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EVIDENCE FOR COOPERATIVE, SEQUENTIAL INTERACTION BETWEEN HIPPOCAMPAL- AND DORSOLATERAL STRIATAL-DEPENDENT NAVIGATION STRATEGIES IN THE MORRIS WATER TASK

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THESIS

Submitted in Partial Fulfillment of the
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Psychology

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Dedication

To Mom and Dad.

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ABSTRACT

The hippocampus and dorsolateral striatum have been found to be critical for spatial navigation based on distal and local cues, respectively. Previous reports from our laboratory have indicated that behavior in the Morris water task may be guided by both cue types, and that rats appear to switch from distal to local cues in a sequential manner within a given trial. In two experiments rats with hippocampal or dorsolateral striatal lesions were trained and tested in water task paradigms that involved translations or removal of the cued platform within the pool or translations of the pool itself with respect to the distal reference frame. Results show that the hippocampus is critical for orienting to distal cues at the beginning of the trial, while the dorsolateral striatum is critical for terminal swim segments based on the location of the cued platform. In addition, results also support the theory that the hippocampus, but not the dorsolateral striatum, is critical for directional responding. These results are important for understanding the cooperative interactions between these brain regions involved in learning and memory.

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Introduction

Strategies used by animals to navigate to a goal have been studied extensively over the past century. John Watson (1907) was the among the first to study the various strategies used by rats to make their way though a complex land maze, and to what degree various levels of sensory deprivation could disrupt these strategies. Since then there have been many theories that have tried to explain what behaviors are exhibited by an animal while in a maze and, more importantly, what is being learned in the process. From a behaviorist perspective Hull (1934) proposed that rats learn to solve a maze based on stimulus-response (S-R) behaviors and habit formation. For example, in a standard land maze a rat might simply learn to make a sequence of left or right turns as it encounters various choice points en route to the goal location. In this case it could be argued that what is learned is the route to the goal, as opposed to the specific location of the goal. This view was challenged by others who believed that rats learned to solve mazes by developing a "cognitive map" of the environment and recalling and updating this map when it was placed in the maze on later trials (Tolman, 1948). According to this view, animals "learn the lay of the land" which would include the precise spatial location of the goal. These conflicting points of view can be summarized in the statement "getting there versus knowing where" (Whishaw, Cassel, & Jarrard, 1995). This debate continued until Restle (1957) appeared to resolve the issue by showing that place learning dominated in certain conditions (i.e. in a well-lit room with ample visual cues) while response learning dominated in other conditions (i.e. in a dimly-lit room with few visual cues). Yet another possibility was pointed out in response to these findings: What Tolman, Ritchie, and Kalish (1946b) failed to rule out in their place learning experiments was the possibility that rats were learning a directional response. In other words, the rat may simply

be learning to navigate in a certain direction with respect to the distal room cues as opposed to learning the specific location of the goal. Other experiments during this era revealed that this type of directional navigation was learned at faster rate than place navigation using similar maze paradigms (Blodgett, McCutchan, & Mathews, 1949). This is an often-underappreciated footnote to the place versus response debate, and lends support to the idea of learning how to get to the goal, as opposed to learning where the goal is. The purpose of this Master's thesis is to describe the specific interactions between navigation/learning strategies in the Morris water task, as well as the neurobiological bases of these strategies.

Behavior in the Morris Water Task

An important advancement that renewed interest in the study of spatial navigation was the development of the Morris water task (MWT) (Morris, 1981, 1984). This task simplified maze paradigms because rats are excellent swimmers and are also highly motivated to escape from the water. As a result, training for the rat proceeds rapidly compared to standard land mazes that required food deprivation and days, or often weeks, of training to observe consistent and accurate responding. The water task is also very versatile and can be manipulated in several ways to test competing hypotheses regarding performance, an important feature to the present set of experiments.

In the first water task experiments conducted by Morris (1981), a circular pool was filled with water and an escape platform was either just above (visible) or just below (hidden) the surface of the water. Rats were released from various points around the perimeter of the pool in conditions where the visible or hidden escape platform either stayed in the same location across all trials, or moved randomly between trials. All of the rats learned strategies for locating the platform quickly and directly with one exception: the group with the moving

hidden platform took considerably longer to find the platform than the other three groups. Two conclusions were drawn from this initial experiment: First, the visible platform acted as a stimulus which controlled navigation regardless of whether it was in a fixed location or moved from trial to trial. Swimming directly toward a single, conspicuous cue is referred to as *cued navigation* or *beacon piloting* and only requires that an animal learn to approach the cue; spatial learning is not a necessary requirement for this strategy. Second, when the platform was hidden from view, rats were able to locate it only when it remained in a fixed location in the pool. Morris concluded that the rats in this condition could only have learned the location of the platform relative to the various cues in the extra-maze environment, as there were no disambiguating, proximal cues in the pool. The poor performance of the rats in the condition in which the hidden platform changed locations on each trial is expected since the spatial location of the platform is unreliable; it also demonstrates that there were no detectable cues from the hidden platform that could be used for direct navigation. A final observation worth mentioning is the finding that rats released from novel starting points were able to swim directly to a fixed platform even if they had only been released from one starting location during training. This phenomenon was termed *instantaneous transfer*, and has been cited by many researchers as support for the theory of cognitive mapping in rats (Morris, 1981) as well as humans (Jacobs, Laurance, & Thomas, 1997). Taken together, these results were considered support for the idea that rats can easily locate a goal purely on the basis of its fixed spatial relationship to a constellation of distal visual stimuli. This type of strategy is referred to as *place navigation* or *place learning* because the rat is thought to be learning to navigate to a specific place in the environment, or learning precisely where the platform is located.

