Disorienting the Rat: Do Lesions to the DTN and LMN Cause Rats (*Rattus norvegicus*) to Lose Their Way?

by

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Abstract

The dorsal tegmental nucleus (DTN) and lateral mammillary nucleus (LMN), and the connections between them, are thought to produce the head direction cell signal, which is associated with animal navigation. Bilateral lesions to these structures disrupt head direction (HD) cell firing in downstream areas of the primary HD cell circuit. Rats with bilateral lesions to the DTN (Experiments 1 and 2) or LMN (Experiment 3) were compared to sham controls in a series of tasks where directional sense is thought to be important. The tasks included a directional water T-maze problem where rats were trained to travel in a constant direction from two different locations to locate a hidden platform and two variants of a foraging task where rats were trained to return to a home location after finding food. Rats with lesions to the DTN were impaired across all tasks. Rats with lesions to the LMN were impaired on the variable-hole version of the foraging task, but showed only transient deficits on the water maze task (However, only a small number of rats sustained an acceptable level of damage to the LMN). These data demonstrate the importance of the direction system on these tasks.

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Table of Contents

Page

List of Abbreviations

ADN: Anterior dorsal nucleus of the thalamus

AP: Anterior-Posterior

AVH: Angular head velocity

DTN: Dorsal tegmental nucleus

DV: Dorsal-Ventral

HD: Head-Direction

IBO: Ibotenic Acid

LMN: Lateral mammillary nucleus

ML: Medial-Lateral

NMDA: N-Methyl-D-Aspartate

PoS: Postsubiculum

List of Figures

Disorienting the Rat (*Rattus norvegicus*): Do Lesions to the DTN and LMN Cause Rats to Lose Their Sense of Direction?

Research on the mechanisms associated with navigation can allow for a deeper understanding of how animals (ourselves included) know where they are, where they are going, and why they lose their way (Dudchenko, 2010). Navigation itself refers to an organism's ability to guide itself from one physical location to another. As a human, this ability may be associated with finding a car in a crowded parking lot or making the daily commute to work. From the perspective of a rat, as an experimental subject, navigation may be synonymous with the ability to travel from the start to the end of a laboratory maze. Also, from an evolutionary standpoint, navigation is undeniably important for animal survival (in terms of escape from predators, foraging, and mating). Thus, navigation merits scientific study from both biological and psychological perspectives.

With that said, much of the research related to navigation had been conducted using animal models (and often the rat), largely using techniques and approaches that are associated with comparative psychology (Dudchenko, 2010; Wang & Spelke, 2002). Although humans and non-human animals navigate differently, analogous mechanisms are thought to exist in humans for the basic navigation mechanisms that are observed in non-human animals (Dudchenko, 2010; Wang & Spelke, 2002; Wiener et al., 2011). Consequently, many comparative psychologists suggest that insights can be made about human navigation by observing animals as humble as the rat and ant (Wang & Spelke, 2002).

Directional and positional information are both required for animals to be successful navigators (Taube, 2007). For instance, for an animal to return home, after foraging for some time, a representation (however rudimentary) of its orientation with respect to its home position

is needed to head home (i.e., the animal needs to be able to both recognize and find its goal location using information available from external stimuli or by tracking its movements on a moment to moment basis). Animals primarily use two strategies to navigate in environments: (1) Path integration and (2) landmark navigation (Frohardt, Bassett, & Taube, 2006; O'Keefe & Nadel, 1978; Taube, 2007). Path integration refers to a mechanism where the animal's brain continuously updates a "home vector" on an outward journey so that a direct path to "home" may be made at anytime (Dudchenko, 2010; Frohardt et al., 2006). Many terrestrial animals are thought to rely on path integration using egocentric motion cues when external cue information, associated with the basic senses (especially visual), is unavailable or obscured (Taube, 2007). Landmark navigation can take two basic forms: Piloting, which refers to an animal's use of the configuration of landmarks to guide its navigational choices (Frohardt et al., 2006; O'Keefe & Nadel, 1978) and beacon homing, where the animals makes a conditioned response to the presence of a specific cue (such as following a single sensory beacon) (Wiener et al., 2011).

As scientists, our long-term goal is to develop a model that describes basic animal navigation, and ideally, such a model would characterize the basic behavioural strategies used by animals alongside the neural mechanisms thought to underlie such strategies. From the perspective of the generalist, we are interested in understanding how the brain is involved in orientation. This is not a new idea; scientists as early as Charles Darwin (1873) and many current scientists, like Paul Dudchenko (2010), have postulated that specific mechanisms in the brain are involved in getting lost and oriented.

Early researchers debated between the relative ease with which place versus response learning (i.e., learning relative locations versus a series of motor responses, respectively) occurs in the rat when navigating elevated mazes (O'Keefe & Nadel, 1978; Restle 1957; Tolman,

Ritchie, & Kalish, 1946). For example, Tolman et al. (1946), in an attempt to separate place and response learning, found that rats could accomplish both; however, place learning was accomplished more easily. Interestingly, Tolman (1948) suggested that such navigation is largely accomplished through the use of cognitive maps, map-like representations in the brain that included the relative relationships between environmental landmarks and potential routes to goals. Notably, Tolman's ideas would receive support many years later with the discovery of cells in the mammalian brain that fire in a location specific manner (see O'Keefe & Nadel, 1978). Restle (1957), in a review of the place versus response debate, concluded that the type of learning that dominates is situational. In short, Restle suggested that in well-lit and cue-heavy environments, place learning dominates; but in dark and cue-poor environments, response learning occurs most readily. Later studies revisited this debate, highlighting that although place learning was apparent early in training, rats often switch to relying on response strategies with extended training (Packard & McGaugh, 1996).

The possibility of direction learning was largely ignored in the above work despite the fact that Blodget, McCutchan, and Matthews (1949) reported that direction learning and response learning were easier for rats relative to place learning and that in much of the research supporting the notion of place learning place and direction information had been confounded (e.g., Tolman et al., 1946). Since then, many scientists have suggested that rats have a relative sense of both direction and place; and some researchers have observed that those senses are, in part, responsible for spontaneous alternation behaviours (a tendency to enter unvisited maze arms across trials) (Douglas, 1966; Sherrick, Brunner, Roth & Dember, 1979). More recently, Skinner et al., (2003) replicated the findings of Blodgett et al. (1949) and, in a set of experiments, concluded that rats readily solve direction and response tasks, but have difficulty using place

information in discrimination problems. Later experiments, by Skinner and colleagues, provided further support for the notion that direction and response learning occur more readily in rats than place learning (See Skinner, Horne, Murphy, & Martin, 2010; Stringer et al., 2005; Whyte, Martin, & Skinner, 2009). Furthermore, other researchers have demonstrated that direction learning predominates place learning in various water maze tasks (where an animal must learn to locate a hidden platform) that have typically been associated with place learning (for example, see Hamilton, Akers, Weisend & Sutherland, 2007; Hamilton et al., 2008).

Navigation and Direction in the Animal Brain

Modern scientific techniques have elucidated that mechanisms exist within the brain that may control or at least support spatial navigation. Specifically, cortical areas (the parietal cortex and the entorhinal cortex) and many interconnected structures within the limbic system are highly associated with navigation, housing representations of place, direction, velocity, and possibly distance (Clark & Taube, 2012; Dudchenko, 2010; Hafting, Fyhn, Molden, Moser, & Moser, 2005; Muir & Taube, 2002; O'Keefe & Dostrosky, 1971; Stackman, 2010; Taube, 2007). Several cell types, whose activity is associated with and thought to be critical for animal navigation behaviours, can be found in this system (Dudchenko, 2010; Stackman, 2010). However, correlations between the activity of such cells and performance on spatial tasks are not often strong, are sparse, or have proven difficult to replicate (Muir & Taube, 2002; Muir & Taube, 2004). The most commonly discussed cell types of this category are: the place cell, the grid cell, and the head direction cell (Stackman, 2010; Taube, 2007).

Place cells, initially discovered by O'Keefe and Dostrovsky (1971), are prominently observed in the hippocampus and are said to exhibit location-specific firing, independent of animal orientation (Muller, Bostock, Taube, & Kubie, 1994; Muller, 1996; Moser, Kropff, &

Moser, 2008). Basically, individual place cells are active when an animal passes through the cell's preferred environmental sub-field (denoted a place field), and cell activity generally peaks in the center of such a field (Muller, 1996; O'Keefe & Nadel, 1978). A similar cell type has been found in the entorhinal cortex that exhibits firing fields at environmental borders; this cell type is aptly known as the border cell (Solstad, Boccara, Kropff, Moser, & Moser, 2008). Populations of place cells in the hippocampus appear to represent spatial environments equally (Moser et al., 2008). Thus, not surprisingly, the discovery of place cells and how they behave was initially associated with Tolman's (1948) notion of the cognitive map, and their presence in the hippocampus gave rise to the belief that the hippocampus may house the cognitive map (O'Keefe & Nadel, 1978; Weiner et al., 2011). Beyond this, some researchers have suggested that place cells function as cells that discriminate between various environmental contexts through remapping processes (Jeffery & Anderson, 2003).

Grid cells were first discovered by Fyhn, Molden, Witter, Moser and Moser (2004) in the entorhinal cortex and were later described in detail by Hafting et al. (2005). Much like place cells, grid cells are sensitive to position; however, individual grid cells have multiple firing fields and fire in a hexagonal pattern or as series of equilateral triangles encompassing entire environments (or at least micro-environments) (Fyhn et al., 2004; Hafting et al., 2005). Grid cells are the most recently discovered among the major cell types described here, and although their functional significance is debatable, they exhibit many of the firing properties akin to both head direction and place cells, and they are thought to play a role in representing distances in environments and in path integration (Dudchenko, 2010; Hafting et al., 2005; Taube 2007).

The head direction cell, first observed by James Ranck in 1984 in the postsubiculum, favours a specific head direction (a 'preferred direction'), firing maximally when an animal's

head faces said direction (Clark & Taube, 2012; Stackman, 2010; Taube, 2007; Taube, Muller, & Ranck, 1990a; Weiner & Taube, 2005). Head direction cell activity is direction specific, independent of the animal's location, and thus can be viewed as an interesting complement to place cell activity (Stackman, 2010). Head direction cells exhibit several firing properties that are worth noting. When compared to place cells and grid cells, head direction cells are the first to develop and it has been proposed that their activity may be responsible for development of the other navigation cell types within the brain (i.e., they may set up the spatial network as a whole) (Langston et al., 2010; Wills et al., 2010). Within environments, head direction cells maintain their preferred firing directions to a great degree, and when an animal is actively traveling to novel environments their preferred directions are largely maintained (Clark & Taube 2012; Taube & Burton, 1995). In contrast, passive transfer leads to a consistent and equal shift in head direction cell firing across cells, seemingly to a random degree (Dudchenko, 2010). The preferred firing range of a head direction cell is on average 90 degrees; however head direction cells have been observed with ranges as low as 60 degrees and as high as 150 degrees and such preferred ranges are thought to be controlled by both external (allocentric) and internal (idiothetic) cues (Taube, Muller, & Ranck, 1990b; Taube & Burton, 1995; Taube, 2007). Head direction cells are not responsive to vertical head movements, only those made in the horizontal (or yaw) plane and thus are thought to define an animal's horizontal reference plane (Taube, 2007). Interestingly, when traversing through a vertical environment, head direction cells seem to treat the vertical plane as its horizontal plane (see Calton & Taube, 2005; Stackman, Tullman, & Taube, 2000); but, if an animal is upside down (say, crawling across a mesh ceiling), head direction cell activity is largely abolished (Calton & Taube, 2005; Stackman et al., 2000). This abolishment in activity is thought to be a result of an occlusion in the otolith organ, an important

component of the vestibular system (Calton & Taube, 2005). Beyond this, head direction cells maintain their firing direction in the dark; however, some drift in the preferred firing direction of cells occurs with time (Goodridge, Dudchenko, Worboys, Golob, & Taube, 1998).

