explained above.

A landmark-based strategy may likewise be unavailable in the water T-maze task due to the plexiglass walls surrounding the maze, which prevent the rats from seeing distal cues directly opposite the start arm while in the water, thereby forcing reliance on directional heading. This may account for the relative difficulty rats have in solving the rotation problem in the water T-maze, compared to the Direction condition of the response reversal task, where distal landmarks are available throughout the trial. It is also possible that the order in which maze positions are presented is a factor: in the water T-maze, trials at the two maze positions were intermixed in a pseudorandom order, never exceeding two trials from the same position in a row, while in the response reversal task rats were trained at a consistent maze position until they reach criterion, before rotating the maze. Even if landmarks are visible from in the water T-maze, making S-R learning possible, it could be that the intermixed order of trials discourages this strategy by making it more difficult to form a landmark-response association. Consistent trials such as in the response reversal task, on the other hand, would make this association relatively easier to form, making recruitment of an S-R strategy more likely.

Landmark-based S-R learning also accounts for the discrepancies in the literature on whether or not response reversal learning depends on HD signalling. If the start arm is not rotated between reversals, and no other context change occurs, neither directional heading nor landmark association is available as a learning strategy, explaining the consistent lack of facilitation in control conditions (Skinner et al., 2014; Wright et al., 2009; Wright et al., 2018). However, if the start arm is rotated, even in the same room, both strategies become available, as both start orientation and the landmark opposite the start arm change, becoming confounding variables. The current study adds to mounting evidence that response reversal learning is not fully dependent on the HD cell signal, though it may be supported by it. It is possible that landmark association is a secondary strategy recruited when directional heading information is unavailable; the opposite is equally possible. It could also be true that these two strategies work in conjunction, with heading information bolstering landmark acquisition or landmarks guiding heading-based navigation. As of yet, response reversal procedures have not been able to separate the two components, though it would be possible to do so by integrating moveable landmarks into the testing procedure. By placing one of two distinct landmarks (e.g. a towel on a coat rack vs. a wooden shelf) opposite the start arm and moving it to maintain that position when the maze is rotated and swapping it for the other landmark for half of the rats, a 2x2 design could assess the contribution of both start point orientation (Control vs. Direction) and landmark availability (Change vs. No Change) on acquisition across four reversals. This, or a similar task, could be used to finally determine how much, if any, of the variance in response reversal learning is dependent on start point orientation alone, regardless of landmark acquisition.

Though the results of the current study, as well as previous research (Wright et al., 2018), suggest that response reversal learning is not dependent on the HD cell signal, there is an alternative explanation; namely, that there is an additional route or mechanism by which the HD signal can bypass the LMN and/or ADN. The existence of this secondary pathway is somewhat speculative, as its exact route has yet to be determined; nevertheless, the complexities of the HD cell circuit make this theory difficult as difficult to disprove as it is to confirm. HD cells exist in a dozen different brain regions, and numerous reciprocal and auxiliary connections have been noted in addition to the primary DTN→LMN→ADN→PoS pathway shown in Figure 1 (Blair et al., 1998; Butler & Taube, 2017; Clark & Taube, 2012; Taube & Muller, 1998; see Taube, 2007 for review), as well as being connected to the equally complex network of place cells, grid cells and angular head velocity cells (Giocomo et al., 2014; Harland et al. 2017; Moser, et al., 2015; Muller, 1996; Taube, 2007). Given all of this interconnectedness and complexity, it would be difficult to argue against at least the possibility of a secondary signaling pathway.

On the other hand, there are clear behavioural results, including those of the current study, showing that a secondary signaling mechanism, if one exists, cannot fully compensate for an interruption of the central pathway, evidenced by the impairments seen in the water T-maze and radial arm maze tasks by both the LMN and ADN lesioned rats. Similar impairments on spatial tasks are often seen following lesions to the LMN or ADN, but are often small compared to the effects of DTN lesions (Bassett et al., 2007; Dwyer et al., 2013; Peckford et al. 2014; Vann, 2005; 2011), which can be explained by the use of tasks which do not heavily task the HD system, but which could also be a result

of alternative signaling pathways. The water T-maze task used in the present study is known to be a HD cell signal dependent task, yet both LMN lesioned and ADN lesioned rats eventually reached criterion, as did DTN lesioned rats (Dwyer et al., 2014), indicating that learning can still occur following disruption of the central HD signalling pathway. If these central pathway lesions cannot abolish learning in tasks dependant on the HD signal, it stands to reason that the remaining non lesioned HD cells in neighbouring brain regions are still functional enough to send some heading information on to the hippocampus, even if it is not as precise as the information from the primary pathway. This reasoning is also supported by the consensus in the literature that the HD signal begins in reciprocal connection between the LMN and DTN, but is primarily directed from the DTN to the LMN and on to other downstream structures; though the DTN seems to be the "first stop" along the HD cell circuit, and is associated with the largest behavioural deficits, both regions receive input from the angular head velocity cell circuit and are involved in the origin of the HD cell circuit (Butler and Taube, 2017; Taube, 2007). Therefore, simultaneous lesions to both the LMN and the DTN would be required to abolish the HD cell signal completely, and lesions to one or the other maintains the possibility of a reduced HD signal which could still be communicated to the hippocampus.

The existence of such a neurological "failsafe" is evolutionarily sound, and not dissimilar to the ability of rodents to adopt alternative learning strategies when the preferred strategy becomes unavailable. In the life of a wild animal, the ability to successfully navigate their environment could determine access to food, mates, nesting sites, etc., making it a key factor of survival, and several studies have shown that

directional heading is a critical factor for spatial navigation (Dwyer et al., 2014; Harland et al., 2017; Peckford et al., 2013; Skinner et al., 2003; Skinner et al., 2010; Whyte et al., 2009). Therefore, it is a logical conclusion that the ability to maintain heading information - the HD cell circuit - has been conserved through evolution, and the widespread presence of HD cells in a dozen different brain regions indicates just that. The more regions HD cells are present in, and the more routes by which heading information (even if minimal) can be integrated into the hippocampus, the less likely that damage or functional defects to one brain region will negatively affect navigation, and therefore survival.

This is not to say that evidence supporting an alternative HD signaling pathway necessarily opposes the idea that response reversal learning is not dependent on HD signalling. As is often the case, what initially seem to be opposing explanations may in fact be complimentary. It is possible that start orientation supports response reversal learning, but is not critical for acquisition, and that an additional HD signal pathway is capable of partially ameliorating the effects of lesions to the LMN or ADN. If this is the case, it is plausible that the minimal heading information passed through a secondary pathway, while not enough to completely offset the effects of lesions to the primary pathway in tasks which heavily tax the HD system, such as the water T-maze or radial arm maze tasks, may be enough to cancel out any minor impairment that result from said lesions on a task which is not fully reliant on the HD system, such as response reversal learning.