Since the publication of this first MWT paper, there have been a number of experiments designed to dissociate place and response strategies in both land and water navigation tasks, and in general, these and other strategies involved in navigation have received a considerable amount of attention from researchers. With regard to the instantaneous transfer phenomenon, it has been argued that even though the novel release points used by Morris (1981) had not been used during training, they still could not be considered purely novel due to the fact that all rats experienced views of the distal cues from almost the entire pool during initial training trials to some degree before learning to navigate consistently to the platform location. To test this idea, Sutherland, Chew, Baker, and Linggard (1987) trained rats with physical and/or visual access to only the half of the pool where the escape platform was located. On test trials the rats were released from novel start points on the side of the pool to which access was restricted during training. Only those rats that had previous swimming and visual access to the entire environment were able to accurately swim to the hidden platform. Thus, Sutherland et al. (1987) failed to support Morris' assertion when truly novel release points were employed. Similar results have been reported in humans using the Virtual Morris Water Task (VMWT), indicating this effect is generalized across species and task demands (Hamilton, Driscoll, & Sutherland, 2002). Additional experiments by Sutherland and colleagues (1987) were conducted in which the lights were turned off during the initial or middle segments of the swim or while the rat was on the escape platform, in order to disrupt access to the environmental cues. Those rats that were unable to see the room cues during the middle portion of their swims were impaired compared to rats that had the lights turned off during the beginning of their swims or while on the platform. On the basis of these findings and the results from the novel release point

tests, Sutherland and colleagues favored the interpretation that animals learn how to swim to the platform within a range of familiar views experienced during training rather than learning precisely where the platform was located. That is, the information that supports place navigation in the MWT is obtained while the rat is actively swimming in the environment.

To further assess the role of visual cues in the environment, experiments have also been conducted in the MWT to observe individual differences in place responding and passive latent learning strategies. Latent learning in the MWT was first described in experiments where rats were repeatedly placed on the hidden platform immediately before trials, and for some groups the platform location they were placed on was in the same location in the pool that it was to be located during training. The rats that had been placed on the correct platform location prior to swim trials demonstrated faster navigation to the hidden platform than controls (Sutherland & Linggard, 1982; Keith & McVety, 1988). In a more recent experiment, rats were rated as "good" or "poor" place learners based on their performance in the standard version of the hidden platform water task. The rats were then trained in a new environment involving a latent learning paradigm where they were placed on the hidden platform prior to being released into the pool. No correlation was found between the good and poor place learners and the good and poor passive latent learners unless a polarizing cue (in this case the door to the room) was covered (Devan, Petri, Mishkin, Stouffer, Bowker, Yin et al., 2002). This finding again highlights the importance of movement in place navigation; however, it also suggests that animals are capable of learning the precise location of the platform in the room. Recent results from our laboratory question where animals actually learn the precise platform location during passive placement (see below), and the ability of rats to learn the platform location after passive placement is

generally considered to be much weaker than the capacity to learn how to navigate to the platform (see Chew, Sutherland, & Whishaw, 1989).

Behavior in Other Task Environments

Regardless of whether learning in the MWT is primarily related to learning how to get to a goal versus learning where the goal is located, it is generally agreed that the distal visual cues are the critical features of the environment. Some researchers argue that navigation in the hidden platform task requires that the animal possess a cognitive map, while others suggest that animals navigate to the platform on the basis of its fixed spatial relationship to distal cues. Implicit in the latter is that animals learn where the goal located relative to the available cues. Another type of navigation strategy that has received recent attention is the previously mentioned directional responding. One such experiment was conducted where response, direction, and place learning were compared in a T-maze that was rotated from trial to trial. In the response group, rats were required to make the same egocentric response at the choice point (i.e. always turn right) regardless of the orientation of the maze. In the direction group, rats were required to turn to the same direction with regards to the extra-maze cues (i.e. always go to the west) regardless of maze orientation. The place group was required to navigate to the same spatial location with regards to the extra-maze cues regardless of maze orientation. Results showed that the response and direction groups learned the correct response quickly, while the place group still had not learned the correct response after 300 trials. The only condition where the place group was able to learn the correct response was when the maze was manipulated so the start locations were sufficiently unambiguous (Skinner, Etchegary, Ekert-Maret, Baker, Harley, Evans et al., 2003). This would indicate that a place response is rather difficult for rats to learn and perhaps what is

often referred to as place responding in the literature could instead be thought of as directional responding, although place navigation would still represent a possible strategy that animals could utilize. It is important to note that place and directional responding cannot be dissociated unless the apparatus is moved in such a way as to put these two strategies into competition. In order for this manipulation to be achieved, the apparatus must be translated within the environment, as noted by Skinner and coworkers (2003). For example, Packard and McGaugh (1996) trained rats on a plus maze where the start and reward arms were held constant. On days eight and 16 rats were started from the arm opposite the one used during training. Control rats exhibited place learning on the early test trial and response learning on the late test trial. It was concluded from this experiment that place learning develops faster than response learning, and this place learning persists even when there is a switch in strategies to response learning (as exhibited by the control rats). A directional response, however, would yield the same results as the place response that is described in this experiment. Since directional responding has not been ruled out in previous experiments that have investigated place responding, further investigation into this type of navigational strategy is necessary.

It is worth noting that the results reported in Skinner et al. (2003) were found in both an open-field task in addition to the T-maze. Some researchers argue that the results of land mazes are difficult to interpret because rats often naturally alternate between choices in land-based tasks, but not in water tasks (Whishaw & Pasztor, 2000). With this in mind, similar directional responding paradigms have been developed for the MWT. One experiment involved rats that were trained with the hidden platform in a constant location. When the pool was moved within the room so that the trained (absolute) location with regard to the extra-

maze cues was put into competition with the trained (relative) location within the pool, rats overwhelmingly demonstrated a directional response to where the platform had been located within the pool (the relative location), as opposed to a place response to where the platform had been located with regard to the distal room cues (absolute location). These results were found regardless of whether the platform was hidden or marked by a cue (Weisend, Klein, Hoesing, Astur, Koerner, McDonald et al., 1995; Hamilton, Akers, Weisend, & Sutherland, 2007). Further investigation revealed that directional responding persisted even in situations that would be expected to favor place responding (Hamilton, Akers, Johnson, Rice, Candelaria, Weisend et al., 2008), and that rats switch from a place response strategy to a directional response strategy across three days of training in the MWT where the pool was filled to the top, in order to reduce the influence of the pool wall (Hamilton, Akers, Johnson, Rice, Candelaria, & Redhead, 2009). This set of results brings into question the longstanding assumption that distal cues in the environment are the primary source of control in the MWT and other tasks. It would appear that other cues in the proximal reference frame also play a role in navigation strategies used to locate a goal.