Within a population of head direction cells, different cells often represent different directions; and importantly, any given population of head direction cells is thought to represent its full 360-degree firing range equally (Taube et al., 1990a). However, only one direction can be represented at any given moment. So, not only can a head direction cell be seen as having a preferred firing direction, but a population itself can, at anytime, be considered tuned to a particular direction (Taube et al., 1990a; Taube et al., 1990b). Thus head direction cells are thought to underlie our so-called sense of direction (Stackman, 2010; Taube et al., 1990; Weiner & Taube, 2005). Head direction cell firing at this level is thought to work as an attractor-hill network, where head direction cells are thought to be linked together so that while one head direction cell is firing maximally, cells tuned to similar firing directions are excited the most while other cells are inhibited from being active (Clark & Taube, 2012; Sharp, Blair, & Cho, 2001a). Such a system should never indicate that an animal is facing more than one direction at any given time (Sharp et al, 2001a).

Early work established that head direction cell firing can become anchored to or controlled by landmarks (Taube et al. 1990b; Taube, 1995). Specifically, head direction cell firing is often directly associated with prominent landmarks (like a cue card on a maze wall); and when such landmarks are moved, head direction cells adjust their firing in accordance with the landmark's new position (Taube et al., 1990b; Taube, 1995). For example, if a prominent cue card's position is rotated 60 degrees along a circular maze wall, head direction cells will tend to adjust their preferred firing directions 60 degrees in the same direction. Such cue control with

head direction cells has been observed to happen within minutes of exposure to a prominent visual cue (Goodridge et al., 1998). However, as noted above, firing is observed in the absence of visual cues, implying that head direction cells are not simply controlled by visual stimuli (Goodridge et al., 1998). Notably, tactile and odour cues are thought to help with maintaining preferred firing directions or may be used as landmarks themselves (Goodridge et al., 1998; Taube, 2007); but ideothetic motion-related cues are thought to play a large role in firing maintenance as well (Taube, 2007).

Head direction cells exist in many brain areas, mostly within what is known as the Papez circuit (For a diagram of this circuit see Figure 1, modeled after Taube, 2007). Consequently, the head direction cell signal is replicated in each of the areas with head direction cells (Dudchenko, 2010; Taube, 2007). We do not know why the signal is replicated in many brain areas, or whether each different region plays a distinct role in the processing of orientation (See Taube, 2007 and Weiner & Taube, 2005 for more comprehensive reviews of this circuit).

Information in the primary head direction circuit is thought to originate in the vestibular nuclei and is then processed serially through the Nucleus Prepositus, the Dorsal Tegmental Nucleus (DTN), the Lateral Mammillary Nucleus (LMN), the Anterior Dorsal Nucleus of the thalamus (ADN), and the Postsubiculum (PoS), terminating in the hippocampal formation (Clark & Taube, 2012; Sharp et al., 2001a; Taube, 2007; Weiner & Taube, 2005). Among these structures, the ADN has the highest density of head direction cells; specifically, approximately 60% of its cells are classic head direction cells (Taube, 1995). The known approximate relative proportion of head direction cells in each other structure is as follows: 12.5% of the DTN (Sharp, Tinkleman, & Cho, 2001b), 25% of the LMN (Stackman & Taube, 1998), and 25% of the Postsubiculum (Taube et al., 1990a).

Importantly, head direction cells have been found outside the primary circuit in varying numbers. For example, Cho and Sharpe (2001) have reported that 10% of the retrosplenial cortex consists of classic head direction cells. However, head direction cell firing outside the primary circuit is thought to be derived from firing within the primary circuit (Taube, 2007). The exact contribution of each structure to an animal's direction sense, the relative behavioural function of each of these structures, and why the directional signal is replicated in multiple brain regions are not well understood (Taube, 2007). However, some evidence suggests the PoS plays a significant role in cue control over head direction and place cell firing (Goodridge & Taube, 1997). Furthermore, head direction cells in different structures are known to have different relative preferred firing ranges and firing rates (Stackman, 2010; Stackman & Taube, 1998). Specifically, head direction cells in structures that are more upstream in the Papez circuit (like the LMN) have been shown to have wider firing ranges and higher peak firing rates than head direction cells in structures that are more downstream (like the ADN and PoS), which appear to have more fine tuned firing ranges (See Stackman, 2010 for a graphical representation of this) (Stackman, 2010; Stackman & Taube, 1998).

The DTN and LMN are highly inter-connected and contain another cell type, the angular head velocity (AHV) cell that appears to be related to the head direction cell (Sharp et al., 2001a; Taube, 2007). AHV cells are sensitive to (i.e., their activity is correlated with) the speed of head turns in the horizontal plane (Bassett & Taube, 2001; Sharp et al., 2001b). These cells exist in two known forms: a symmetric type (which fires regardless of where the head moves) and an asymmetric type (which is associated with firing in either clockwise or counter-clockwise head turns specifically) (Bassett & Taube, 2001). Studies suggest that approximately 75% of the cells

in the DTN (Bassett & Taube, 2001; Sharp et al., 2001b) and approximately 50% of the cells in the LMN are AHV cells (Stackman & Taube, 1998).

Electrophysiological recordings have illustrated that bilateral lesions to early structures within the head direction cell-circuit abolish head direction cell activity downstream (Bassett, Tulman, & Taube, 2007; Blair, Cho, & Sharp, 1999; Goodridge & Taube, 1997; Taube, 2007; Sharp & Koester, 2008). For example, Goodridge and Taube (1997) showed that bilateral lesions to the ADN eliminated head direction cell activity in the PoS but not vice versa. Importantly, bilateral lesions of the LMN and the DTN have been shown to disrupt head direction cell firing in the ADN and PoS (Bassett et al., 2007; Blair et al., 1999; Sharp & Koester, 2008). These data, accompanied with the observation that there appears to be a transition between the representation of head velocity and head direction (as seen with the presence of head direction and AHV cells) at the level of the DTN and LMN, have led most researchers to suggest that the reciprocal connections between the DTN and LMN act as the origin of the head direction cell signal in the brain (Bassett et al., 2007; Blair et al., 1999; Sharp et al., 2001b; Sharp & Koester, 2008).

Much like other researchers (See Stackman, 2010), we believe that by combining lesioning techniques with behavioural tasks known to be associated with orientation, we may examine the behavioural contribution of the individual brain structures in the head direction cell circuit. If head direction cells in the Papez circuit are representative of direction sense, then lesions to components of this circuit should theoretically eliminate the rats' sense of direction and we would expect to see deficits on tasks where orientation is important. This should particularly be true for structures upstream in the circuit (like the LMN and DTN) that are necessary for downstream activity. However, only a small number of studies have adopted this

approach to examining the head direction cell circuit; this is especially true with respect to the DTN and LMN.

Two known studies, outside of our lab, have attempted to examine the behavioural contribution of the DTN on orientation related tasks. In the first study, Frohardt et al. (2006) showed large impairments in heading and accuracy in rats with bilateral electrolytic lesions to the DTN, and only mild deficits in ADN lesioned animals, on a foraging task with and without visual cues (where an animal is trained to leave and return to a home location after foraging for food, a task that is thought to rely heavily on a sense of heading). Similarly, in a more recent study Clark, Rice, Akers, Candelaria-Cook, Taube & Hamilton (2013) showed large deficits in navigational ability in rats with electrolytic lesions to the DTN on several variants of the Morris water maze task, including one that was largely directional. In our lab deficits have been observed on a directional water maze task and a variable hole foraging task (described below) in rats with electrolytic lesions to the DTN relative to sham controls (unpublished data).

Few studies have shown observable behavioural deficits on orientation-related tasks with lesions to the LMN. Vann (2005) showed no deficits on a T-maze alternation task and mild deficits on a working memory task in the water maze in rats with ibotenic acid-induced lesions of the LMN versus sham controls; and Vann (2011) again showed mild deficits on similar tasks in LMN lesioned rats (again using ibotenic acid (IBO)) versus sham controls. In contrast, Winter, Wagner, McMillin, and Wallace (2011) observed large impairments on a water maze task and a food foraging task in rats with electrolytic mammillothalamic tract lesions versus sham controls.

Since it is believed that reciprocal connections between the DTN and LMN are responsible for generating the head direction signal in the Papez circuit and since it has been postulated that head direction cell and AHV cell activity is representative of a direction sense in

animals, we hypothesize that without a DTN or LMN deficits in behaviour will be observed in tasks that have a directional component. Accordingly, rats with bilateral lesions to the DTN or LMN were trained on three tasks to examine whether success on the tasks is reliant on an animal's sense of orientation.

The first task was a directional water maze task, where rats are trained to swim in a constant direction regardless of maze position (adapted from Whyte et al., 2009). In a series of experiments, Peckford, McCrae, Thorpe, Martin and Skinner (2013) indicated that success on this task was based on rats using direction to solve the problem rather than location or specific cue information. In one experiment, rats that were initially trained to swim in a constant direction from two maze positions were later trained from two new positions where they were required to either make the same response based on start point orientation or start point location. Rats that were required to make the same response based on start point orientation were not disrupted in their performance while rats that were required to make the same response based on start point location exhibited an initial disruption in performance. In another experiment, the training room was made dark prior to placing rats in the maze. Rats trained to respond using orientation cues were not disrupted when visual, scent, and auditory cues were made obscure by the experimenters. However, rats required to respond using location cues were disrupted. The authors showed that some initial exposure to the training room (in the light) was required for rats to successfully use orientation cues. Taken together, these results suggest that rats learn the directional water maze task by making a conditional discrimination based on orientation rather than relying on start point location or distal visual cues. Such results are consistent with data from appetitive dry-land tasks (Skinner et al., 2003), aversive water maze tasks (Whyte et al.,

2009) and a response reversal learning paradigm (Wright, Williams, Evans, Skinner, & Martin, 2009).

The second and third tasks were variants of a foraging task (Whishaw & Maaswinkel, 1998), where rats were trained to return home after finding food on an open field maze. In one version of the task, a single home hole was used. In the other, the home hole varied from trial to trial. Similar tasks have been used with success in other behavioural experiments examining the head direction cell circuit (see Frohardt et al., 2006). In the absence of visual cues, such tasks are thought to rely on path integration processes (Whishaw & Maaswinkel, 1998). However, even in bright conditions, where rats presumably solve the task using visual cues, the types of errors animals make could reveal deficits in orientation. For example, animals with impaired heading (i.e., animals with lesions to the DTN or LMN) might be expected to approach holes adjacent to the correct hole, after foraging, more often than sham controls.

Rats were given bilateral lesions to either the DTN (Experiments 1, 2) or LMN (Experiment 3) and were tested against sham controls on the water maze task (all experiments), or in one of two variants of a foraging task (Experiments 2 and 3). Since, the DTN and LMN (and the connections between them) appear critical in the generation of the head direction signal, it was hypothesised that lesioned animals in all experiments would exhibit impaired performance compared to sham controls on these tasks, and that their behaviour would indicate that our tasks are at least partially reliant on an animal's sense of orientation.

Experiment 1

The aim of this experiment was to replicate previous work from our lab, examining the effects of electrolytic lesions of the DTN on direction learning and to examine the behavioural effects of neurotoxic lesions to the DTN (using IBO) in our water maze task. Rats were placed

into three groups: a sham control group, a neurotoxic lesion group (where cell bodies are selectively killed while fibers of passage remain intact), and an electrolytic lesion group (non-selective lesions, where all matter surrounding an electrode tip is destroyed). Performance of the three groups of rats was compared on the directional water T-maze maze task. The high combined percentage of HD and AHV cells in the DTN, the DTN's apparent importance for HD cell activity throughout the Papez circuit, and previous behavioural analyses, indicate that the DTN is important in the brain's orientation system. Thus, we hypothesized that lesions to the DTN, regardless of type, would produce deficits in the water maze task.

Method

Subjects

Thirty-two male Long-Evans rats (*Rattus norvegicus*) were purchased from the Charles River Company (St. Constant, Quebec, Canada) and housed individually at Memorial University of Newfoundland, on a 12:12 light/dark schedule (with lights on at 0700). Rats were fed standard laboratory animal feed (LabDiet) and water ad libitum prior to experimental trials and throughout the experiment. The rats' weights were recorded upon arrival (and then twice weekly, with very few exceptions), and prior to the experiment rats weighed on average 306 g (s = 15 g). The rats were usually given one or two objects in the home cages for enrichment, which could include wooden blocks, plastic toy bones (Nylabone), paper cups or plastic tubes. All procedures were approved by Memorial University of Newfoundland's Institutional Committee on Animal Care and followed the Canadian Council on Animal Care guidelines.