The primary goal of the current study was to further illuminate the connection between the neurological HD cell circuit and the behavioural importance of heading

direction in the acquisition of spatial problems. We did so by performing neurotoxic lesions to the LMN and ADN, both major components in the central signaling pathway of the HD cell circuit, and assessing performance on three tasks thought to depend on directional heading: the water T-maze task, the response reversal task and the radial arm maze task. Our results show that both the LMN and the ADN are critical for the solution of spatial problems involving orientation angles of 90° or less in which alternative learning strategies cannot be used, confirming their dependence on the HD system, i.e., the rotation problem in the water T-maze task and the Adjacent condition of the radial arm maze. These results confirm that behavioural deficits following lesions to the HD system are in line with the cell signalling patterns and lesion-induced disruptions seen in neurological studies. Our results also show that lesions to the LMN and ADN have less of an effect on spatial tasks which are not wholly dependent on heading direction for solution, such as the direction water T-maze problem, the Separate condition in the radial arm maze task and the response reversal task, illuminating the behavioural specificity of the HD cell circuit and illustrating the importance of using behavioural tasks which not only heavily tax the HD system, but which also preclude the recruitment of other learning strategies which could confound the results.

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Table 1. Percentage of Tissue Damage to LMN in Lesioned Rats.

Animal Number	Percentage of Tissue Damage			Included
	Right	Left	Total	
2	15.184	15.184	15.184	No
3	14.123	13.063	30.026	No
4	57.592	49.110	53.351	Yes
5	09.883	03.521	6.7020	No
6	09.883	15.184	12.533	No
7	20.485	38.508	29.496	No
8	56.532	62.893	59.712	Yes
9	18.364	33.207	25.786	No
10	44.869	44.869	44.869	No
11	63.953	65.013	64.483	Yes
12	09.883	04.582	7.2321	No
13	100.000	100.000	100.000	Yes
17	10.942	10.943	10.943	No
18	59.712	57.592	58.652	Yes
19	60.772	67.134	59.182	Yes
20	85.157	81.976	83.567	Yes

Table 2. Percentage of Tissue Damage to ADN in Lesioned Rats.

Animal Number	Percentage of Tissue Damage			Included
	Right	Left	Total	
1	61.281	70.668	65.975	Yes
2	09.025	07.942	9.657	No
5	28.429	20.217	24.323	No
9	65.975	55.415	60.695	Yes
10	09.657	03.791	6.724	No
13	90.614	89.440	90.027	Yes
16	0.271	05.596	2.662	No
21	4.964	26.083	15.523	No
23	97.653	96.480	97.067	Yes
25	78.881	85.921	82.401	Yes
28	24.910	17.870	21.390	No
31	42.509	78.881	60.695	Yes
32	23.736	12.004	17.870	No
34	17.870	03.249	7.3105	No
37	58.935	63.628	61.282	Yes
38	97.653	82.401	90.027	Yes
39	19.043	24.910	21.976	No

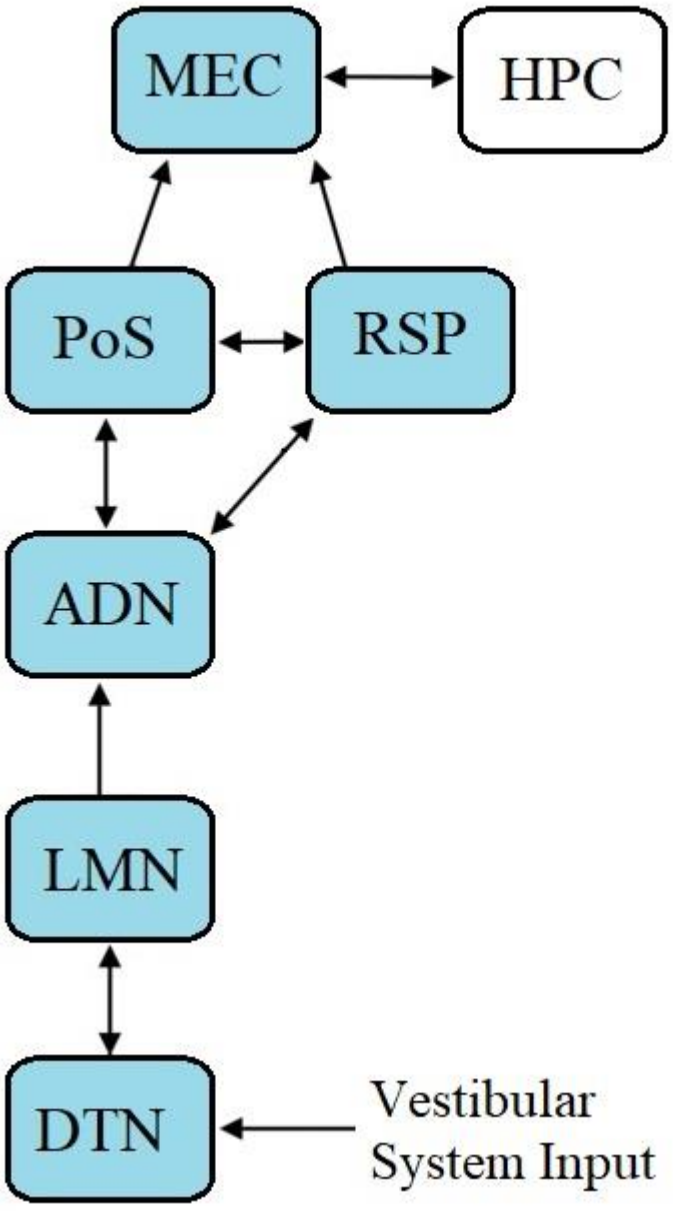

Figure 1. Diagram showing the primary signal pathway of the head direction cell circuit. Shaded regions contain head direction cells. DTN, dorsal tegmental nucleus; LMN, lateral mamillary nucleus; ADN, anterodorsal nucleus of the thalamus; PoS, postsubiculum; RSP, retrosplenial cortex; MEC, medial entorhinal cortex; HPC, hippocampus.

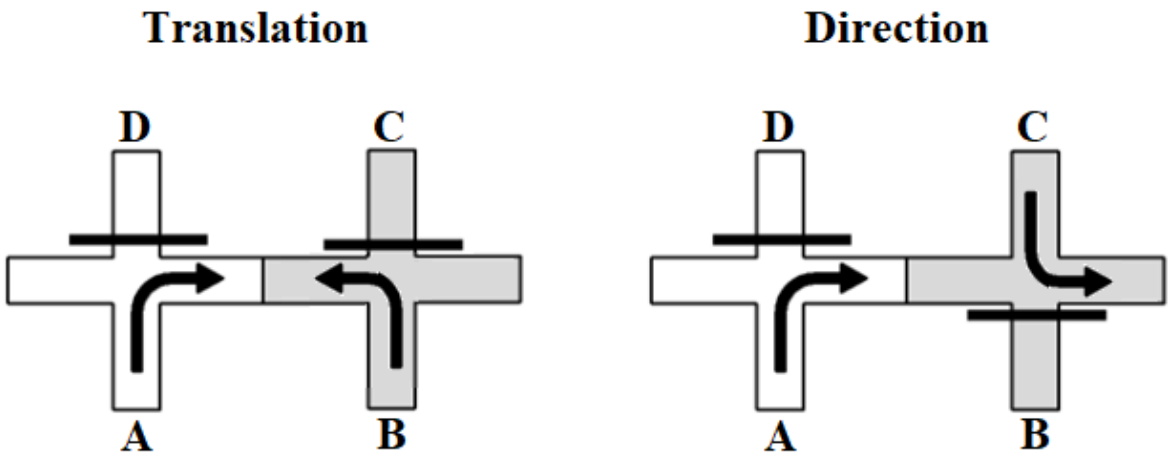

Figure 2. Schematic diagram of the T-maze apparatus used by Skinner et al., 2003 showing primary (white) and secondary (grey) maze positions. Translation rats were trained to a specific location from start positions A and B, or D and C (not shown). Direction rats were trained to travel east from start positions A and C, or west from start positions B and D (not shown).