Differentiating Distal and Proximal Cues

Although the precise nature of how distal cues control navigation in the water task is far from resolved, most researchers agree that the behavioral and psychological processes involved in the hidden platform version of the MWT can be dissociated from those involved in the visible platform task. Furthermore, there are compelling neurobiological dissociations of place and cued navigation that will be discussed below. The neurobehavioral dissociation between place and cued navigation has served as a point of departure for numerous experiments given that place and cued navigation are distinct strategies that are supported by

different neural circuitry. Whether the stimuli critical to these strategies compete for control of navigation, influence behavior independently and in parallel, or cooperatively contribute to behavior, is unclear.

In order to address the degree to which a cued-platform controls navigation in normal rats, Redhead, Roberts, Good and Pearce (1997) ran a series of water task experiments where a submerged platform could be found using a beacon attached to the platform or by using the extra-maze cues to guide behavior. When the beacon was co-localized with the platform, it came to control navigation strategies more than the extra-maze cues as indicated on test trials where the beacon and platform were removed and navigation to the platform location was disrupted. This phenomenon is known as overshadowing; in this case the proximal beacon cue overshadowed the distal room cues. A blocking effect was noted in an additional experiment where learning to navigate to a cued-platform disrupted performance on later trials where the cued-platform was moved to a new location in the pool. The cued-platform navigation strategy effectively blocked the ability to use the extra-maze cues to place navigate (Redhead et al., 1997).

Recent studies have also indicated that intra-maze cues can come to control behavior even when the cue is unstable. Roberts and Pearce (1998) found that an intra-maze cue that was located a constant distance and direction from a hidden platform could come to control navigation to the platform even when the platform moved around the pool. In addition, more accurate searching was found for the group that was trained with the unstable cue compared to a group that was trained with a stable cue. Perhaps the better performance of the unstable cue rats could be explained by some strategy other than place or response learning. This would contradict the findings of Biegler and Morris (1993) that showed that persistent

responding was only found for the group of rats that had been trained with the landmark consistently marking the goal location.

The foregoing discussion indicates that proximal intra-maze cues and the distal extra-maze cues in the water task compete for control of behavior in the water task. Several results (e.g., Whishaw, Mittleman, Bunch, & Dunnett, 1987), however, demonstrate that rats can learn to navigate to the platform location when the cued platform is removed during probe trials. More recently, Hamilton, Rosenfelt, and Whishaw (2004) found that rats use distal (extra-maze) cues to guide initial heading and then switch to the use of the visual platform (intra-maze) cue to navigate towards the goal. This switch in strategies is evident in kinematic analyses of behavior, as the rat's swim speed slows during head scanning after an initial trajectory away from the pool wall; this head scan may reflect an attempt to locate or estimate the distance to the cued platform. This *shift point* behavior was quantified by conducting experiments in which the visible platform was either removed from the pool, relocated within the pool, or when test trials were conducted in a novel environment. In the first two cases, initial heading was to the trained platform location. In the last case initial heading was disrupted, indicating that extra-maze cues are critical to the selection of initial heading. In all cases where the visible platform was present, heading beyond the shift point was accurate, indicating that intra-maze cues (in this case, the visible platform) control the final segment of the swim (Hamilton et al., 2004). Similar experiments in humans where eye movements were tracked while subjects navigated to a hidden platform in the VMWT revealed that those individuals who learned the task exhibited eye movements directed towards the distal cues in the environment during the first few seconds of the trial. Eye movements after establishing a trajectory were then focused on the inside of the pool,

including the pool wall and the area immediately surrounding the subject's point of view (Hamilton, Johnson, Redhead, & Verney, 2009).

In a related experiment, Hamilton et al. (2007) trained rats to swim to a cued platform and then moved the pool to a novel position in the room. For half the rats the cued platform remained in the same absolute spatial location in the room and for the other the platform was moved with the pool such that it remained in the same relative location (direction) in the pool. Rats tested with the cued platform in the same absolute location took significantly longer to navigate to the platform than the group tested with the platform in the same direction (relative location) in the pool. In many cases, the rats in the absolute group navigated to the relative location first even though the platform was marked by a conspicuous cue, whereas the rats in the relative group navigated directly to the platform. Together, these findings and the results of Hamilton et al. (2004, 2009) indicate that distal and proximal cues control navigation within a single trial, and that each source of control is responsible for different components of navigation to the cued platform; Distal cues control initial heading and proximal cues co-localized with the goal control the final segment of the swim. If this theory is accurate, then there must be some mechanism, or some neural correlate, involved in dynamically switching from one source of control to another within a particular trial.

Neurobiology of Spatial and Cued Navigation

Another aspect of spatial navigation that has received considerable attention is the study of the neurobiological underpinnings of learning and memory. O'Keefe and Dostrovsky (1971) used electrodes to record the activity of single neurons and found that specific groups of pyramidal cells in the hippocampus were rarely active except when the rat was restricted to a small region in an environment. These "place cells" became highly active

when the rat was exploring an open field and it was shown that certain neurons were associated with a specific place within the open field. This was taken as support for the idea that a cognitive map is indeed formed in the brain, specifically in the hippocampus (HPC). This led to the publication of *The hippocampus as a cognitive map* by O'Keefe and Nadel (1978), which pointed to the HPC as the neural substrate for allocentric spatial localization and thus central to using relations between cues to locate places in space. This sparked a line of research intended to describe the exact function of the HPC and how it is involved in spatial navigation.

The Role of the Hippocampus

The HPC is believed to be critical for place navigation based on experiments that have demonstrated that lesions to the HPC disrupt water task learning (Morris, Garrud, Rawlins, & O'Keefe, 1982; Sutherland, Whishaw, & Kolb, 1982). Based on these results, researchers have concluded that the HPC is responsible for learning relationships among a constellation of distinct stimuli. It is generally agreed that the inability to learn about spatial relationships is responsible for this deficit. More recently, experiments using technology to image immediate-early gene (IEG) expression as a measure of HPC activity have found that *Arc* RNA expression in the HPC was correlated with learning in the hidden platform MWT, but not in the cued platform version of the MWT (Guzowski, Setlow, Wagner, & McGaugh, 2001).