Surgery

The day before surgery, animals were taken off ad libitum feeding to promote the effectiveness of the anaesthetic. Twelve rats were given neurotoxic lesions to the DTN, twelve

rats were given electrolytic lesions to the DTN, and eight rats were used as sham controls. Rats were anesthetized via injection of a 0.24M chloral hydrate solution (1 ml/100 g, i.p.) and placed in a stereotaxic instrument (Model 900, KOPF). Neurotoxic lesions to the DTN were initially produced by injecting 0.3 µl of IBO (10 mg/1 ml), mixed in phosphate buffered saline (Sigma Chemical, St. Louis, MO), into each hemisphere using a 1 µl Microliter Syringe (Hamilton Company) and Micro Injection Unit (Model 5000, KOPF). The volume of IBO was reduced to 0.15 µl after five rats died shortly after the injection. The coordinates used were modified from those used by Frohardt et al. (2006) (AP coordinates: 11.7 mm posterior to bregma; ML coordinates: ± 0.3 mm; DV coordinates: 7.0 mm below the brain's surface). The syringe was lowered at a 20° angle to avoid damage to the superior sagittal sinus. The IBO was injected at a constant rate over 6 min, and the needle was left in position for an additional 5 min after the injection to prevent backflow. Electrolytic lesions to the DTN were produced by inserting a stainless steel electrode (NE 100, Rhodes Mechanical instruments) into the sites described above and by passing a 3.0 mA current for 15 s through the electrode. Sham controls were anesthetized, placed in the stereotaxic instrument and had holes drilled into the skull over the lesion sites; however, they did not receive injections. For these sham surgeries, 2 were lowered needle shams, 2 were lowered electrode shams, and 4 were unlowered shams. For the lowered shams, to control for mechanical damage, a needle or the electrode was lowered to the surgical site in the brain. Following surgery, the rat's wounds were sutured and rats were placed under a heat lamp, where they rested until they were fully awake.

Subjects Post Surgery

Six rats (one sham control and five neurotoxic lesions) died during (or shortly after) surgery. Although the cause of death is unknown in these rats, we assume that the sham rat died

as a result of an overdose of anaesthetic and the lesioned rats died as a result of too much neurotoxin being infused into the DTN. Rats were given a minimum of one week to recover post surgery and were monitored daily during this period.

Apparatus and Materials

The water maze apparatus was a Plexiglas plus maze within a circular metal tank (120 cm in diameter and 31 cm high), with Plexiglas walls extending 31 cm above the metal tank. The maze arms were 11.5 cm wide and 52.5 cm long; and, like the walls of the maze, extended 31 cm above the metal tank. The whole apparatus was placed on a metal frame with wheels to facilitate transportation. The plus maze was converted into a T-maze using a clear Plexiglas barrier, which was snapped to the plus maze with butterfly clips, obstructing access to the arm opposite the start arm, but not obstructing visual access. The water level was kept at approximately 2.5 cm below the top of the metal tank, and the water was kept at room temperature (about 20° C) and was made opaque by adding approximately 250 ml of non-toxic white Tempera paint (Michael Stores, Inc, Irving, TX). The escape platform (11.5 cm in diameter and 26.5 cm high) was constructed from white plumbing tubing attached to a Plexiglas base and filled with sand for stability. The platform was placed approximately 1 to 2 cm below the surface of the water, remaining obscured when viewed from the water's surface.

The training room (528 x 464 x 267 cm) was littered with visual cues. Windows (lined with aluminum foil) covered the north wall. There were two doors, one located on the south wall and one on the east wall. In the southwest corner of the room was a sink and counter top; shelves lined the west wall and half of the east wall, housing various lab supplies. The southeast corner of the room contained a metal garbage can and various lab supplies (a cart, maze parts, and several boxes). The northeast corner of the room contained a coat rack and several storage

lockers and the northwest corner of the room contained a small table with two chairs. Lastly, animal cages were arranged on a table below the shelving on the west wall.

Basic t-tests were used to analyze the data between groups for this experiment. Only probability values less than .05 were accepted as significant.

Procedure

Rats were trained to swim in a constant direction to locate the hidden platform from two maze positions. Half the rats in each group were trained to go east from locations A and C and half were trained to go west from locations B and D as shown in Figure 2. Between trials, the maze was translated (approximately 114 cm) and rotated 180° so that the maze and start point were in different locations in the experimental room. Thus rats were trained to make a conditional discrimination on the basis of their start-point orientation in the room (as per Peckford et al., 2013). For example, if a rat was trained to make a right turn from position A, then a left turn would be the correct response from position C. Training proceeded with eight trials/day until the rats met a criterion of 18/20 or had completed a total of 120 trials. Within each block of eight trials there were four trials from each start position with no more than two trials in a row given from the same maze position. Although the inter-trial interval varied as the experiment progressed, the interval was initially about 10 minutes in length.

On training days, the rats were wheeled upstairs on their housing carts and were brought into the experimental room one squad (n = 8) at a time on a smaller cart. They were placed individually in plastic holding cages, like their home cages, where bedding was replaced with industrial paper towel. Rats that were not being trained, and all home cages, were kept outside the experimental room. For each trial, a rat in its holding cage was carried in a counterclockwise direction to a chair positioned at the start arm, and then the rat was placed in the start arm facing

the wall of the maze. The arms visited by the rat and the latency (in seconds) to locate the hidden platform was recorded for each trial. Rats were considered to have made a choice when their body, minus their tail, was in an arm. A trial was deemed correct if a rat entered the arm containing the platform, and successfully climbed onto the platform, without entering another arm. Once the rat located the platform, it was allowed to sit there for approximately 5 s before being removed from the maze and returned to its holding cage. If a 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; and if two experimenters were in the room, a second experimenter remained seated at the table in the room's northwest corner. Upon completion of a trial, the rat was placed back in its holding cage and carried back to the holding table in a clockwise direction and the next rat began its trial. To reduce background noise, a radio played music from a local radio station during experimental trials.

Histology

Upon completion of this experiment, the rats were euthanized via urethane (15%) injection (1 ml/100 g) followed by decapitation. The brains were removed, submerged in cooled 2-methylbutane (-70°C), and stored in a freezer (which was maintained at a constant temperature of -70°C). Each brain was sectioned in the coronal plane at 30-μm using a standard cryostat and later nissl stained (with cresyl violet) and digitally imaged for lesion site verification. Digital images taken approximately at the level of the central and peri-central subregions of the DTN (9.3 mm posterior to bregma) were analyzed using Image-J software (http://rsbweb.nih.gov/ij/) to measure the percent damage to the DTN. This analysis was made blind to the behavioural data. The following formula (from Clark et al., 2013) was used to assess relative damage: Percent tissue damage = [average area of DTN in control rats ($pixels^2$) - total area of spared DTN tissue

in lesioned rats (pixels^2)/ average area of DTN in control rats (pixels^2)] X 100%.

Results

Histological analyses, performed blind with respect to behavioural data, revealed that one neurotoxic lesioned rat and five electrolytic lesioned rats had significant damage to the DTN (>53%). Damage resulting in apparent cell death (appearing as structural shrinkage or as abnormal staining) or a physical hole near the central region of the DTN was accepted for this analysis. Representative brain sections for this experiment are shown in Figure 3. Since only one neurotoxic lesioned animal sustained significant damage to the DTN (53%), the IBO lesion group was excluded from statistical analysis. Nonetheless, this animal was the most impaired among the animals in the IBO group (reaching criterion on the water maze task in 92 trials). The five electrolytic lesioned rats each sustained greater than 60% damage to the DTN (with damage ranging from 63 – 90%).

As in a previous unpublished experiment from this lab, rats with electrolytic lesions to the DTN were impaired on the water maze task relative to sham controls. Using the criterion of 18/20 trials correct used previously, the difference between the sham control group ($M = 61.40$) and the electrolytic lesion group ($M = 90.80$) only approached significance, $t\,(10) = 2.03$, $p = .07$. However, when a criterion of 16/20 trials correct was used sham control rats ($M = 48.40$) completed the water maze task in significantly fewer trials than DTN lesioned rats ($M = 87.60$), $t\,(10) = 2.69$, $p < .05$ (see Figure 4).

Discussion

Consistent with previous findings from this lab, rats with electrolytic lesions to the DTN were impaired on the directional water maze task. The fact that this deficit was significant only with the less stringent criterion of 16/20 trials correct is probably due to the relatively small

number of rats with acceptable lesions (n = 5). Furthermore, our final sample size was too small to make any definite claims about our neurotoxic lesions. Consequently, it was decided that further experimentation was needed to examine the possible behavioural deficits of neurotoxic lesions to the DTN.

Experiment 2

To further examine the behavioural effects of neurotoxic lesions to the DTN and to obtain a larger sample than in Experiment 1, we repeated the previous experiment using a different neurotoxin (NMDA) and modified the surgical coordinates. Sham- and neurotoxin-lesioned rats were compared on a series of behavioural tasks thought to have an orientation component; the water maze task used in Experiment 1 and two versions of a dry-land foraging task. For the reasons outlined in Experiment 1, it was hypothesized that neurotoxic lesions to the DTN would produce observable deficits on all tasks. It was also hypothesized that the error types observed on the fixed and variable hole versions of the foraging task would reflect deficits in heading as evidenced in a propensity for lesioned animals to return to holes adjacent to the correct hole.

Subjects

Twenty male Long-Evans rats (*Rattus norvegicus*) were purchased from the Charles River Company (St. Constant, Quebec, Canada) and weighed on average 278 g (s = 14 g) at the start of the experiment. The rats were maintained as in Experiment 1 with the following exception: Prior to Phases 2 and 3, the rats were food deprived so that their weights were maintained at approximately 85% of their free feeding weight (which, at the time, was on average 440 g, s = 24 g). For these phases of the experiment, rats were fed 1 g dusted food pellets (BioServ, Frenchtown, NJ) as reinforcement during experimental trials. All procedures

were approved by Memorial University of Newfoundland's Institutional Committee on Animal Care and followed the Canadian Council on Animal Care guidelines.

Surgery

Four rats were used as surgical pilots to test the neurotoxin NMDA (Sigma Chemical, St. Louis, MO) and to verify (and adjust) the surgical coordinates for the DTN. Eight rats were given neurotoxic lesions of the DTN and eight rats were used for sham controls. The surgical procedures for the neurotoxic lesions in this experiment mimicked those outlined in Experiment 1, but with the following differences. Neurotoxic lesions to the DTN were produced by injecting 0.3 µl of 100 mM NMDA, mixed with a phosphate buffered saline, into each hemisphere. The surgical coordinates were slightly modified from those used in Experiment 1 (AP coordinates: 11.7 mm posterior to bregma; ML coordinates: ± 0.3 mm; DV coordinates: 7.1 mm below the brain's surface). Lastly, all sham animals were unlowered shams.

Subjects Post Surgery

Two rats were euthanized due to scratching behaviours (around the ears and the surgical site) and were deemed unfit for training. One of these, a sham animal, completed training in the water maze; the other, a lesion animal, did not. Another lesioned rat died during phase 2 of this experiment. All rats were given a one-week recovery period as in Experiment 1.

Apparatus and Materials

The water maze apparatus and experimental room used in Phase 1 of the current experiment were identical to those of Experiment 1.

The dry-land apparatus used for the foraging task was a large, wooden, white circular table (204 cm in diameter) that was raised 75 cm above the floor. Eight holes (11.5 cm in diameter) were evenly distributed around the perimeter of this table (45° degrees apart and 13.5

cm from the edge of the table). Three wooden food cups (6 x 6 x 4 cm) were arranged in the center of the table in an equilateral triangle with edges 57 cm in length (from the center of the food cups) and oriented with respect to hole 1 (See Figure 5). This maze was used for both Phase 2 and 3 of this experiment and a single food pellet was placed in one of the three small wooden cups (in a pseudo randomized order) for all training trials. Steel wire mesh cages (20 × 25 × 19 cm) were used during training, and these cages were manually attached to metal runners beneath a hole at the periphery of the table. For all trials, the maze apparatus was placed approximately in the center of the training area. Wooden stair-like blocks were positioned in the wire cage at the beginning of each trial to help rats in accessing the table. Once the wire cage was placed beneath a hole, a rat could easily leave the cage by climbing up on the table and return by climbing back down.