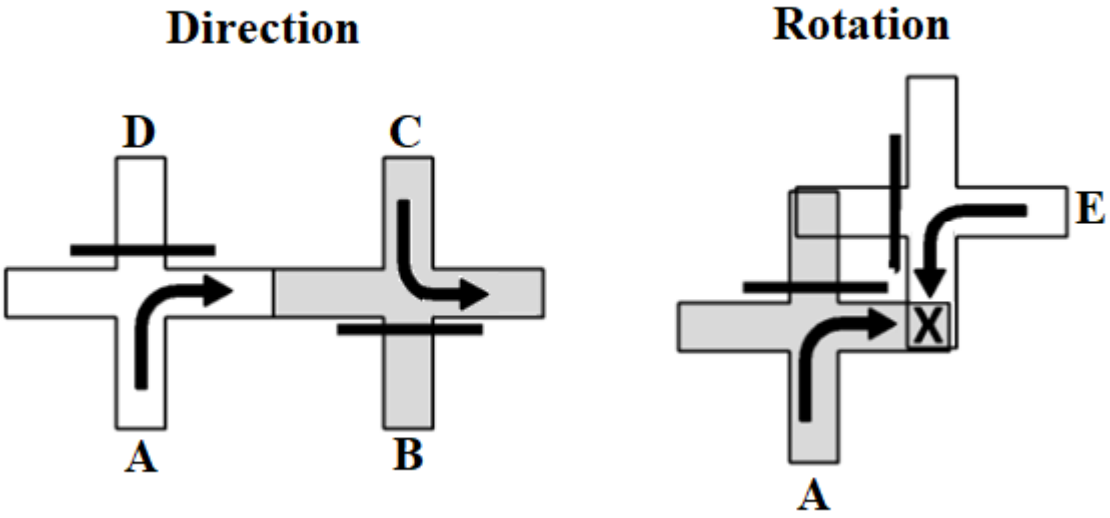

Figure 3. Schematic diagram of the water T-maze apparatus showing the primary (white) and secondary (grey) maze positions of Direction and Rotation rats. Half of the Direction rats were trained to travel east from start positions A and C, while half were trained to travel west from positions B and D (not shown). Of the Rotation rats, half were trained to turn right from position A and left from position E, while half were trained to turn left from position D and right from position F (not shown).

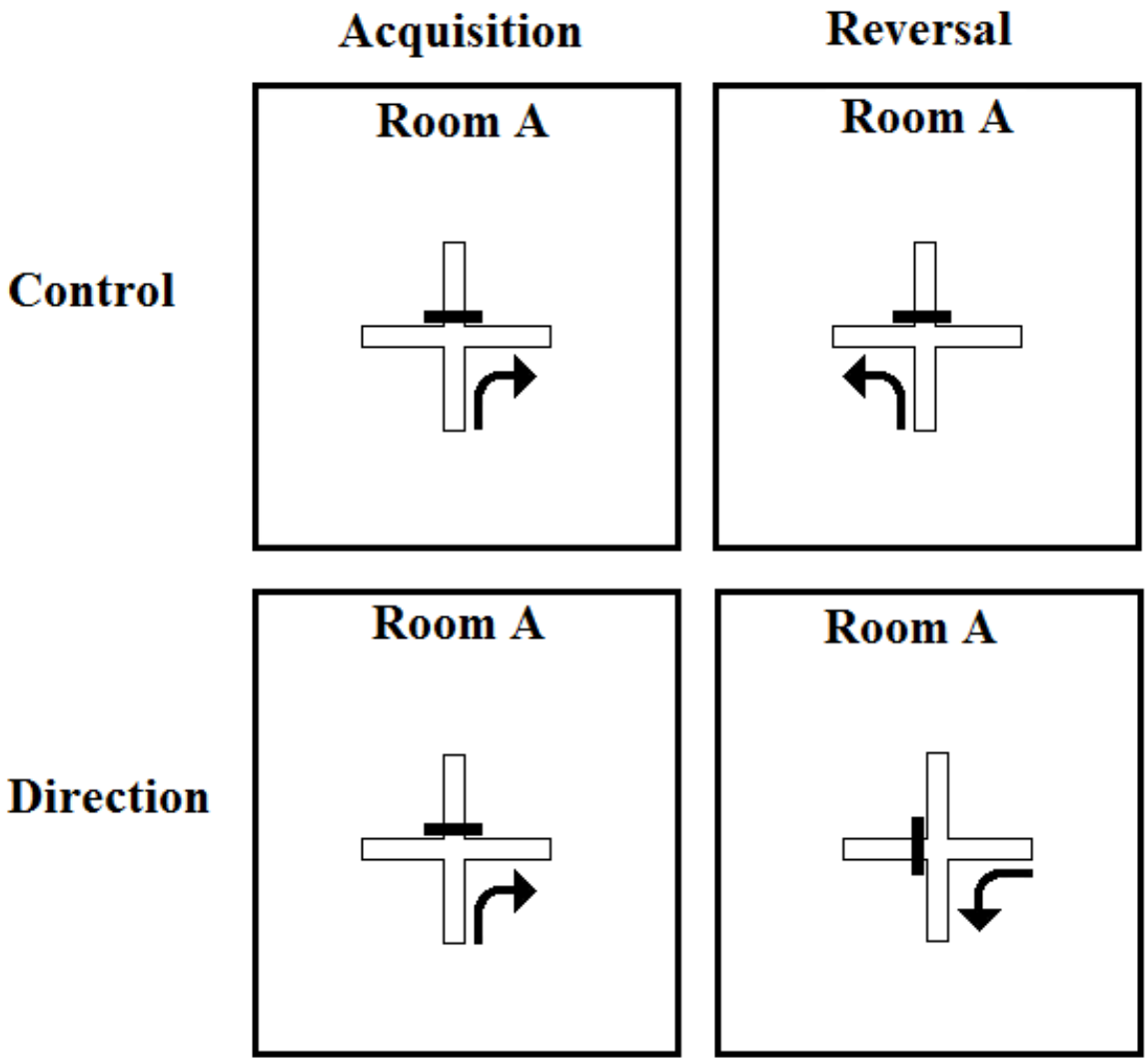

Figure 4. Schematic diagram of the response reversal apparatus showing the responses required for initial acquisition and reversal one of half of the Control and Direction rats. Both groups were trained to turn right on initial acquisition and left on reversal one; the start arm of the maze was rotated 90° between reversals for Direction rats only. The remaining rats were trained to turn left on initial acquisition and left on reversal one (not shown).