Several other studies have been conducted to describe more specifically the involvement of the HPC in spatial navigation. Eichenbaum, Stewart, and Morris (1990) proposed that the HPC is important for behavioral flexibility in the water task based on the finding that rats with damage to the HPC could still learn a place response during training,

but failed to update that response when tested from novel release points compared to controls who were able to quickly adjust to navigating to the platform location from the novel release points. A similar result was found in an experiment where a single cue was tethered to the hidden platform so that when the platform moved to different locations around the pool between trials, it could be found at a constant distance and direction from the cue. On the first trial following platform relocation, rats with HPC lesions were faster than controls at locating the platform. However, control rats showed significant improvement on subsequent trials with the platform in the same location, while performance in rats with HPC lesions failed to improve (Pearce, Roberts, & Good, 1998). The conclusion from this experiment is that damage to the HPC disrupts navigation based on a cognitive map, but correct "heading vectors" remain intact. This argument is disputed by other published reports demonstrating the importance of the hippocampus in a "sense of direction" (Whishaw & Maaswinkel, 1998). Another study involving reversible inactivation of the HPC found that acquisition was impaired when the HPC was offline, but this was due to procedural disruptions rather than cognitive deficits as persistence at the platform location was intact (Micheau, Riedel, Roloff, Inglis, & Morris, 2004). These conflicting accounts of the role of the HPC in navigation highlight the difficulty in dissociating the neurobiological basis of procedural and cognitive aspects of the MWT.

There is a significant body of work that supports the notion that the HPC is not necessary for learning a place response when rats are trained in a specific manner. Whishaw and Jarrard (1996) found that when rats with damage to the HPC are trained to navigate to a platform that is visible and conspicuous, on test trials where the platform was submerged or removed altogether these rats were able to navigate to the location where the platform was

located during training. This would imply that rats with damage to the HPC are able to make some association about where the platform is in relation to the distal extra-maze cues during training even though the platform is visible. Similar results were found in rats where damage was limited to the fimbria-fornix (Whishaw et al., 1995).

Another manipulation used in the MWT involved altering the size of the escape platform and discouraging thigmotaxic behavior. Thigmotaxis refers to swimming around the perimeter of the pool, a strategy that is quickly abandoned by normal rats that come to realize that the platform is located away from the pool wall but persists in rats with HPC lesions. One such experiment involved using a series of escape platforms that encompassed almost the entire pool at the start of training and then became progressively smaller across trials. At the same time, thigmotaxic behavior was discouraged using barriers that blocked access to the pool wall away from the release point. In this situation, performance on probe trials with the platform removed was not found to be significantly different between rats with lesions of the HPC and controls (Day, Weisend, Sutherland, & Schallert, 1999). In a follow-up experiment the platform was located along the pool perimeter. Although a thigmotaxic strategy would allow the rats to locate the platform, rats with HPC damage were unable to switch back to this type of strategy because it had been discouraged during training. Because controls were easily able to switch to this response behavior, it is concluded that the HPC is critical for pliancy, or the ability to change response strategies (Day et al., 1999). This is further exemplified by the fact that the normal thigmotaxic response of rats with damage to the HPC would lead them to easily find the platform if it were located around the perimeter of the pool. Despite the fact that several studies have demonstrated apparently intact spatial navigation abilities in the rat, the dependence of the water task on the HPC is still generally

accepted, and the disruptions in the water task caused by HPC damage are widely considered to be among the most robust and reliable cognitive and learning deficits that can be observed following brain damage (Morris, 1991).

Other maze tasks have been used in addition to the MWT to identify HPC contributions to place learning in rats. For example, McDonald and White (1995) dissociated passive versus active place learning in an eight-arm radial maze. In the passive place learning task, rats spent an equal amount of time in two of the eight arms of the maze, one of which was always baited with a food reward. When rats were later allowed to explore the maze with no food in either arm, rats with damage to the HPC spent significantly more time in the arm where the food had been located compared to controls. In the active place learning task, one arm constantly contained a food reward, but the other arm that did not contain food changed from trial to trial so that the only way that rats could distinguish from the food and no-food arms was on the basis on their location relative to the distal room cues. In this case, damage to the HPC resulted in impairment in the ability to distinguish between arms of the maze. In evaluating the difference between active and passive place learning, McDonald and White describe how the importance of time and movement demonstrated by Sutherland et al. (1987) as well as White and Ouellet (1997) appear to be dependent on the HPC. A long lapse in time on the passive learning trials rules out the potential issue of working memory effects, and a restriction of movement throughout the maze in the passive place task could explain the disruption in the ability of the rats to make cue discriminations. Movement through the maze and shorter time periods might explain why the HPC is necessary for the active learning task.

In a land based T-maze experiment conducted by Stringer, Martin and Skinner (2005) rats with HPC lesions and controls were trained to find a food reward in one arm of the T-

maze with the maze shifted in different configurations as in Skinner et al. (2003). Rats with HPC lesions were not impaired on response navigation but were impaired on direction and place responding compared to controls. These results indicate that the HPC is important for learning not only place navigation, but also for a more general directional response. Similar preliminary results have also been reported in the MWT using paradigms where preference and forced choice between place and directional responding strategies were employed (Rice, Akers, Johnson, Candelaria, Wallace, & Hamilton, 2008).

Another important factor to consider is the amount of damage that is induced in hippocampal lesions and to which specific regions these lesions are confined. Many studies have looked at lesions to the entire hippocampal formation, including Ammon's Horn (CA1-3) and the Dentate Gyrus (e.g., Day et al. 1999) while others have limited lesions to the fimbria-fornix afferents/efferents to the HPC (e.g., McDonald & White, 1995). Although there seems to be no reason to think that behavioral deficits differ in either case, connectivity to surrounding areas could be important. It has been shown that the amount of damage to the dorsal HPC is highly related to spatial learning task deficits while damage to the ventral HPC is only a factor when the region is almost completely destroyed (Moser, Moser, & Andersen, 1993). The data suggest that the dorsal region of the hippocampus receives more sensory input via the lateral entorhinal cortex and thus is more important for spatial learning tasks. In order to produce a maximal disruption in the MWT, it would be important to damage the entire HPC.

Although there has been considerable attention paid to the role of the HPC in spatial navigation, the results of the previously mentioned studies often raise questions about what other brain regions might be involved in these tasks as well. For example, the amygdala was

found to be important for the passive place learning task described by McDonald and White (1995). Damage to regions of the frontal cortex has been shown to impair spatial navigation as well (Sutherland et al., 1982). Because there are numerous strategies that a rat could use to solve the MWT (and other dry land tasks), it is important to investigate what brain regions besides the HPC are involved, particularly because the contemporary place versus cued navigation debate is often framed as the evidence for multiple memory systems in the brain (i.e., Packard, Hirsh, & White, 1989).