The room used for pretraining on the dry-land task (523x 439 x 267 cm) had a variety of visual cues. Windows covered the north wall; and in front of these windows were a desk and a table, each with a computer on them. There were three doors in the room, one on the west wall, one on the east wall, and one on the south wall. Shelving lined the east, south and half of the west wall, housing a variety of laboratory supplies. Underneath the shelving, in the southeast corners of the room were a countertop and a sink; and a table rested alongside the west wall. A cart, housing the animal cages, was positioned in a space near the southwest corner of the room; and the experimenter sat at a table in the northeast corner.

The training room (589 x 465 x 267 cm) for Phase 2 was a large room that had been divided into two training areas with a blue curtain in the center of the room. The cage-holding cart, housing the animal cages, was positioned in a space near this curtain. There were two doors in this room, one on the west wall and one on the north wall. The south wall was lined with

windows and the west wall was lined with shelving, housing various supplies. Two tables rested in the room, one along the west wall and one along the east wall, both in the northernmost half of the room. A long countertop (with a shelf above it, housing supplies) ran along the southernmost half of the east wall, and, various boxes and materials could be found underneath this counter. Beyond this, in the southwest corner of the room, there was a sink. Phase 2 was completed in the south most half of the room and the experimenter sat by the sink in the northeast corner.

The training room for Phase 3 was the smallest of the training rooms used in this study (259 x 589 x 267 cm). There were two doors in this room, one on the north wall and one on the east wall. A cage-holding cart was placed near the door on the north wall. On the west wall, in front of the cart, was a large air conditioner. In the northwest corner of the room there was a sink and, during experimental trials, the experimenter sat by this sink. Lastly, windows lined the south wall and a computer table (with a computer) rested in front of these windows.

Basic t-tests and ANOVAs (with appropriate related analyses) were used to analyze the data between groups for this experiment as outlined below. Only probability values less than .05 were accepted as significant.

Procedure

Phase 1 – Water maze task

Rats were trained to swim in a constant direction to locate the hidden platform from two maze positions. All details of the training procedure for this phase were identical to those outlined in Experiment 1.

Phase 2 – Fixed hole version of the foraging task

During pretraining (and training), the rats were transferred to the steel wire mesh cages in the colony room and then were wheeled upstairs on a holding-cart. For each pretraining trial, a

rat's wire mesh cage was attached to the metal runners on the maze corresponding to hole 1 and then the rat was allowed to begin foraging. A single food pellet was positioned in a single food cup on the center of the maze, and rats were trained (given 2 to 4 trials daily) until they consistently left and returned to the starting hole with the food pellet (i.e., on three consecutive trials on a given day). Initially, to coax the rats from their cages, food pellets were placed near the opening of the hole and incrementally moved closer to the food cup with each pretraining trial. Rats were given a maximum of two minutes to leave their home cage and they were allowed a maximum of three minutes to explore the open field; if they exceeded either of these constraints, the trial was ended and they were immediately returned to the holding-cart. Because the amount of pretraining required was variable, and some rats completed pretraining days before others, all rats were administered refresher trials on the day before regular training began.

Rats were trained to return to a single, fixed hole on the dry-land maze after foraging for a food pellet (located in one of the three food cups). During training, each rat was pseudo-randomly assigned a home hole (1, 3, 5, or 7 as shown in Figure 5). On any given trial, a rat's wire mesh cage was attached to the metal runners on the maze at the appropriate hole and then the rat was allowed to begin foraging. Generally, a rat would exit its cage, forage until it found the food pellet, and then attempt to return home with its reward. After a trial was completed, the rat was returned to the holding-cart and the next rat was prepared for its trial. If a rat did not leave its cage in 120 s, then the trial was ended and the rat was returned to the holding-cart. Any trials where the rat did not leave its cage or did not find food were excluded from data analysis. The latency to retrieve a food pellet, the latency to return home after retrieving a food pellet, the food cups visited prior to retrieving a food pellet, and the holes visited after retrieving a food pellet were recorded. Rats received three trials/day until they reached a criterion of 9/10 trials

correct; and a trial was deemed correct if a rat returned to its starting hole after finding food without a visit to another hole. Training was stopped at 34 trials for rats in the lesioned group that did not meet criterion; this was double the number of trials required by the slowest sham rat. Throughout this phase, to reduce background noise, a radio played music from a local radio station. Between a set of trials (where each rat in a group was run once), the maze was rotated 45° and the food cups were repositioned accordingly; furthermore, after each training session, the maze was cleaned (with soap and water) to minimize the presence of scent related cues. Although the inter-trial interval varied, on average it was 20 minutes in length. Rats that finished early were given refresher trials prior to the onset of Phase 3.

Phase 3 – Variable hole version of the foraging task

The rats were trained to return to a starting hole, which varied from trial to trial, after foraging for a food pellet. This task and the behavioural measures recorded were identical to the fixed hole version of the foraging task with the following exceptions. On each training day, the rats were given one trial from each of holes 1, 3, and 5 in a pseudo-random order (i.e., the rats were trained to forage from multiple starting holes). Rats were trained until they had completed a total of 60 trials. If a rat did not leave its cage in 120 s or if it returned to its cage without a food pellet, then the trial was not included in the data analysis and did not count towards the 60 total trials. As in Phase 2, the inter-trial interval was approximately 20 minutes. 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. "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, Pirzada, Bridger, Tomlin, Thorpe, & Skinner, 2011).

Histology

All histological procedures and analyses of this experiment were identical to the procedures outlined in Experiment 1.

Results

Histological analyses, conducted as in Experiment 1, showed that six neurotoxic lesioned rats sustained a significant amount of damage to the DTN (with damage ranging from 77 – 90%). Figure 3C shows a representative brain section from this experiment. One sham rat was euthanized after water maze testing, so n = 7 shams for the foraging tasks. Of the six rats with >70% damage to the DTN, one died during the fixed hole version of the foraging task so n = 5 DTN rats for the analyses of foraging data.

Phase 1 – Water maze task

Neurotoxic lesions to the DTN, like the electrolytic lesions used in Experiment 1, produced impairments on the direction task in the water maze. Rats with lesions to the DTN (M = 95.00) took significantly more trials to reach the criterion of 18/20 correct trials than sham controls (M = 57.35), t (12) = 2.31, $p < .05$ (see Figure 6).

Phase 2 – Fixed hole version of the foraging task

Rats with neurotoxic lesions to the DTN were also impaired on the fixed hole version of the foraging task. Rats with lesions to the DTN (M = 27.6) took significantly more trials to reach criterion on this simplified foraging task than sham controls (M = 11.0), t (10) = 5.59, $p < .05$ (see Figure 7). The types of errors made by the lesioned rats were categorized as visits to a hole adjacent to, 90° away from, 135° away from, or 180° away from the correct hole (since analyzing memory errors on this task is not appropriate). A one-way ANOVA revealed a significant main effect of error type, F (3, 16) = 10.70, $p < .05$. Dunnett's multiple comparisons test revealed that

animals with lesions to the DTN made significantly more adjacent-hole errors than each of the other error types (all probability values < .05; see Figure 8). Sham animals did not make a significant number of errors on this task, so, their data was excluded from this analysis.

Phase 3 – Variable hole version of the foraging task

Rats with lesions to the DTN were impaired on the variable hole version of the foraging task. A Two-way (Group x Trial Block) repeated measures ANOVA, using mean trials correct as the dependent variable, revealed a significant effect of Group, $F(1, 10) = 16.83, p < .05$. Rats with lesions to the DTN had significantly fewer trials correct than sham controls (see Figure 9A). A second two-way ANOVA (Group x Trial Block), using total errors as the dependent variable, revealed a significant effect of Group, $F(1, 10) = 11.90, p < .05$, and a significant effect of Trial Block, $F(4, 40) = 2.88, p < .05$. This effect of Group shows that rats with lesions to the DTN exhibited significantly more errors than sham controls on this task. The effect of Trial Block likely reflects a reduction of errors across blocks in lesioned rats (see Figure 9B). A third two-way (Group x Error Type) ANOVA on first hole choice errors was used to examine the types of errors made by the rats, and revealed a significant effect of Group, $F(1, 10) = 16.83, p < .05$ and a significant effect of error type, $F(2, 20) = 6.87, p < .05$. For the latter effect, Holm- Šídák multiple comparison tests revealed that all rats made significantly more memory errors than other errors ($p < .05$; see Figure 9C). Separate t-tests comparing lesioned and sham rats across error types revealed that sham and lesioned animals did not differ significantly in the number of memory errors ($t(10) = 1.22, p > .05$) or the number of other errors made ($t(10) = 2.08, p > .05$). However, lesioned animals made significantly more adjacent-hole errors than sham controls, $t(10) = 3.94, p < .05$ (see Figure 9C).

Discussion

Deficits were observed in animals with neurotoxic lesions to the DTN versus sham controls on all tasks. Rats with lesions to the DTN were not incapable of completing the tasks. Rats with lesions to the DTN were capable of reaching criterion in both the water maze task and the fixed hole version of the foraging task, they simply took more trials (on average) to do so in comparison to sham controls. Furthermore, a reduction of errors across blocks was noted in lesioned animals in the variable hole version of the foraging task. These results could be reflective of an intact, albeit impaired, sense of direction. Rats with lesions to the DTN did not make significantly more memory errors than sham controls, implying that the use of visual cues in navigation were not impaired in animals with DTN lesions. It is possible that DTN lesioned rats developed alternate strategies (that do not necessarily require a sense of direction) to solve the tasks. However, DTN lesioned animals made significantly more adjacent-hole errors than any other error type on the fixed hole version of the foraging task and significantly more adjacent-hole errors as compared to sham controls one the variable hole version of the foraging task, implying that some aspect of the orientation or heading system was impaired in the animals with DTN lesions. Our results clearly support the notion that the DTN appears to be important for solving these behavioural tasks.

Experiment 3

The LMN, much like the DTN, is largely comprised of a combination of HD cells and AHV cells. Furthermore, it is believed that reciprocal connections between the DTN and LMN are responsible for generating the head direction signal in the Papez circuit. Thus, we expect to see deficits on tasks with an orientation component in LMN lesioned animals comparable to those observed in DTN lesioned animals. In this experiment, rats with neurotoxic lesions to the

LMN and sham control rats were tested on the water maze task and the variable hole foraging task described in Experiment 2. It was hypothesized that LMN lesions would produce observable deficits on these learning tasks. It was also hypothesized that LMN lesioned animals would show deficits in heading compared to sham controls by making more adjacent-hole errors on the variable hole version of the food foraging task.

Method

Subjects

Twenty-four male Long-Evans rats (*Rattus norvegicus*) were purchased from the Charles River Company (St. Constant, Quebec, Canada) and maintained as in Experiment 2. Rats weighed between 247 – 281 g at the start of the experiment. Prior to Phases 2 and 3, the rats were food deprived so that their weights were maintained at approximately 85% of their free feeding weight. All procedures were approved by Memorial University of Newfoundland's Institutional Committee on Animal Care and followed the Canadian Council on Animal Care guidelines.

Surgery

Sixteen rats were given neurotoxic lesions of the LMN and eight rats were used as sham controls. Surgical procedures for this experiment mimicked those outlined in Experiments 1 or 2 as follows. Neurotoxic lesions to the LMN were produced by injecting NMDA as per Experiment 2, but with coordinates, based on coordinates used by Vann (2005), corresponding to the position of the LMN in the rat brain (AP coordinates: 4.7 mm posterior to bregma; ML coordinates: ± 1.1 mm; DV coordinates: 9.0 mm below the brain's surface). All lesions to the LMN were conducted by lowering the syringe into the brain perpendicular to the surface of the skull. Four sham animals were unlowered shams and four sham animals had an empty needle lowered into the LMN.

Subjects Post Surgery,

Post surgical care procedures were identical to those used in Experiment 1 and 2.