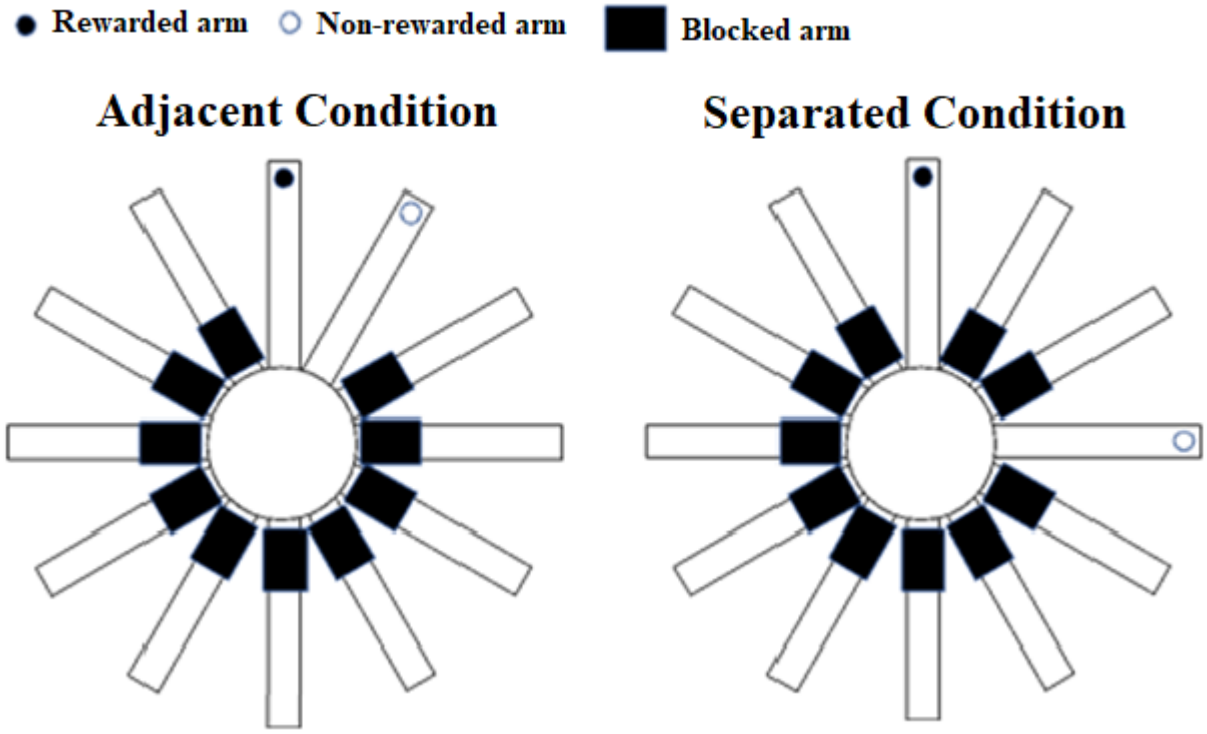

Figure 5. Schematic diagram of the radial arm maze apparatus showing representative maze positions from the Adjacent and Separated conditions. The rewarded arm was assigned in a pseudo-random order and kept constant for each rat, while the non-rewarded arm varied between two position, one to the left and one to the right of the rewarded arm. In the Adjacent condition these arms were immediately beside each other, separated by at least 30°, while in the Separated condition the open arms were at least 90° apart, separated by two blocked arms.

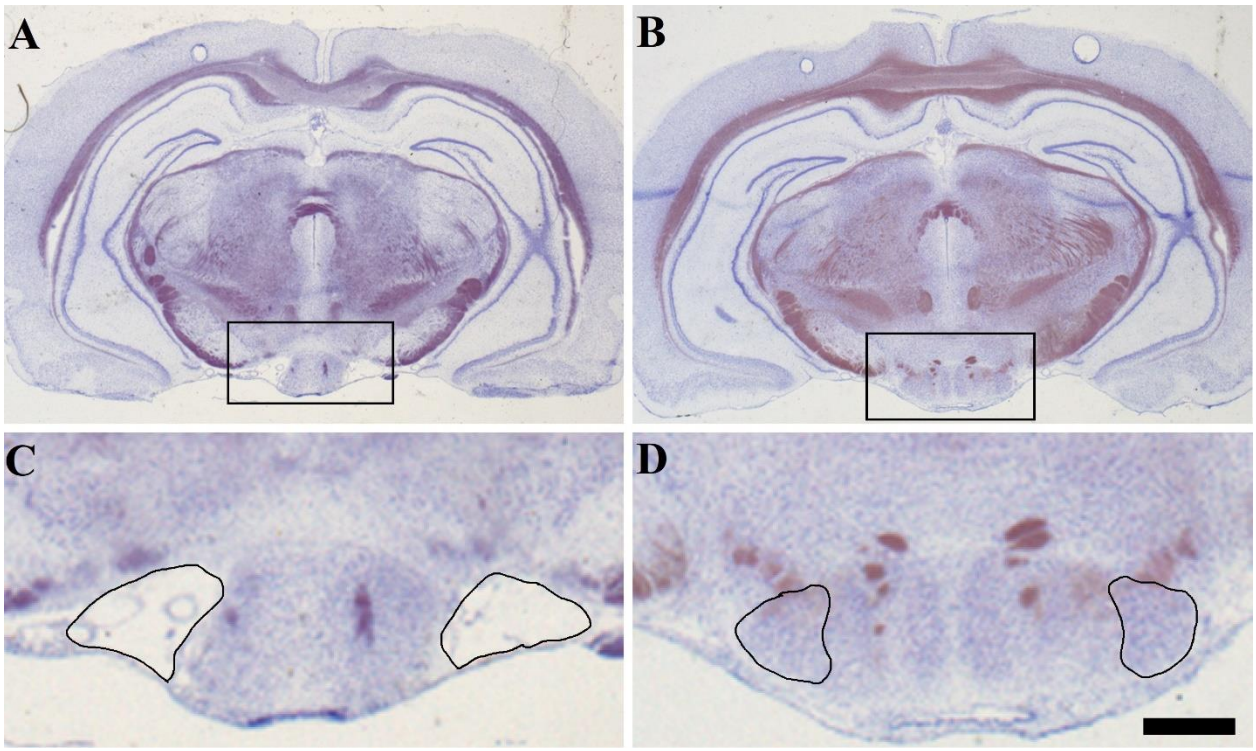

Figure 6. Images A and B show a representative LMN lesioned and sham brain, respectively. Image C shows the clear absence of tissue (outlined) denoting a lesioned LMN. Image D shows an intact LMN (outlined). Scale bar 500μm.