Although it has been noted that damage to the HPC results in impairment on the hidden platform version of the Morris water task compared to controls, this impairment can be mitigated by attaching a cue to the platform marking its location (Morris et al., 1982; Sutherland et al., 1982; Sutherland, Whishaw, & Kolb, 1983). In this cued-platform version of the task the use of distal room cues to find the platform becomes irrelevant because the platform can be found by simply swimming to the cue marking the platform. This does not explicitly rule out the involvement of extra-maze cues, but it does give support to the idea that a possible interaction between navigation strategies exists.

Taking these findings, in addition to the findings that rats with damage to the HPC are not impaired on the cued-platform version of the water task into account, it is clear that navigational strategies demonstrated in the cued-platform task are distinct from the strategies used in the hidden platform task and may be driven by different brain regions. If the hidden platform version is solved using place navigation and place navigation is dependent on the HPC, the next logical step would be to describe what brain regions are critical for navigation in the cued-platform version of the MWT, with an emphasis on the possible interactions between these navigation strategies.

The Role of the Dorsal Striatum

Whishaw and colleagues (1987) found that rats with lesions to the dorsal striatum, analogous to the caudate-putamen in humans, were impaired on both the cue and place navigation versions of the water task compared to controls, but these rats were still able to reach control levels of performance with extended training. On test trials, rats with dorsal striatal lesions used extra-maze cues on a place learning task and a beacon piloting strategy based on intra-maze cues in the cued-platform task, but these rats demonstrate a preference for place responding strategies unless forced to do otherwise. Recent studies have found that lesions of the dorsal striatum disrupt learning in rats using a complex multiple T-maze task (Pistell, Nelson, Miller, Spangler, Ingram, & Devan, 2009).

These findings opened the door to a closer look at the role of the basal ganglia, and in particular the dorsal striatum, in spatial navigation. It has been suggested that the striatum is important for procedural learning and motor responses related to navigation. For example, Kesner and Gilbert (2006) used a match-to-sample task in a cheese board maze and found that damage to the dorsomedial striatum, but not the HPC, disrupted motor responses to the reward location. Several studies have found that cholinergic activity in the striatum is important for the consolidation of memories involved in procedural tasks (White, 1997). If the HPC is thought to be important for flexible learning or pliancy, the striatum has often been described as mediating a "less cognitive, more rigid" memory that could be described as habit formation or S-R learning (White, 1997). The importance of the dorsal striatum in spatial navigation might be explained by the high interconnectedness of this region with two areas of the prefrontal cortex (the posterior orbitofrontal anterior insular cortex and the posterior medial prefrontal anterior cingulate cortex), the medial dorsal thalamus, the

amygdala, and the HPC (White, 1997). The pathway to the striatum from the amygdala and HPC is believed to be important for communicating learning about previous encounters involving reward outcomes. Additionally, cells that code for the direction the rat is facing ("head direction cells") have also been identified in the dorsal striatum (Ragozzino, Leutgeb, & Mizumori, 2000), which point to this brain region as being important in spatial navigation, and, in particular, to adaptive navigation and behavioral flexibility (Mizumori, Puryear, & Martig, 2009). The presence of head direction cells is an important factor to behavioral flexibility because these cells could update the location of the animal within the environment, and thus serve as an egocentric cue that could influence navigation strategy.

To test the effect of striatal lesions on egocentric localization, Cook and Kesner (1988) used four tasks in the eight-arm radial maze that required either egocentric or allocentric responses for reward. On the adjacent arm and left-right discrimination tasks, rats with lesions of the striatum were severely impaired in comparison to controls. These tasks are deemed to be egocentric in nature because the information required to successfully solve them is relative to the organism, not the external cues. On a standard place learning version of the radial arm maze, the striatal lesioned rats performed at the same level as controls. In this case, the solution could be found using the extra-maze cues in the environment. Such place navigation strategies do not appear to be dependent on the dorsal striatum (c.f. Yin & Knowlton, 2004). Electrophysiological studies also demonstrate that neuronal activity in the striatum correlate with egocentric, self-initiated navigation behavior (Wiener, 1993).

Dorsomedial vs. Dorsolateral Striatum

In order to detect specific functions within the dorsal striatum, Devan, McDonald, and White (1999) tested rats with either medial or lateral dorsal striatum lesions on the

hidden and visible platform versions of the MWT. In the hidden platform task, rats with dorsomedial striatal (DMS) lesions took longer to navigate to the platform than rats with dorsolateral striatal (DLS) lesions and controls, and displayed an increase in thigmotaxic behavior. In the visible platform task the platform was moved to a different location each day. Again, the rats with DMS lesions displayed significant increases in thigmotaxic behavior and took longer to reach criterion performance levels than rats with DLS lesions and controls. On a competition test between the two tasks (hidden versus visible platform), rats with DMS lesions swam to the visible platform more frequently than controls. This effect was not found in rats with lesions to the DLS. A final experiment using a land maze found reduced thigmotaxis across all lesion groups, ruling out fear or anxiety due to the aversive nature of the water in the MWT as an explanation for this specific behavior. The conclusion from these experiments is that the connections of the DMS with limbic and prefrontal regions are the driving force behind integrating cognitive information with S-R behavior.

Other studies have dissociated functions of the DMS and DLS and found that lesions of the DMS impaired performance on delayed non-matching (DNM) tasks in a radial-arm maze regardless of the length of delay while no impairment was found on a serial reaction time (SRT) task. DLS lesions produced the opposite effect in both tasks (Mair, Koch, Newman, Howard, & Burk, 2002). Win-stay and win-shift strategies have also been studied specifically in rats with DMS lesions (Sakamoto & Okaichi, 2001). This study found that the DMS was critical for the win-stay place task, but not for the win-shift place, win-stay cue, or win-shift cue tasks. The conclusion based on these findings is that the lateral sensorimotor areas of the striatum (DLS) are critical for responses to external stimuli while the limbic-

connected regions of the DMS are important for responses that involve working memory. In addition to dissociations between the DLS and DMS, heterogeneity within the DMS has been described in rats with lesions to either the anterior or posterior DMS. Results indicate that the posterior region of the DMS appears to play a critical role in place navigation, while the anterior region does not (Yin & Knowlton, 2004). Taking these findings into account, the current study involved lesions of the DLS in order to induce a deficit in cued-platform navigation in the Morris water task.