Apparatus and Materials

The water maze and foraging apparatus were the same as used in Experiment 2. The training room for the water maze task was the same as used in the previous two experiments. The pretraining room for the foraging task (478 x 338 x 253 cm) was located in the basement of the facility and had a single door on the north wall. There was a sink was in the southwest corner and a table in the northwest corner of the room. The cage-holding cart, some boxes, and laboratory supplies, rested along the west wall. The experimenter sat in the northeast corner of the room at a desk. The training room for the variable hole version of the task was the pretraining room used in Experiment 2 for the foraging task.

Like with Experiment 2, basic t-tests and ANOVAs (with appropriate related analyses) were used to analyze the data between groups for this experiment as outlined below. Only probability values less than .05 were accepted as significant.

Procedure

Phase 1 – Water maze task

The procedure for the water-maze task was identical to that used in Experiment 2.

Phase 2 – Variable hole version of the foraging task

The procedure for this foraging task, and the pretraining associated with this task, was identical to that of Experiment 2.

Histology

The histological procedures of this experiment were the same as the procedures used in Experiments 1 and 2 with the following exceptions. Digital images of the LMN (taken at about

4.80 mm posterior to bregma) were analyzed using Image-J software to measure the percent damage to the structure. The following formula was used to assess relative damage: Percent tissue damage = [average area of LMN in control rats (pixels^2) - total area of spared LMN tissue in lesioned rats (pixels^2)/ average area of LMN in control rats (pixels^2)] X 100%.

Results

Histological analyses revealed that damage to the LMN was minimal in most rats. Only three neurotoxic lesioned rats sustained >30% damage the LMN (with damage ranging from 30 – 100%). All other lesioned rats were removed from the behavioural analyses. Damage resulting in apparent cell death (appearing as structural shrinkage or abnormal staining at the lesion site) or a physical hole near the LMN was accepted for this analysis. Figure 10 shows a brain section from a sham control (A) and a section from the animal with 100% damage to the LMN (B).

Phase 1 – Water maze task

Rats with lesions to the LMN showed a transient deficit on the direction task in the water maze. Lesioned rats had significantly fewer trials correct on the first day of training than sham controls, t (9) = 3.36, $p < .05$ (see Figure 11A). However, rats with lesions to the LMN (M = 44.20) and sham controls (M = 43.75) reached criterion in a similar number of trials, t (9) = 0.84, $p > .05$ (see Figure 11B).

Phase 2 – Variable hole version of the foraging task

Rats with lesions to the LMN were impaired on the variable hole version of the foraging task. A two-way (Group x Trial Block) repeated measures ANOVA, using mean trials correct as a dependent variable, revealed a significant effect of Group, F (1, 9) = 45.56, $p < .05$. Rats with lesions to the LMN had significantly fewer trials correct than sham controls (see Figure 12A). A second two-way ANOVA (Group x Trial Block), using total errors as a dependent variable,

revealed a significant effect of Group, F (1, 9) = 51.76, $p < .05$. Rats with lesions to the LMN made significantly more errors than sham controls (see Figure 12B). A third two-way (Group x Error Type) ANOVA on first hole choice errors was used to examine the types of errors made by the rats, and revealed a significant effect of Group, F (1, 9) = 43.81, $p < .05$ and a significant effect of error type, F (2, 18) = 13.13, $p < .05$. For the latter effect, Tukey post-hoc comparisons revealed that rats made significantly more memory errors than adjacent-hole errors ($p < .05$) and other errors ($p < .05$; see figure 12C). Separate t-tests comparing lesioned and sham rats across error types revealed that rats with lesions to the LMN made significantly more memory errors (t (10) = 3.41, $p < .05$), other errors (t (10) = 2.97, $p > .05$) and adjacent-hole errors (t (10) = 3.19, $p < .05$) than sham controls.

Discussion

Prior to histological analyses, we observed no obvious differences between our LMN lesioned animals and sham controls on the water maze task. Thus we opted to exclude the (simpler) fixed hole version of the foraging task used in Experiment 2 and trained the rats on the (more difficult) variable hole version of the foraging task.

Post histology, we observed a transient deficit on the water maze task with LMN lesioned animals. On the first day of training, LMN lesioned animals appeared impaired versus sham controls on the water maze task. However, with continued training, no deficits in performance were observed. In contrast, we observed impairments on the variable hole foraging task in animals with lesions to the LMN in comparison to sham controls. Specifically, we observed more memory errors, other errors and adjacent-hole errors than sham controls in our lesioned animals. Although lesioned animals made more adjacent-hole errors than sham controls as predicted, the impairments here are more suggestive of general impairments than impairments

specifically associated with heading. It is quite possible that the difficulty of the variable hole foraging task makes it more sensitive to disruption than the simpler water maze task. However, our small sample size and the relative size of our lesions make it difficult to make any definite claims about the role of the LMN. Thus, our results can only hint that the LMN plays a significant role in solving the behavioural tasks outlined here, but further examination will be necessary to confirm this and to determine whether heading specifically is impaired.

General Discussion

The pattern of behaviour observed in sham controls across experiments and tasks was typical for unlesioned animals. In all experiments, control rats rapidly met our stringent criterion of 18/20 trials correct on the water maze task. Thus, control rats showed that they could readily learn to travel in a consistent direction from two distinct locations to reach a hidden platform, a result that is consistent with previous research (Hamilton et al., 2008; Peckford et al., 2013). Likewise, control rats in Experiment 2 readily learned to return to a fixed home location after foraging in the open field, rapidly meeting a criterion of 9/10 trials correct.

In Experiments 2 and 3, sham controls exhibited relatively few errors and the pattern of error types was comparable to that observed by other researchers on the variable hole version of the foraging task (Frohardt et al., 2006; Martin et al., 2005; 2011). Furthermore, control rats on the fixed hole version of the foraging task made a negligible number of errors. The most commonly observed error type on the variable hole foraging task in both Experiments 2 and 3 was the memory error. Such errors are thought to reflect proactive interference associated with the use of environmental cues in navigation; rats tend to visit holes that have been associated with escape on previous trials and likely use environmental cues to recognize these escape routes (Martin et al., 2011). Control rats made few errors otherwise, implying that their heading remains

relatively intact while foraging. Their heading could be maintained by using external or internal cue information; however, the prevalence of memory errors implies that allocentric information or external cue (i.e., visual) information is used.

It was hypothesised that lesioned animals in all experiments would exhibit impaired performance compared to sham controls on these tasks, and that their behaviour would indicate that our tasks are at least partially reliant on an animal's sense of orientation. In line with our hypothesis, Experiments 1 and 2 demonstrated that DTN lesioned animals are impaired on tasks with an orientation component. Rats with electrolytic lesions (Experiment 1) and rats with neurotoxic lesions (Experiment 2) were both impaired on the water maze task as evidenced by an increased number of trials needed to meet criterion. Rats with neurotoxic lesions (Experiment 2) were impaired on both the fixed hole and the variable hole versions of the foraging task. Importantly, DTN lesioned animals made a significant number of adjacent-hole errors on both versions of the foraging task. The present results are consistent with those observed in our own lab, using electrolytic lesions (unpublished data), and by other researchers (Clark et al., 2013; Frodhardt et al., 2006). Altogether, these results confirm that the DTN plays a significant role in the directional water maze task and both foraging tasks used in this study, providing further support that rats use their sense of orientation on these tasks.

Although rats with lesions to the DTN were impaired across tasks, improvements were seen with increased training. Most rats with lesions eventually met criterion on the water maze and the fixed hole version of the foraging task. Furthermore, the performance of lesioned animals improved on the variable hole foraging task as evidenced by the reduction in errors across training blocks. These successes may imply that an animal's direction sense is not completely removed with lesions to the DTN and that some alternate path for information transfer exists in

the head direction cell circuit. However, the observed improvements may be indicative of the rat's ability to develop alternative strategies for task success. For example, rats on the variable hole foraging task may have developed a strategy of systematically checking holes on the periphery of the maze after making an incorrect choice (a thigmotactic strategy); a strategy that would reduce the total number of errors over training. Furthermore, much like controls, DTN lesioned animals made a large (and similar) number of memory errors, implying that their ability to use information related to visual cues to orient themselves remained intact. This ability could explain their eventual success on the directional water maze task. For example, although they may have been impaired in their ability to make a conditional discrimination with respect to their heading on in the water maze, DTN lesioned animals may have adopted a beacon homing strategy to succeed at this task.

For LMN lesioned rats, in Experiment 3, a transient deficit was observed on the water maze task (as evidenced by their performance on the first day of training) but impairments were observed on the variable hole version of the food foraging task (as evidenced in the mean correct trials, the total number of errors, and the greater number of memory, adjacent-hole, and other errors). These results suggest that the LMN is at least somewhat important for success on these tasks and is, in part, consistent with previous research (See Vann, 2005; 2011; Winter et al., 2011). Vann (2005; 2011) observed small impairments in LMN lesioned animals on a T-maze alternation task and working memory tasks in the water maze, although these results arguably have little to do with the processing of orientation information. However, Winter et al. (2011) observed severe impairments in LMN lesioned animals on a foraging task similar to that used here and by Frodhardt et al. (2006).

Although LMN lesioned rats reached criterion on the water maze task in a similar number of trials as sham rats, no improvement across trial blocks was seen on the variable hole version of the foraging task with these animals. This discrepancy in performance across tasks is perplexing but it likely can be attributed to differences in task difficulty or differences in strategy use in appetitive versus aversively motivated tasks (Dudchenko, Goodridge, Seiterle, & Taube, 1997). The water maze task used here is a two-choice task (where the animal must choose to go left or right), and conceptually, such a task is relatively easy, requiring only a general sense of heading or a simple beacon homing strategy to complete. Conversely, the variable hole version of the food foraging task is an eight-choice task, with choices equally distributed throughout the horizontal plane. Such a task would clearly be more difficult, where more cues may be necessary for task success and where impairments in the fine tuning of heading may be more observable (with the ability to classify error types). Consequently, the second task may be more sensitive to deficits in orientation; and so, we see deficits with LMN lesioned animals (who may only be mildly impaired in some aspect of orientation processing) on this task. The LMN lesioned animals made significantly more memory, adjacent-hole and other errors than sham controls on the variable hole version of the foraging task. Such results imply that our LMN lesioned animals were impaired on a general level. Cells in the LMN could be responsible for these impairments; however, this could be the result of our small sample size or the accuracy of our lesions.

Our small sample size (of 3 rats) in the LMN lesion group, accompanied with the fact that a great deal of sparing occurred in our LMN lesioned animals, makes it difficult to make sweeping conclusions about the role of the LMN in the tasks used here. It is possible that enough damage was done to the LMN in our experimental group to produce deficits on the more difficult foraging task, but enough of a functioning LMN (and consequently a functional orientation

system) remained to successfully complete the water maze task. Thus, the conclusions made in this thesis with respect to the LMN lesions are tentative and we cannot confidently say that our hypothesis with respect to the LMN was supported. At best, the results hint that the LMN many be important in some aspects of the tasks used here (which may or may not be related to the directional aspects of these tasks). Future research should attempt to replicate and expand upon the outcomes of this thesis to elucidate to relative role of the LMN. Furthermore, the apparent sensitivity of the variable hole foraging task makes it a great candidate task for future experimentation in this field.

The head direction cell signal is thought to originate in the reciprocal connections between the LMN and the DTN and bilateral lesions to both of these structures result in disruptions in head direction cell activity in downstream structures (Bassett et al., 2007; Blair, et al., 1999; Goodridge & Taube, 1997; Sharp & Koester, 2008; Taube, 2007). Furthermore, the large proportion of AHV cells in the DTN and LMN alone suggests that these structures are important for a sense of heading (Bassett & Taube, 2001; Sharp et al., 2001b; Stackman & Taube, 1998). With all of this considered, we expected equivalent deficits in animals with lesions to the DTN and the LMN in the tasks outlined in this work. However, this was not the case. Instead, we observed clear deficits in animals with lesions to the DTN on both the water maze task and the foraging task, but very little impairment in rats with lesions to the LMN on the water maze task. This discrepancy is likely the result of sparing in our LMN lesions, but could also be indicative of redundancy in the head direction cell circuit or may be reflective of the relative difference in the number of AHV cells present in the DTN and LMN (approximately 75% of the DTN and 50% of the LMN is AHV cells).The LMN may play a different role in navigation and orientation than expected, other structures in the head direction circuit may be able to

compensate for damage to this structure, or there may be an alternate pathway for the DTN to relay orientation information throughout the brain. Future studies are necessary to assess these possibilities. Furthermore, the DTN may simply be more important than the LMN and other structures in the head direction circuit for direction sense; and the head direction cell circuit may be arranged in a hierarchy (from general to specific) with regards to orientation sense.