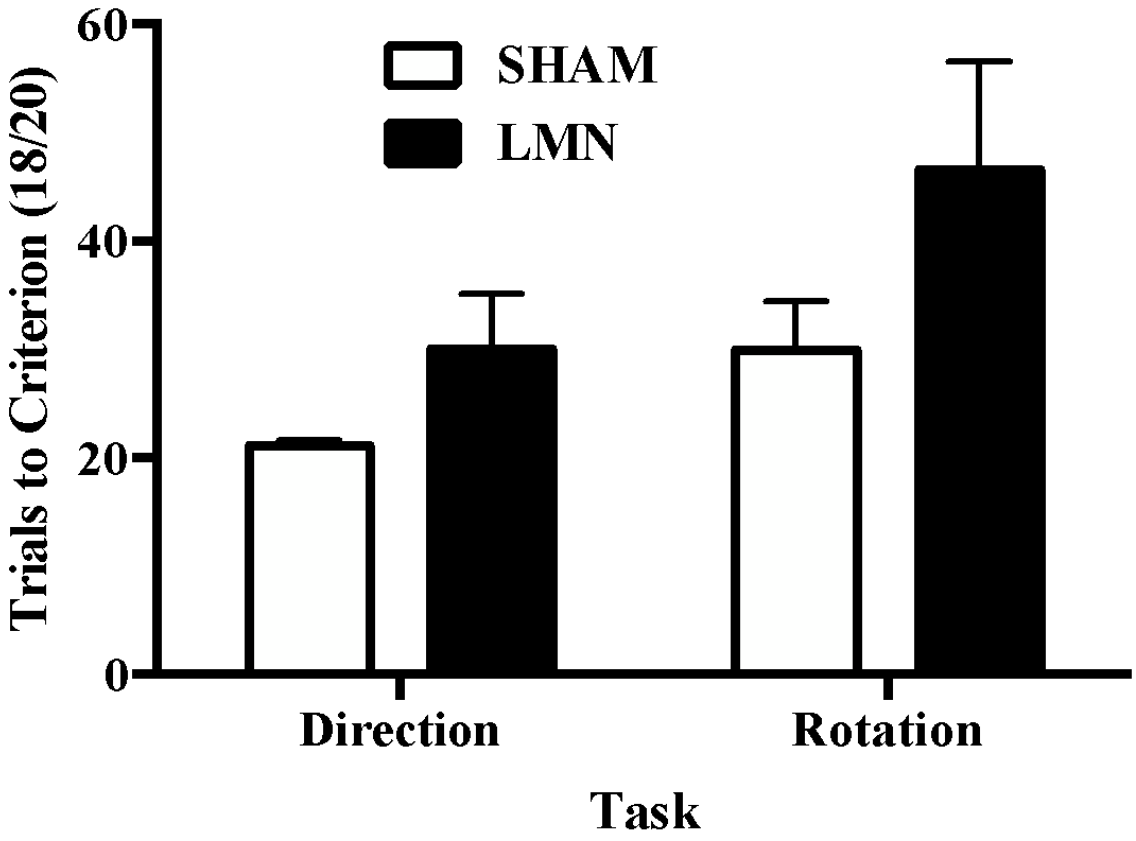

Figure 7. Mean (+SEM) trials to criterion of LMN lesioned and sham rats in the Direction and Rotation conditions of the water T-maze task in Experiment 1.

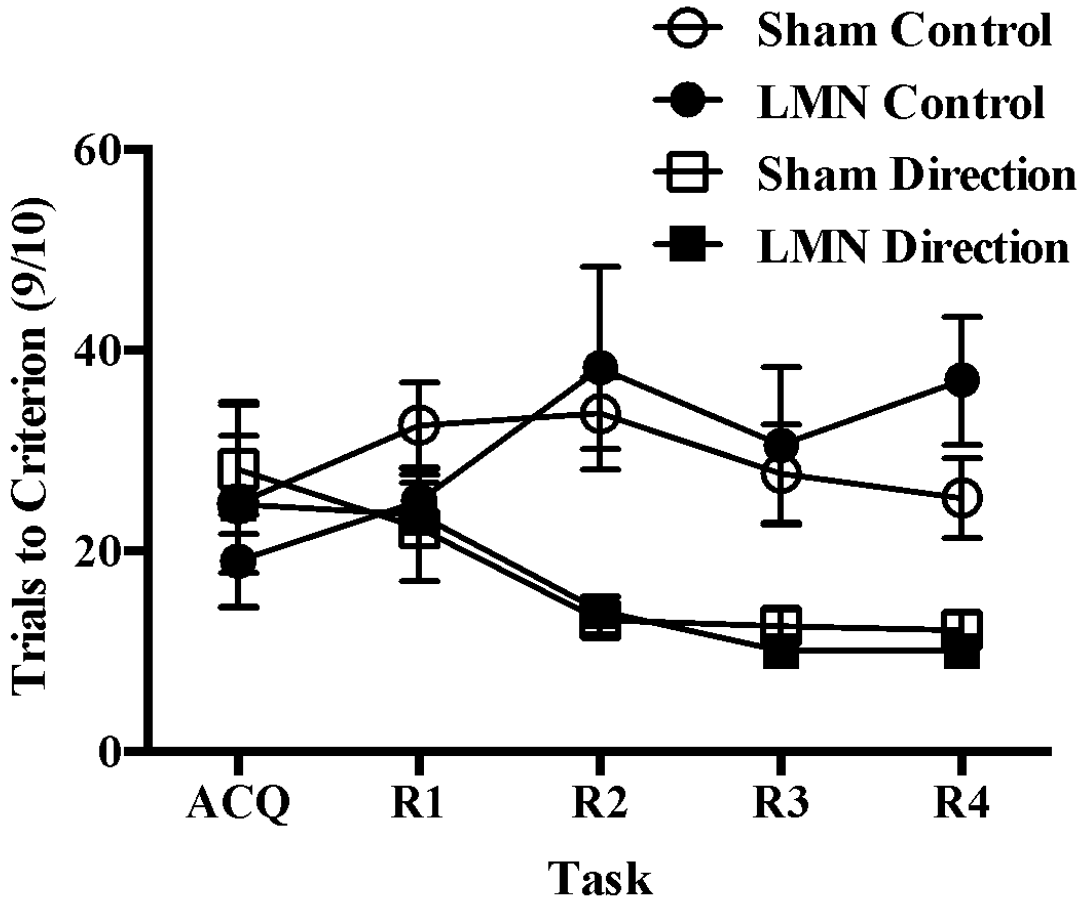

Figure 8. Mean (±SEM) trials to criterion of LMN lesioned and sham rats in the Direction and Control conditions across acquisition and four reversals in the response reversal task of Experiment 1.