Many of the findings discussed thus far are based on single dissociation studies where brain lesions or pharmacological manipulations are limited to a specific brain region and then deficits in several different tasks are described. The difficulties in making conclusions based on single dissociation studies are discussed by Sutherland and Hamilton (2004) as an issue of false dichotomies. For example, just because a rat with damage to the HPC is able to solve the cued-platform version of the water task does not mean that a functional HPC is not involved or is inactive during the same task in a normal rat. Similarly, a rat with damage to the DLS may not be impaired on the hidden platform version of the water task, but it is erroneous to assume that this area of the brain is inactive or uninvolved in place navigation strategies. Because of this fact, it is important to use double dissociation paradigms in order to make more accurate statements about HPC and DLS functions involved spatial navigation. Several double dissociation studies have been conducted in order to address this issue.

Double Dissociations between HPC and DLS

In one experiment rats with lesions to either the fimbria-fornix or the dorsal striatum were tested on two tasks in a radial-arm maze (Packard et al., 1989). In the win-shift task, each arm of the maze was used as a reward location once. When a rat revisited an already

rewarded arm, it was scored as an error. Compared to controls, rats with fimbria-fornix lesions displayed significantly more errors while rats with striatal lesions were unaffected. In the win-stay task, a single light above the correct arm signaled the reward location. This would require a response towards the light that might also involve a previously visited arm. Fimbria-fornix lesions had no effect on this task, while dorsal striatal lesions resulted in significantly more errors. Packard and colleagues concluded that multiple memory systems are active in the brain for these two tasks, and these memory systems may be in competition with each other. The win-shift task might involve cognitive mapping, working memory, and/or contextual retrieval while the win-stay task might involve taxon learning, reference memory, and/or habit formation (Packard et al., 1989).

Further evidence for the multiple memory systems hypothesis described above came from another study that was conducted with similar lesion conditions in the MWT. In the spatial task, rats were required to respond to the same location in the pool regardless of the cue used to mark the platform location. In a visual discrimination task, rats were required to respond to the appearance of the cue regardless of the cue location within the pool. Rats with fimbria-fornix lesions were impaired on the spatial but not the visual discrimination task; those with lesions to the dorsal striatum were impaired on the visual discrimination but not the spatial task (Packard & McGaugh, 1992). The impairment observed in rats with dorsal striatal lesions on the visual discrimination task could be due to the connections of this brain region with the visual cortex (Faull, Nauta, & Domesick, 1986). To further evaluate the role of the visual cortex in spatial navigation, Whishaw (2004) conducted experiments on rats with visual cortex lesions. Results show that non-spatial training given before or after lesions of the visual cortex improved the deficits seen in place navigation for these rats. When using

a matching-to-place task, rats with visual cortex lesions were severely impaired compared to controls. This would indicate that although the visual cortex is critical for spatial navigation, extended training should rule out visual impairment as a possible explanation for the results reported by Packard and McGaugh (1992).

Another double dissociation was observed using two tasks in the radial-arm maze that were meant to mimic egocentric-procedural or allocentric-declarative information processing. In the procedural task, one arm at a time was available to the rat. After entering the arm and eating a food reward, another arm was opened. The rats simply had to learn to enter the next available arm, as the others were closed. Rats with DMS lesions took longer to learn this task as evidenced by their inability to increase speed of response across trials. Although acquisition of this task was impaired, once this information was learned in normal rats, retention was not impaired after lesioning the DMS. In the declarative task, rats had to attend to arms in a specific sequence in order to gain access to a reward. Rats with HPC lesions were impaired on this task until they were able to use an egocentric strategy to learn the sequence. Striatal lesioned rats were able to solve this task using allocentric information to learn the sequence (DeCoteau & Kesner, 2000).

A delayed matching-to-sample task was designed to test the role of the striatum in a more general memory for direction information in the radial-arm maze. Results indicated that control rats favored a directional response as opposed to a turning response in order to solve the task. When these rats were given lesions to the DMS or the hippocampus and then retested, there were initial deficits in both of the lesion groups. The conclusion from these results is that the hippocampus and DMS are both involved in short-term directional information (DeCoteau, Hoang, Huff, Stone, & Kesner, 2004). These results parallel the

findings of Stringer et al. (2005), but it is important to point out that in radial-arm maze tasks, movement is much more constrained compared to open field tasks or the water task, making conclusions about directional responding difficult to interpret.

Cooperation between Memory Systems

If it is, in fact, the case that there are multiple memory systems active in the HPC and dorsal striatum, the next step would be to determine the degree to which these systems cooperate. McDonald and White (1994) observed rats with lesions to either the dorsal striatum or fornix on both the hidden and cued platform version of the MWT. They found that lesions to the dorsal striatum did not disrupt learning on either task, but when the visible platform was moved to a novel location within the pool these rats swam first to the previously trained location. Fornix lesions disrupted learning on the hidden platform task but not the cued-platform task. When the cued-platform was relocated, they swam directly to it, despite the fact that they had never been reinforced for navigating to that specific location during training. McDonald and White concluded that in the absence of the dorsal striatum, spatial information comes to control navigation strategies even when swimming to a single proximal cue would be the simplest solution. On the other hand, when the hippocampus is damaged, intra-maze cues come to control navigation strategies more than extra-maze cues. It is important to note that the deficits observed in this study, particularly in the dorsal striatum lesioned rats, were only observed when the competing response was introduced. This result falls in line with previous studies of rats with dorsal striatum damage (Whishaw et al., 1987).