Evidence for a behavioural hierarchy in the head direction cell circuit can be seen when considering the results of other studies in combination with this work. Firstly, Stackman (2010) reported that the preferred direction of head direction cells becomes finer tuned in downstream areas of the head direction cell circuit. Secondly, studies by Clark et al. (2013), Frodhart et al. (2006), and the present study seem to hint that lesions to the DTN produce severe impairments, while lesions to the LMN and ADN produce milder impairments on tasks that have a directional component. A detailed series of experiments, examining the relative contribution of multiple structures in the head direction cell circuit, with a large group of animals, would be needed to test this hypothesis and could be the subject of future work in this area.

Future studies could examine whether structures in the head direction cell circuit play a significant role on tasks where orientation is thought to be important. For example, such procedures could be applied to a response reversal learning paradigm where direction is used as a discriminative stimulus as in Wright et al. (2009). We would predict that animals with lesions to structures like the DTN and LMN would have difficulty using direction as a discriminative stimulus in such a paradigm.

It was hypothesised that DTN and LMN lesioned animals in all experiments would exhibit similar impairments in performance compared to sham controls on our tasks because both of these structures and the connections between them appear important in the generation of the

head direction signal and thus, presumably navigation. In light of the results, this hypothesis was partially supported. The data from Experiments 1 and 2 suggest that the DTN is critical for the tasks examined here, but the data from Experiment 3 are inconclusive (due to the small number of subjects). Peckford et al. (2013) suggested that rats make a discrimination based on their orientation prior to the choice point in the water maze task (i.e., if heading North, make a right turn; if heading South, make a left turn). This present study implies that such a strategy, and the strategy that rats use on the foraging task, are at least partially dependant on the DTN and possibly the LMN. The exact role of the structures in the head direction cell circuit and their relative importance in animal orientation remains to be determined.

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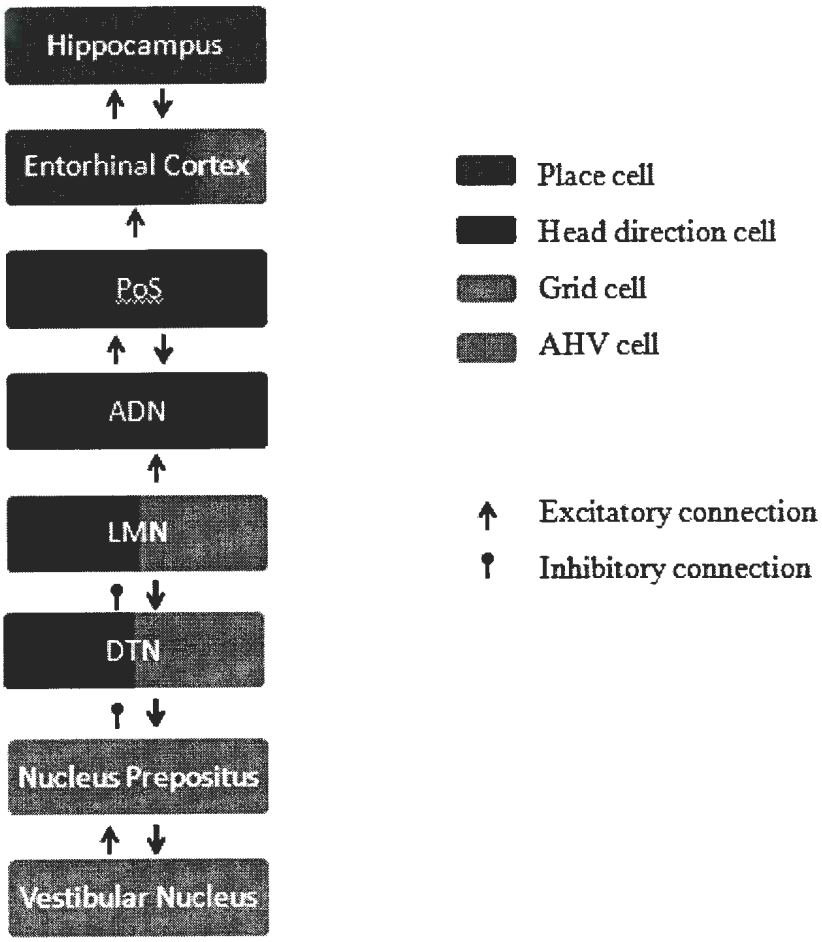


Figure 1: Major connections in the head direction cell circuit (Modeled after Taube, 2007). The diagram indicates (with its colour key) the brain areas where head direction, grid, place and AHV cells have been identified. Colouring is not representaive of the proportions of cell types in the brain per strcutre.

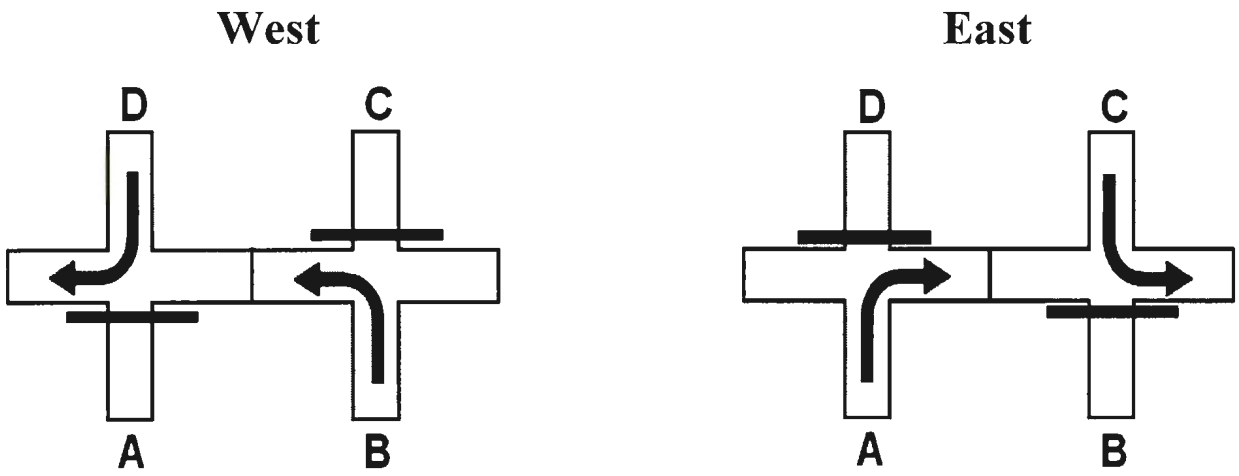


Figure 2: Schematic diagram of the water maze task. Rats were trained to swim in a constant direction regardless of start location. For each experiment, half of the rats were trained to go west from B and D and half of the rats were trained to go east from A and C. Solid black lines indicated the presence of the barrier used to convert the plus maze into a T-maze and solid black arrows indicate the correct path (per trial) from the various start locations.

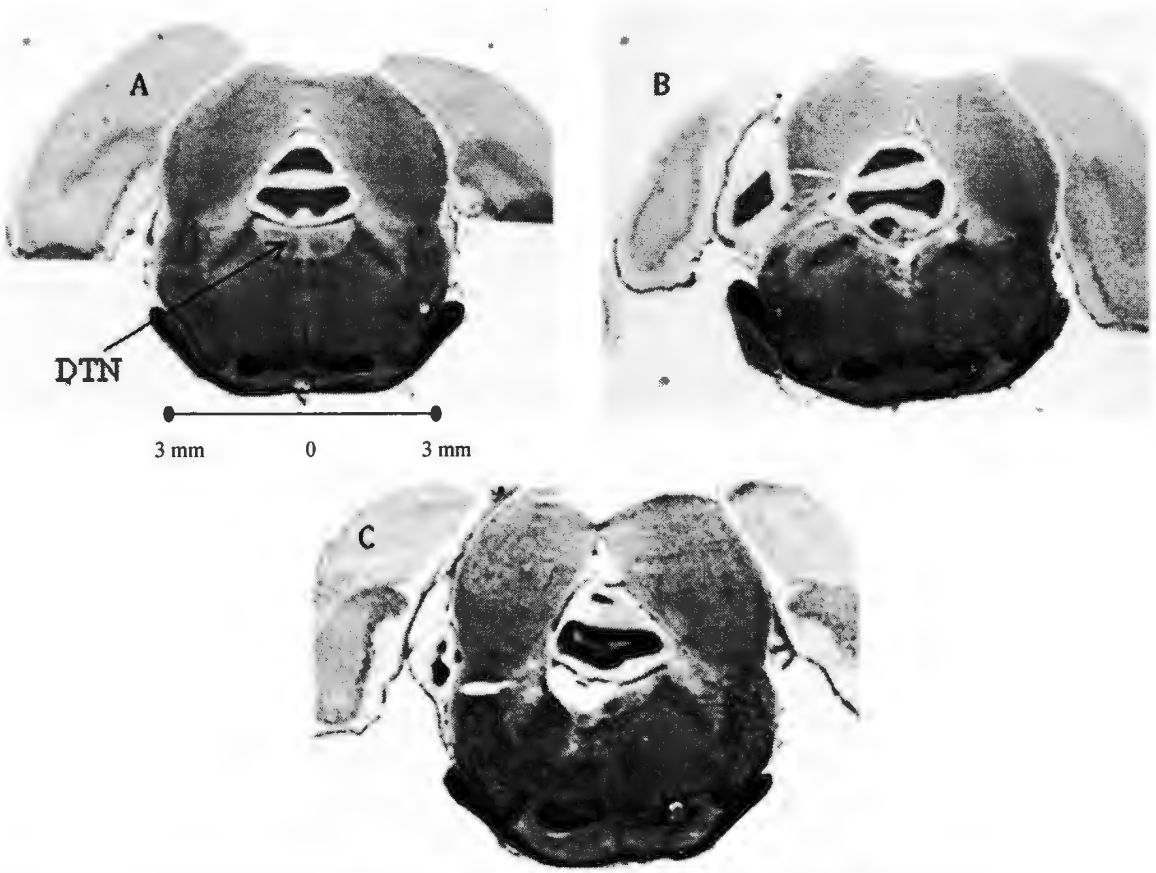


Figure 3: Representative histological sections of DTN lesioned animals and sham controls for Experiments 1 and 2. Panel A shows a brain section from a sham control with an intact DTN, panel B shows a brain section from an animal with an electrolytic lesion to the DTN (Experiment 1), and panel C shows a brain section from an animal with a neurotoxic lesion to the DTN (Experiment 2).

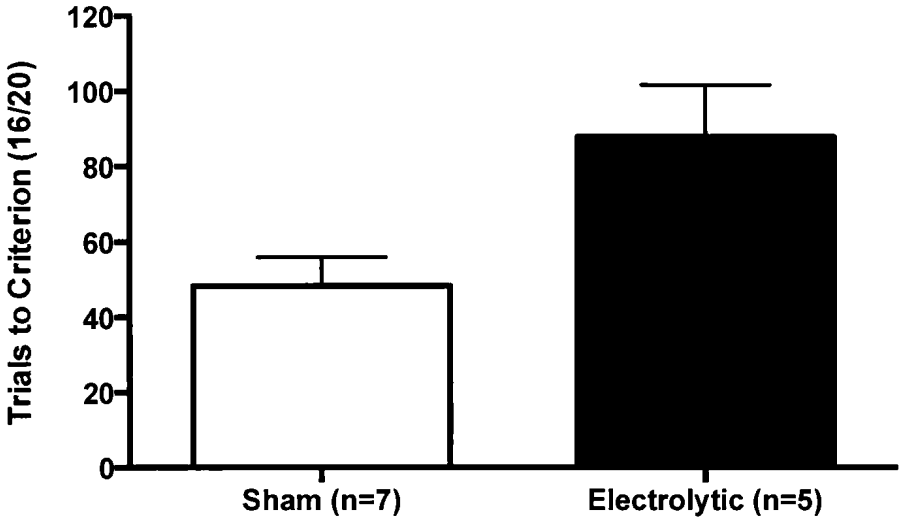


Figure 4: Mean (± SEM) trials to criterion for DTN lesioned rats and Sham controls on the directional water maze task in Experiment 1. Rats with electrolytic lesions to the DTN ($M = 87.60$) took significantly more trials to reach a criterion of 16/20 correct trials than sham controls ($M = 48.40$), $t\ (10) = 2.69$, $p < .05$.