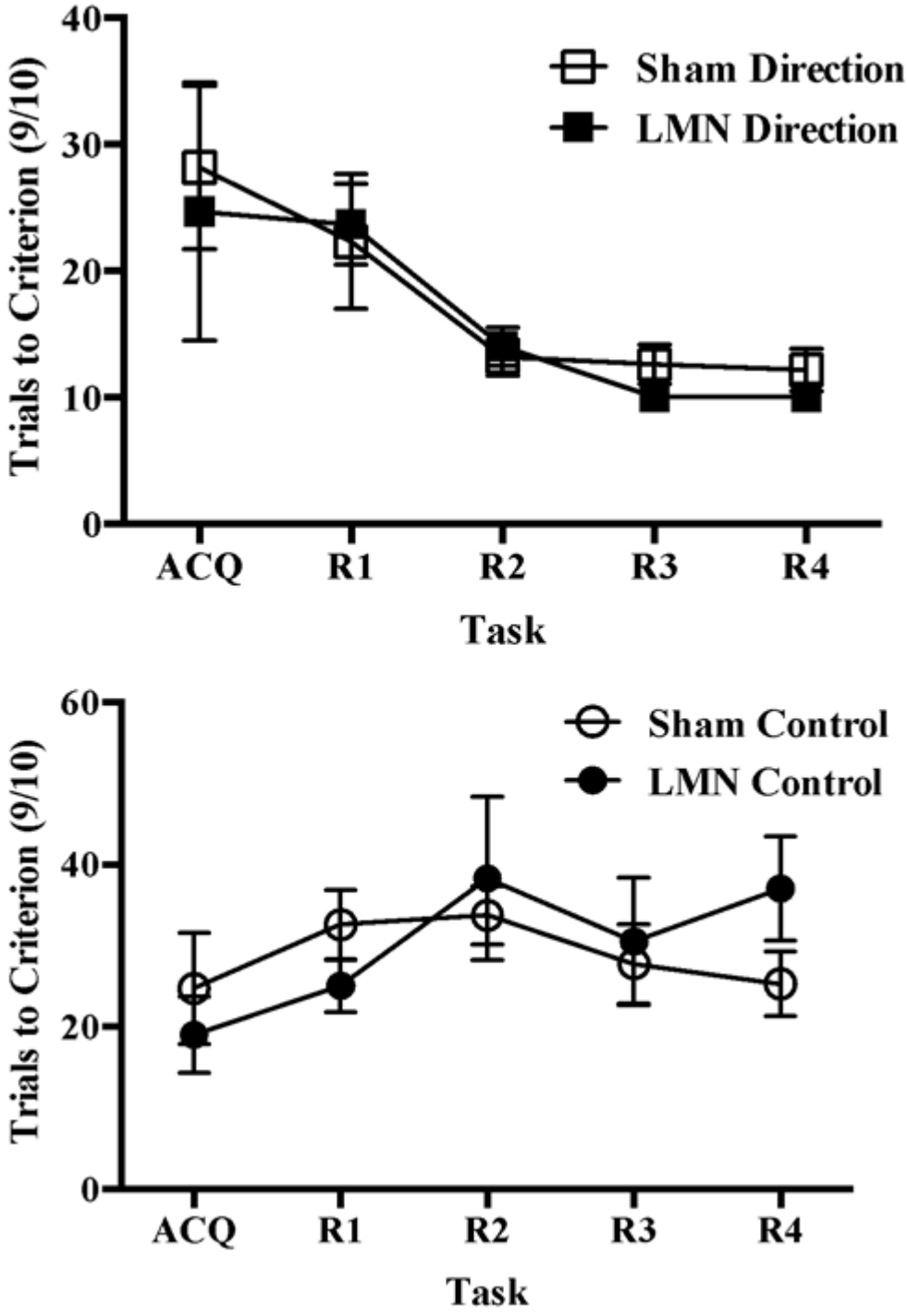

Figure 9. Mean (±SEM) trials to criterion of LMN lesioned and sham rats in the Direction (top panel) and Control (bottom panel) conditions across acquisition and four reversals in the response reversal task of Experiment 1.

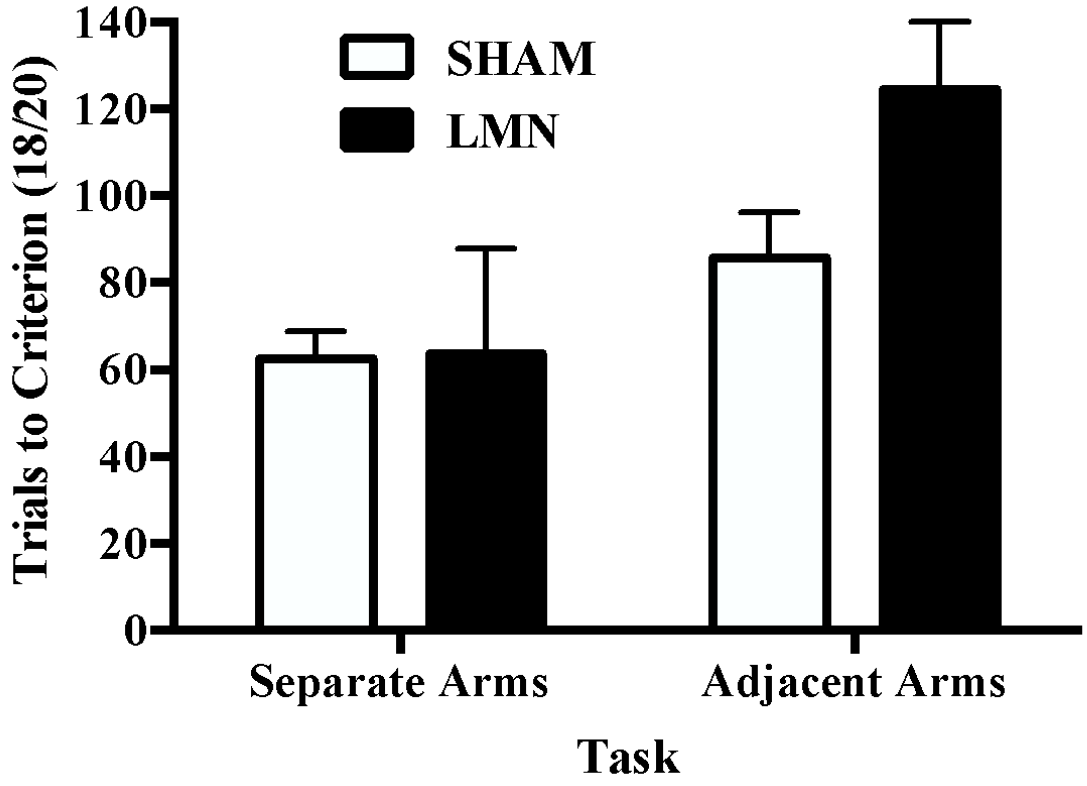

Figure 10. Mean (+SEM) trials to criterion of LMN lesioned and sham rats in the Separate and Adjacent conditions of the radial arm maze in Experiment 1.

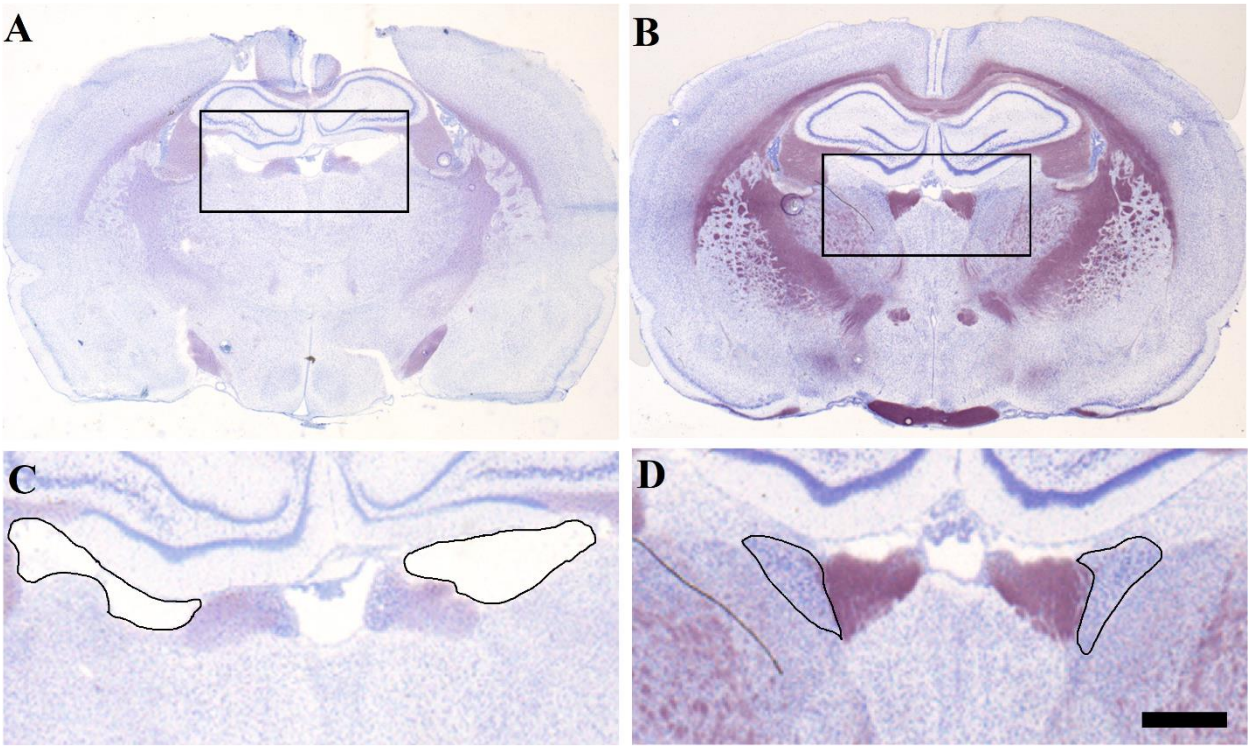

Figure 11. Images A and B show a representative ADN lesioned and sham brain, respectively. Image C shows the clear absence of tissue (outlined) denoting a lesioned ADN. Image D shows an intact ADN (outlined). Scale bar 500μm.