Other research has suggested that the hippocampus and dorsal striatum interact in a more cooperative manner. By looking at the direction and angles of departure for rats with

fimbria-fornix and dorsal striatal lesions on both the hidden and cued-platform versions of the water task, Devan, Goad, and Petri (1996) found that rats with fimbria-fornix lesions were using some other strategy besides place navigation to find the hidden platform in that particular task. Furthermore these rats also spent less time in the quadrant where the platform had been during training compared to controls, and demonstrated impaired flexibility when the platform was moved elsewhere in the pool. An interesting finding was reported in rats with striatal lesions; during the probe trial where the platform was removed from the pool, these rats exhibited a strong preference for the trained platform quadrant in the pool but failed to cross the exact location where the platform had been located. This finding raises two concerns. First, it is apparent that the dorsal striatum plays a role in learning the procedural components of both the hidden and cued-platform versions of the water task. Second, specific analyses of trajectory and persistence measures are important to further understand navigation strategies and related behavior observed in the water task.

To further investigate the different learning systems that appear to be mediated by the HPC and dorsal striatum, Packard and McGaugh (1996) trained rats on a plus maze where the start and reward arms were constant. On days eight and 16 rats were infused with lidocaine into either the hippocampus or striatum to render these areas inactive during test trials. All rats were then started from the arm opposite from the one used during training. As noted earlier, control rats exhibited place learning on the early test trial and response learning on the later test trial. Inactivation of the hippocampus resulted in no preference for either strategy on the early test trial and response learning on the later test trial. Dorsal striatal inactivation resulted in place learning on both test trials. It was concluded from this experiment that place learning develops faster than response learning and this place learning

persists even when there is a switch in strategies to response learning (as exhibited by the control rats). Similar findings have been reported in humans using functional magnetic resonance imaging (fMRI) while subjects were navigating a virtual eight-arm radial maze (Iaria, Petrides, Dagher, Pike, & Bohbot, 2003). As stated previously, directional responding could not be ruled out as an explanation for these results.

In addition to using lesion and pharmacological manipulations to investigate the role of the HPC and striatum and how these brain regions interact, immediate-early gene expression (IEG) has also been useful in describing brain function related to spatial navigation. The advantage of using these techniques is the ability to simultaneously observe activity in several brain regions in the rat. Several studies have used IEG expression as a way to measure activity in the HPC, DMS, and DLS immediately following activity in land or water tasks. Colombo, Brightwell, and Countryman (2003) measured cAMP response element-binding (CREB) protein and *c-Fos* activation in the HPC (including CA1, CA3, and DG) and in the striatum (DMS and DLS) to observe neuronal activation in response to learning a place or response strategy in a cross maze. CREB and *c-Fos* was significantly higher in all HPC regions for place learners, and CREB was also higher in the DMS and DLS for response learners. *C-Fos* expression in the DLS and DMS did not differ between strategies, however. Another study using yoked controls and measuring the IEGs *c-Fos* and *c-Jun* found increased *c-Jun* in the CA1 and CA3 regions of the HPC in rats trained in a place version of the MWT. Similar increases were found in the CA1 region of place learning rats for *c-Fos,* but only nominal *c-Jun* expression was noted in the DMS for a cued platform version of the MWT (Teather, Packard, Smith, Ellis-Behnke, & Bazan, 2005). More recently, Gill, Bernstein, and Mizumori (2007) examined *c-Fos* and Zif268 activation in the HPC and

dorsal striatum (including the DLS and DMS) of rats trained in a radial arm maze. Response learning induced increases in *c-Fos* expression in both the DMS and DLS, and was related to post-training probe trial performance. No effects for *c-Fos* expression were noted in the hippocampus. Similarly, Zif268 expression did not differentiate place or response learning in either the HPC or the striatum. Although there are discrepancies in the literature concerning IEG expression and learning strategies, it is clear that the HPC, DLS, and DMS are recruited to some degree in both cue and place learning tasks. This could be taken as support for cooperative interactions between these brain regions, as neuronal responses have been found in both task types regardless of the expected strategies. There is also the possibility that these areas are active in the presence of cues that the animal experiences during place and cued navigation.

Focus of This Thesis

Putting together the findings of the various experiments and theories discussed to this point, it appears that the learning systems represented in the HPC and dorsal striatum work cooperatively when a rat is navigating in a given space (Hamilton et al., 2004). The idea that there is a switch from place (or directional) navigation to response navigation within a given trial of the Morris water task is the focus of the current research. To date, no research has been done to follow up the findings of Hamilton et al. (2004) to examine the neurological basis of this response switching behavior.

In order to determine the unique contributions of the HPC and dorsal striatum involved in this phenomenon, it is proposed that rats with lesions to the hippocampus and DLS be trained and tested in the cued-platform version of the Morris water task as in Experiment 2 in Hamilton et al. (2004). Of particular interest are the kinematics displayed by

the lesioned rats compared to controls and the findings of Hamilton and colleagues. In addition, the initial trajectories and changes in head scanning behavior are also of interest. To follow up the findings of Experiment 1, a second experiment is proposed to examine naïve rats in the same lesion conditions on a task where the pool is shifted as in Hamilton et al. (Experiment 2, 2007) where the nature of the control provided by extra-maze cues during cued-navigation can be assessed after HPC or DLS lesions. By assessing specific behaviors and responses in these two variations of the water task, the results will add important knowledge regarding how HPC- and DLS-based memory systems interact in the service of behavior.

Experiment 1

Experiment 1 was designed to evaluate the role of the HPC and the DLS in spatial navigation behaviors in a cued platform variant of the MWT. Rats with lesions to the HPC or DLS, as well as sham controls, were trained to navigate to a submerged platform with a cue marking the platform location. After initial training all rats were given a brief probe trial in which the platform and cue were removed from the pool. Rats were then given additional training, followed by a test trial where the platform and cue were moved to another location within the pool.

If the initial swim trajectory is indeed dependent on the HPC, then it was hypothesized that HPC lesioned rats would be disrupted in the platform removal probe trial compared to DLS lesioned rats and controls on a measure of minimum distance traveled to the trained platform location. These results would be consistent with the findings of Devan and White (1999) where multiple hidden platform probe trials after cued platform training revealed longer latencies for rats with fornix/fimbria lesions compared to rats with either DLS or DMS lesions or controls. When the cued platform is relocated, it was hypothesized that DLS rats will be impaired compared to HPC and control rats when comparing tendencies to swim to the old platform location before navigating to the new location. If HPC rats navigate solely based on the platform cue, then the shift should not affect their strategy. Control rats were expected to show an initial trajectory to the old platform location that is quickly corrected by a shift-point head scan (Hamilton et al., 2004). DLS rats were expected to navigate to the old platform location based on the distal cues before searching for the new location. This has been observed in rats with lesions of the DLS but not the DMS (Devan & White, 1999).