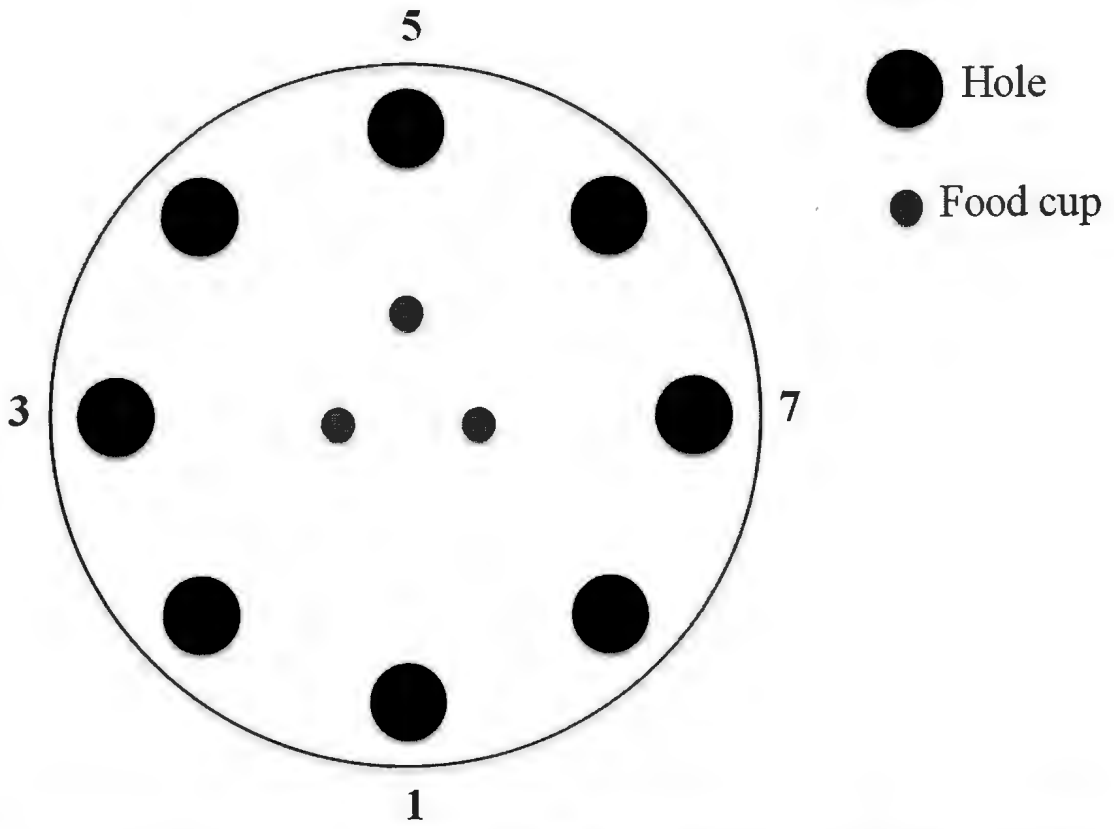


Figure 5: Schematic diagram of the fixed and variable hole foraging tasks. Black circles represent holes in the wooden table where wire mesh (home) cages may be attached to metal runners. Rats were trained to locate and return home with a food pellet (1g) placed in one of three food cups (grey circles). Holes 1, 3, 5 and 7 were used start locations as described in Experiments 2 and 3.

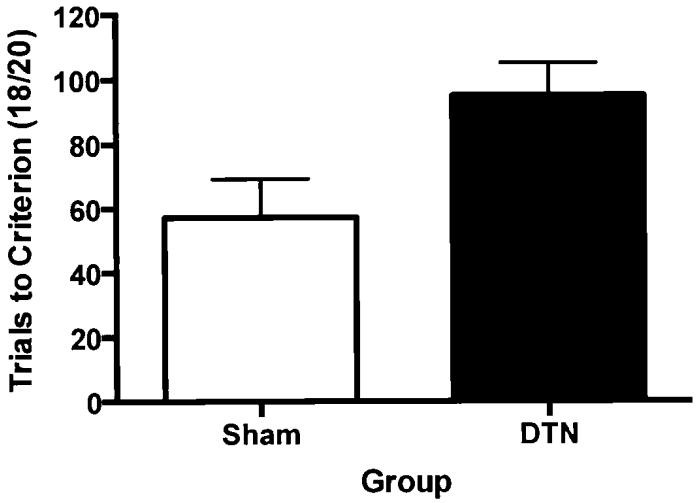


Figure 6: Mean (± SEM) trials to criterion for DTN lesioned rats and Sham controls on directional water maze task in Experiment 2. Rats with neurotoxic lesions to the DTN (M = 95.00) took significantly more trials to reach criterion than sham controls (M = 57.35), t (12) = 2.31, $p < .05$.

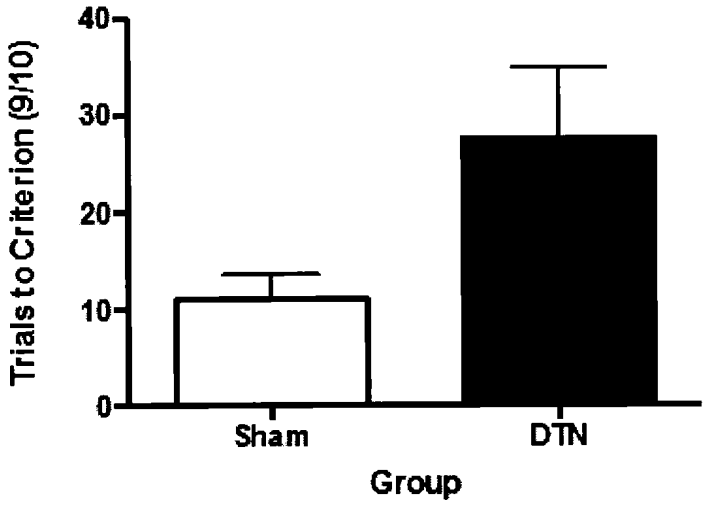


Figure 7: Mean (± SEM) trials to criterion for DTN lesioned rats and Sham controls on the fixed hole foraging task in Experiment 2. Rats with lesions to the DTN ($M = 27.6$) took significantly more trials to reach criterion than sham controls ($M = 11.0$), $t\,(10) = 5.59$, $p < .05$.

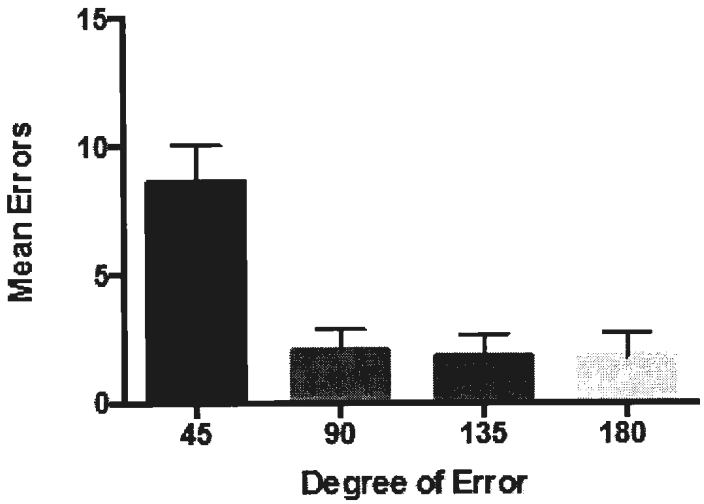
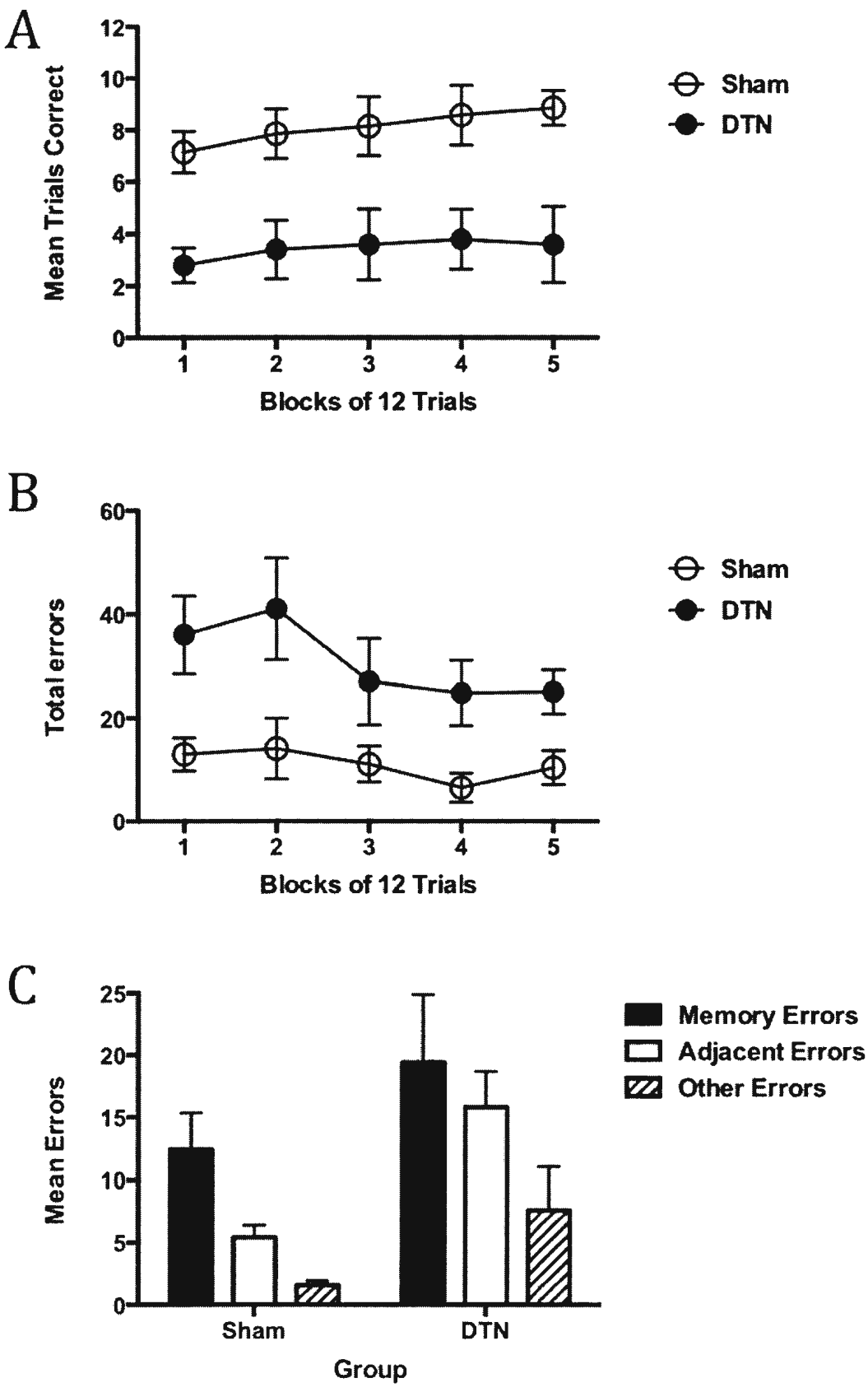


Figure 8: Mean (± SEM) number of errors for each error type (as defined by degree of error) for DTN lesioned animals on the fixed hole version of the food foraging task. A one-way ANOVA revealed a significant main effect of error type, $F(3, 16) = 10.70$, $p < .05$. Dunnett's multiple comparisons test revealed that rats with lesions to the DTN made significantly more adjacent-hole errors than all other error types (all probability values < .05).