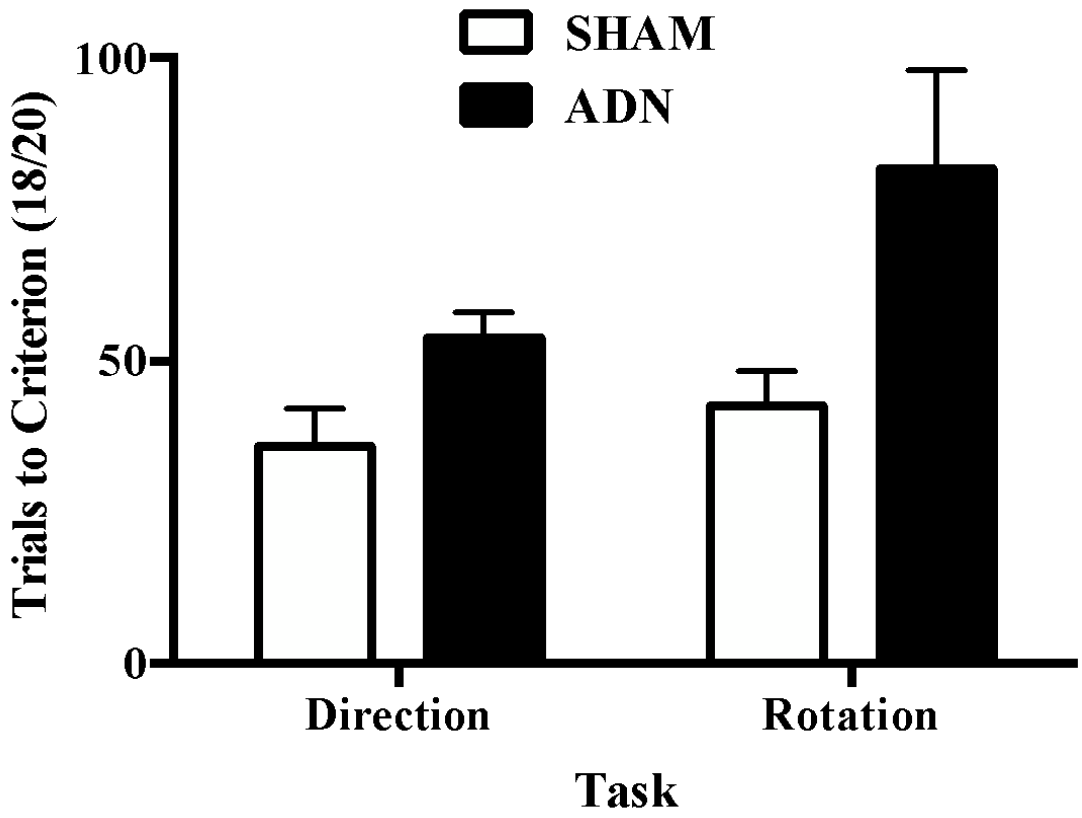

Figure 12. Mean (+SEM) trials to criterion of ADN lesioned and sham rats in the Direction and Rotation conditions of the water T-maze task in Experiment 2.

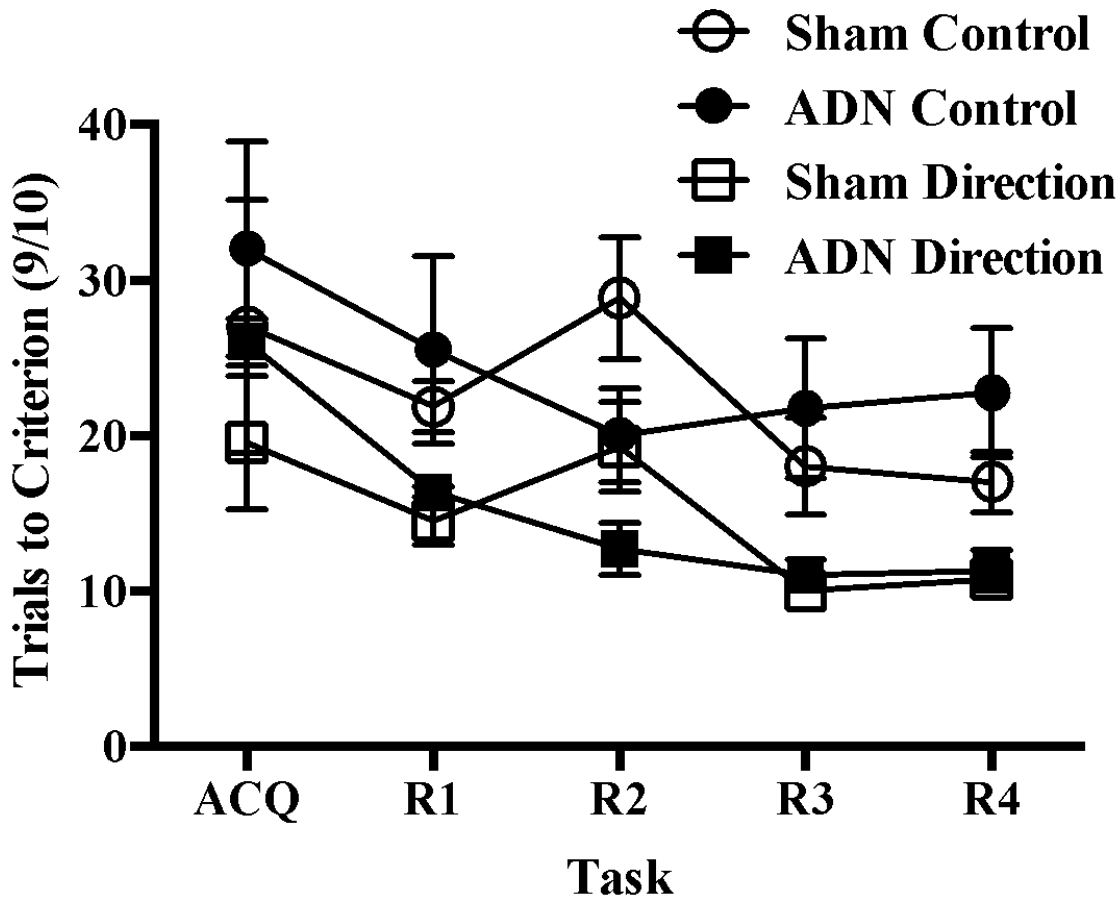

Figure 13. Mean (±SEM) trials to criterion of ADN lesioned and sham rats in the Direction and Control conditions across acquisition and four reversals in the response reversal task of Experiment 2.