A
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6
4
2
0
1
2
3
4
5
Blocks of 12 Trials
Sham
DTN
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Total errors
60
40
20
0
1
2
3
4
5
Blocks of 12 Trials
Sham
DTN
C
Mean Errors
25
20
15
10
5
0
Sham
DTN
Group
Memory Errors
Adjacent Errors
Other Errors

Figure 9: Mean (± SEM) trials correct (panel A) and total errors (panel B) across blocks, and mean (± SEM) number of errors for each error type (panel C) for DTN lesioned animals and sham controls on the variable hole foraging task in Experiment 2. Rats with lesions to the DTN exhibited fewer mean trials correct (A), $F(1, 10) = 16.83$, $p < .05$, and more total errors (B), $F(1, 10) = 11.90$, $p < .05$, than sham controls. Rats with lesions to the DTN made more adjacent-hole errors than sham controls (C) $t(10) = 3.94$, $p < .05$.

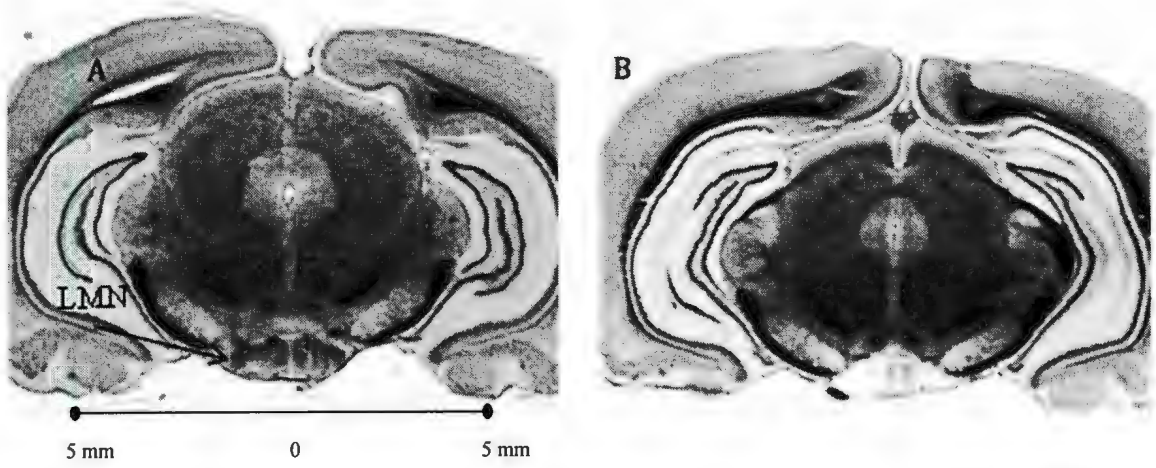


Figure 10: Example histological sections of LMN lesioned animals and sham controls for Experiment 3. Panel A shows a brain section from a sham control with an intact LMN and panel B shows a brain section from an animal with a neurotoxic lesion to the LMN.

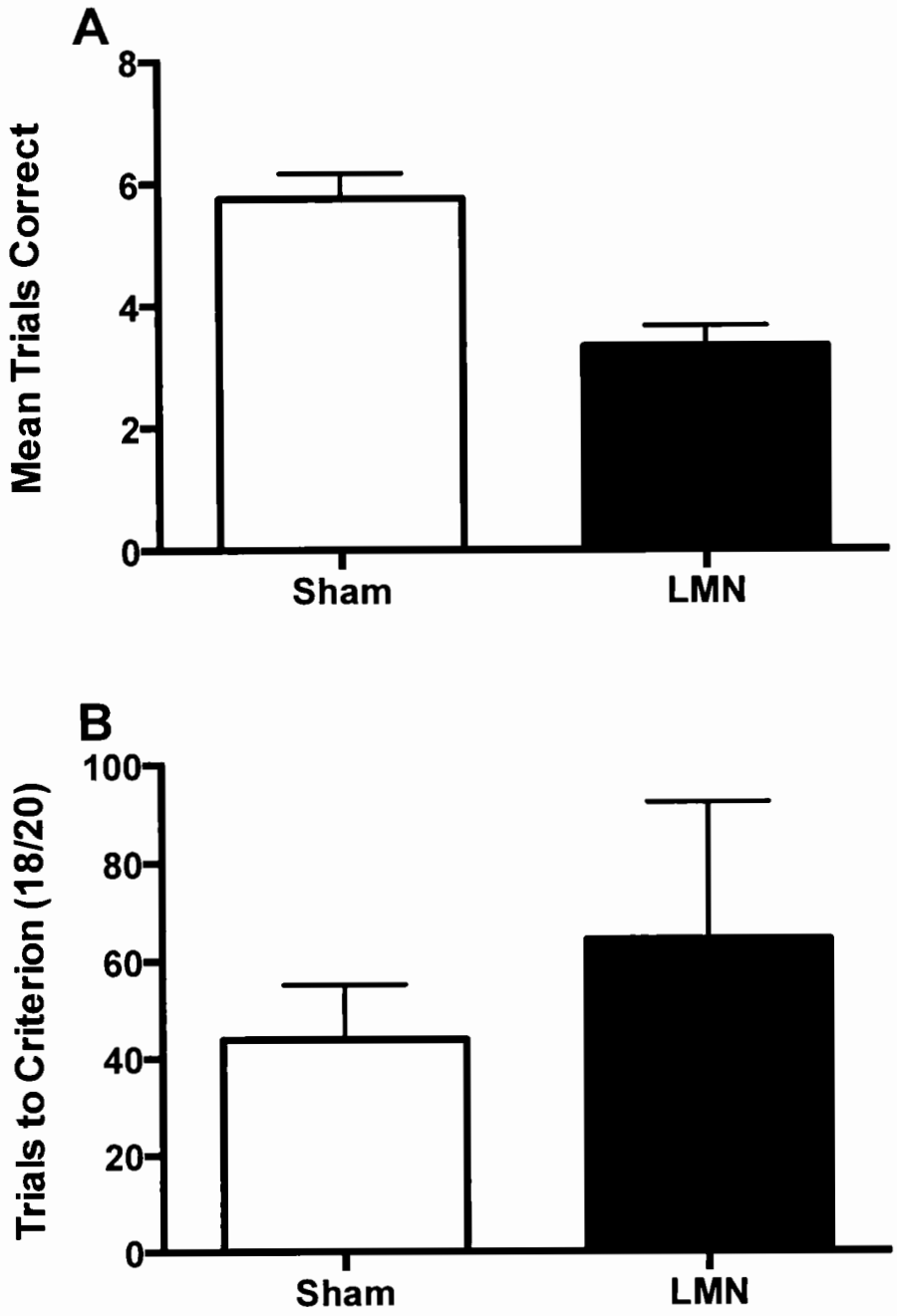
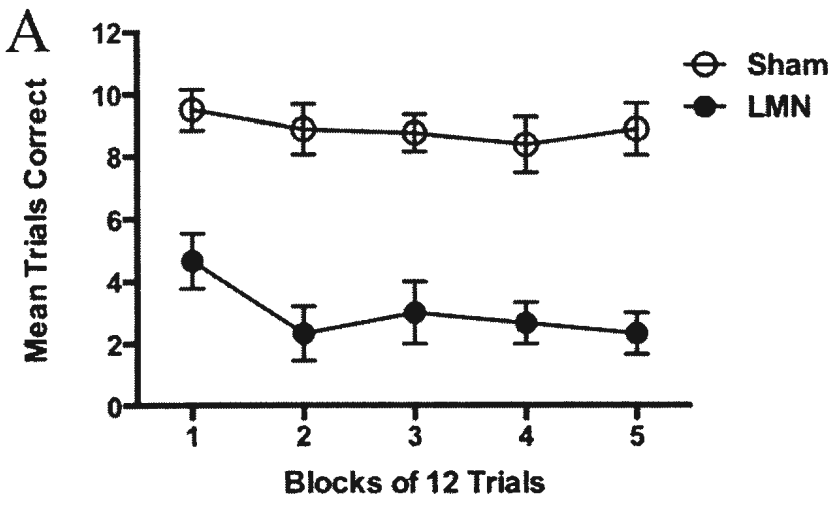
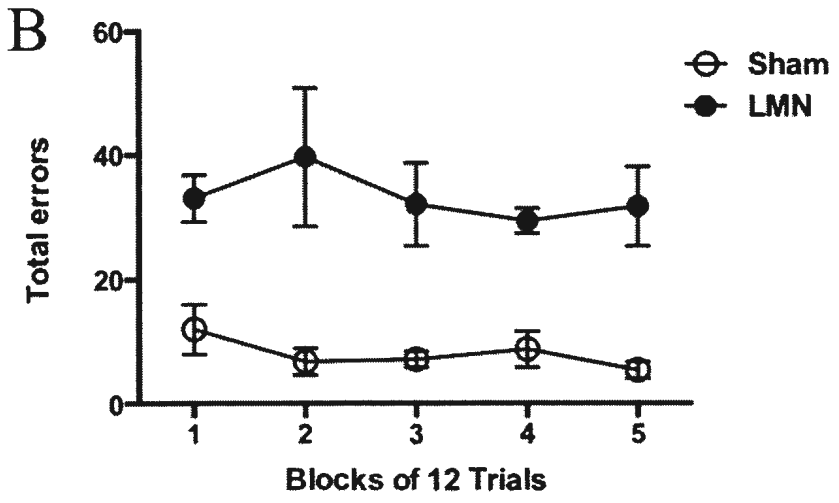


Figure 11: Mean (± SEM) trials correct on the first day (panel A) and mean (± SEM) trials to criterion (panel B) for LMN lesioned rats and sham controls on directional water maze task in Experiment 3. Panel A shows that LMN lesioned animals were impaired on the first day of training, $t(9) = 3.36$, $p < .05$, (i.e., on the first eight trials) and panel B shows that they did not differ from sham controls in terms of reaching criterion.


A
Mean Trials Correct
12
10
8
6
4
2
0
1
2
3
4
5
Blocks of 12 Trials
Sham
LMN


B
Total errors
60
40
20
0
1
2
3
4
5
Blocks of 12 Trials
Sham
LMN

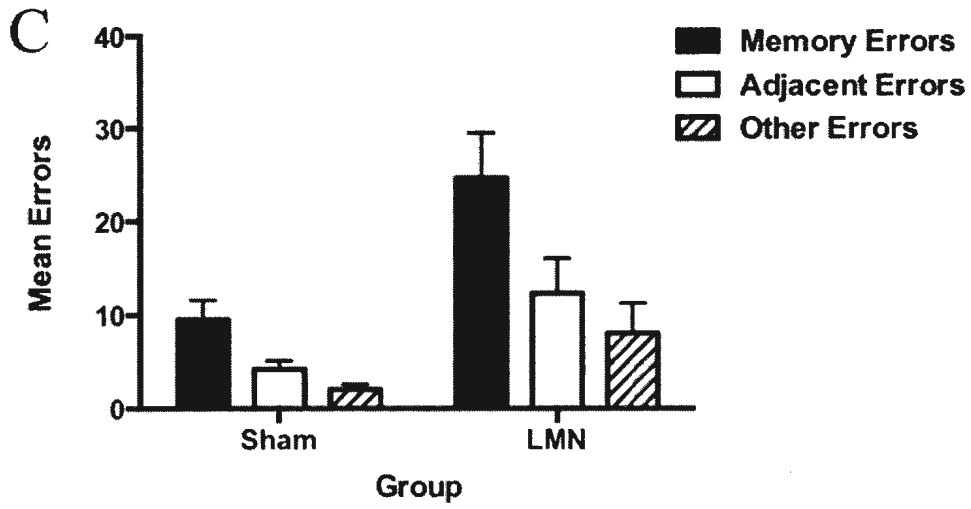

C
Mean Errors
40
30
20
10
0
Sham
LMN
Group
Memory Errors
Adjacent Errors
Other Errors

Figure 12: Mean (± SEM) trials correct (panel A) and total errors (panel B) across blocks, and mean (± SEM) number of errors for each error type (panel C), for LMN lesioned animals and sham controls on the variable hole foraging task in Experiment 3. Rats with lesions to the LMN exhibited fewer mean trials correct (A), $F(1, 9) = 45.56$, $p < .05$, and more total errors (B), $F(1, 9) = 51.76$, $p < .05$, than sham controls. Rats with LMN lesions made more errors of all types as compared to sham controls (C) $F(2, 18) = 13.13$, $p < .05$ (see text for post hoc comparisons).