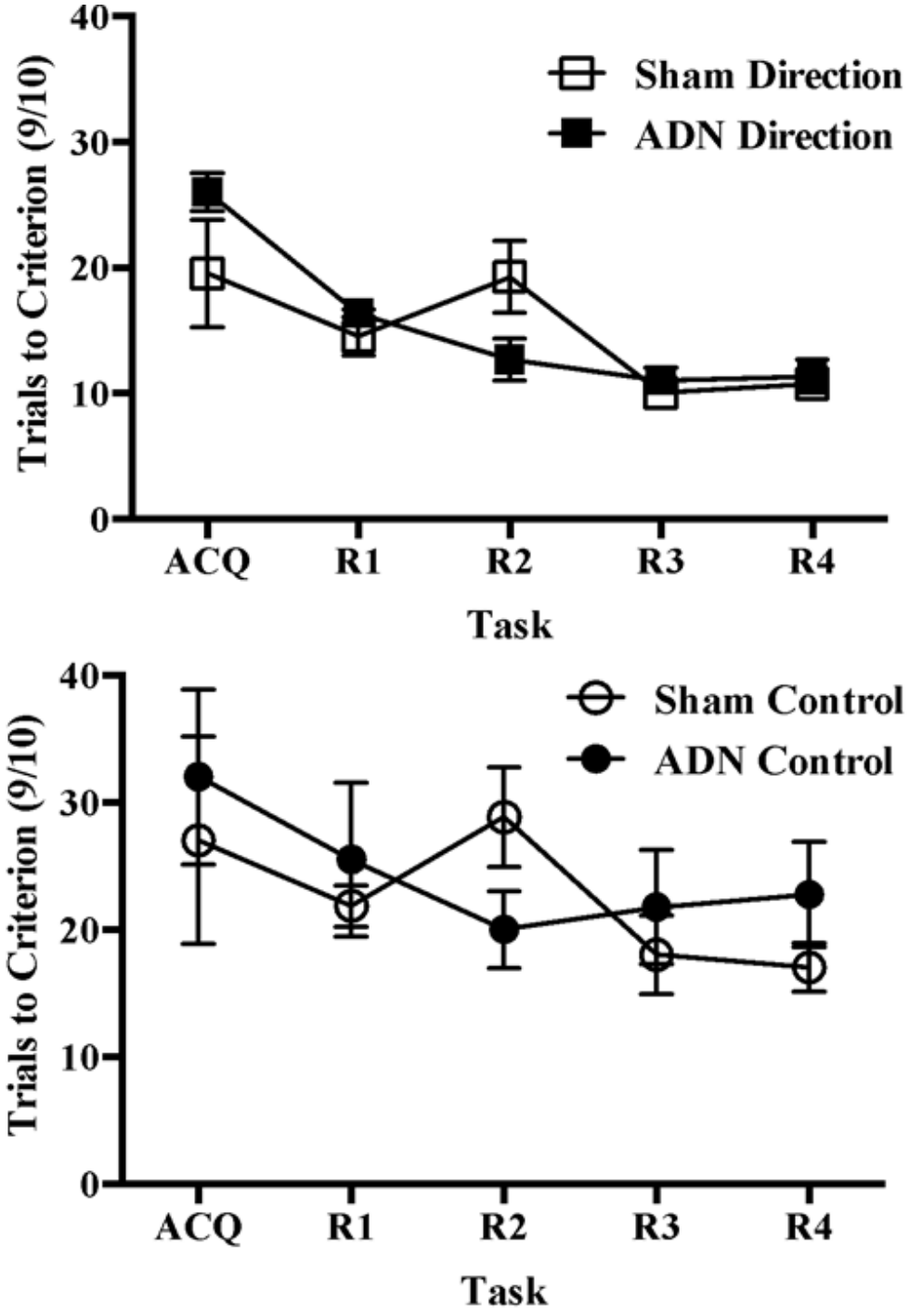

Figure 14. Mean (±SEM) trials to criterion of ADN lesioned and sham rats in the Direction (top panel) and Control (bottom panel) conditions across acquisition and four reversals in the response reversal task of Experiment 2.

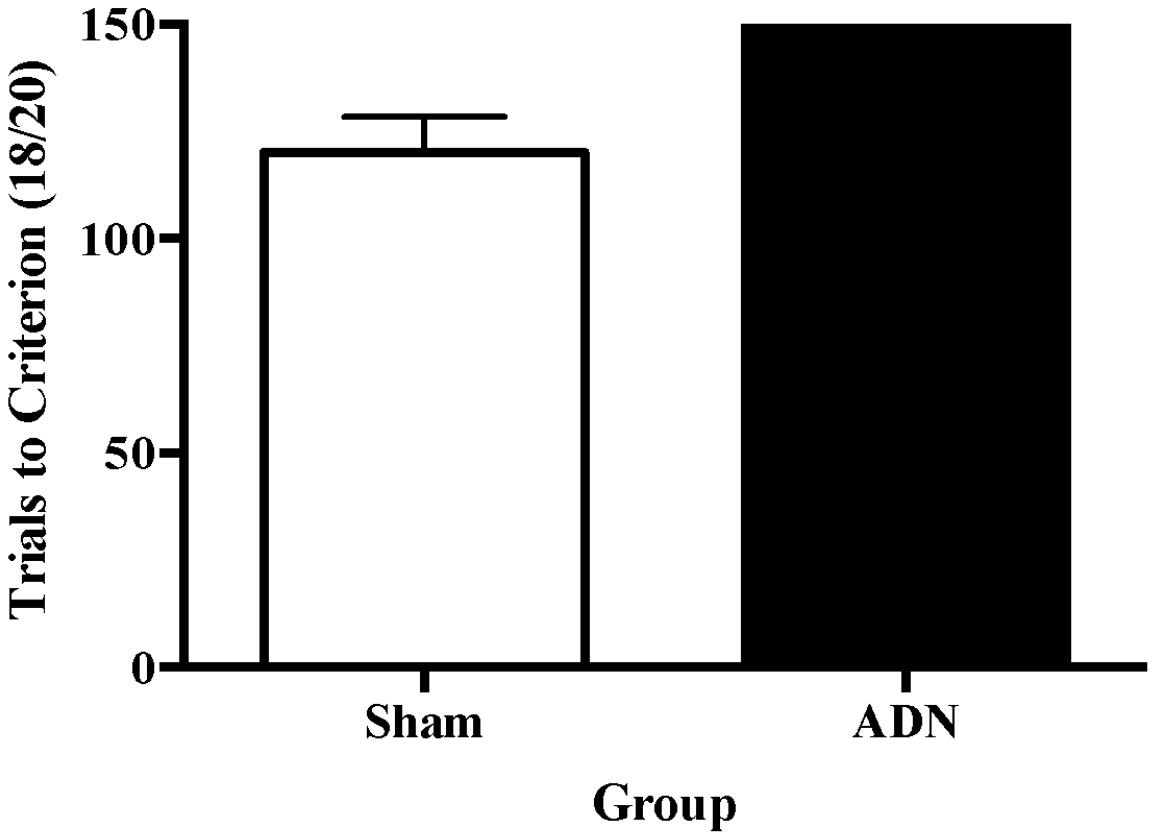

Figure 15. Mean (+SEM) trials to criterion of ADN lesioned and sham rats in the radial arm maze task of Experiment 2.