With regard to kinematics when the platform is removed, previous research has shown that normal rats navigating to a visible platform have a steady acceleration that is broken by a small deceleration spike early on during the trial. This spike is believed to be a point where the rat is shifting strategies from an initial trajectory based on the distal room cues to a direct trajectory to the proximal platform cue (Hamilton et al., 2004). It was expected that this shift point behavior would not be evident for both the HPC and DLS rats compared to controls. HPC rats were expected to learn to navigate strictly based on the platform cue, thus eliminating the shift point because there is no change in strategy throughout the trial. Likewise, DLS rats were expected to learn the platform location based on the distal room cues.

Methods

Subjects. Subjects were 25 naïve male and female hooded Long-Evans rats bred at the Psychology Department Animal Research Facility at the University of New Mexico, originally from Charles River Laboratories stock (Charles River Laboratories, Wilmington, MA) that were at least 90 days old at the beginning of training. All rats were individually housed in plastic cages and maintained on a 12-hour light-dark cycle with food and water available *ad libitum.* All behavioral testing was conducted during the light phase, and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of New Mexico.

Surgery. Rats were randomly assigned to one of three surgical conditions: HPC lesions (n = 9), DLS lesions (n = 8), or sham surgeries (n = 8). All animals were anesthetized with Isoflorane (Phoenix Pharmaceuticals, St. Joseph, MO) and mounted into a stereotaxic frame (David Kopf Instruments, Tujunga, CA). All flat skull stereotaxic coordinates are

derived from the atlas of Paxinos and Watson (2005) using bregma of the skull surface as the reference point. Neurotoxic lesions were produced in the HPC by injecting a 7.5mg/mL solution of N-methyl-D-aspartate (NMDA) in phosphate buffered saline (PBS) through a 30-gauge cannula attached to a Harvard mini-pump. Ten total infusions were made in each rat, five per hemisphere. The coordinates in centimeters for each are AP: -0.31, -0.41, -0.5, -0.53, -0.6; ML: ±0.15, ±0.3, ±0.3, ±0.52, ±0.5; DV: -0.36, -0.4, -0.4, -0.73, -0.73, respectively. Injections were infused at 0.15 μL/min for two minutes and forty seconds and the cannula was left in the brain for three minutes to allow diffusion of the solution. After surgery, each rat with HPC lesions were given 0.1 mL of buprenophine subcutaneously as an analgesic, and diazepam (1 mL/kg) was administered by intraperitoneal injection at the first sign of wakefulness.

Neurotoxic lesions were produced in the DLS by injecting a 7.5mg/mL solution of NMDA, in the same manner as with the HPC lesions. Four total infusions were made in each rat, two per hemisphere. The coordinates in centimeters for each are AP: +0.15, +0.2; ML: ±0.32, ±0.4; DV: -0.44, -0.48, respectively. Sham rats received identical surgical procedures, but no cannulae were lowered into the brain tissue. Animals were allowed at least 14 days to recover from surgery before behavioral testing began.

Histology. Following the completion of all behavioral testing rats in the HPC and DLS groups were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (55 mL/kg) and perfused transcardially with 0.9% saline followed by 4% paraformeldahyde. Brains were removed from the skull and stored in a 20% sucrose solution for at least seven days. The brains were then frozen overnight in an -80° freezer. Frozen coronal sections were cut with a cryostat at 40 μm through the regions of interest and every

fifth section was mounted and stained with cresyl violet. Stained sections were examined microscopically to verify the extent of lesion damage.

Apparatus. A circular pool measuring 1.5 m in diameter and 48 cm high was placed on a 48 cm tall wooden frame. The escape platform was constructed of plastic and was 25 cm high with a 16 cm X 16 cm top surface that was covered with wire mesh so that it could be grasped easily by the rats. The platform cue was a black sphere (9 cm in diameter, 11.5 cm in height) attached to a metal rod that extends 11.5 cm above the platform surface. The pool was filled to a depth of 26 cm with cold (~21 C°) water so that the platform surface was ~1 cm below the water surface. The water was made opaque by adding approximately 2 oz. of powdered white tempura paint. The testing room contained several distinct distal visual cues that form a complex geometry. All behavior was videotaped via a ceiling mounted camera and digital camcorder. The digital video was then transferred to a Linux workstation for analysis.

Design and procedure. Experiment 1 was divided into an initial training and probe trial, followed by additional training and a test trial. During training, all rats received two trials per day with the cued platform located in the middle of the eastern quadrant of the pool for half of the rats in each lesion condition, and in the western quadrant for the other half. During each daily session rats were released one time each from one release point closest to the platform (either NE or SE for the eastern platform location, NW or SW for the western location) on trial 1 and one release point far from the platform (either NW or SW for the eastern platform location, NE or SE for the western location) on trial 2. The particular release points were selected randomly so that each rat was released an approximately equal number of times from each of the four possible release points over the course of the experiment. Each

rat was released into the pool facing the pool wall and given 60 s to navigate to the platform. Once the rat reached the platform, it was given 5 s to remain on the platform before being returned to the holding cage. If a rat failed to navigate to the platform in 60 s, it was placed on the platform by the experimenter for 5 s and then returned to the holding cage. This training continued until all rats had reached a criterion of three consecutive days of direct swim paths to the platform from the far release points.

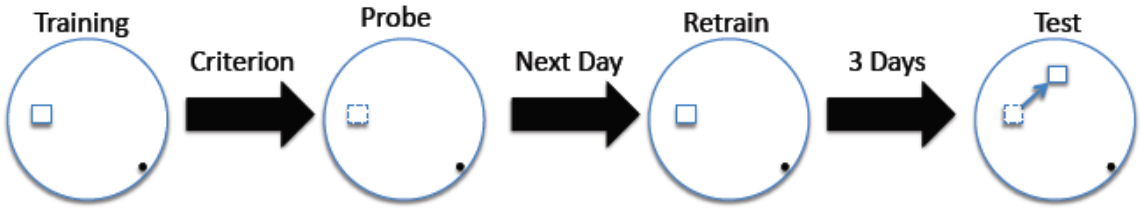

Figure 1. **Representative procedures for Experiment 1. Small dot indicates prelease point, dashed box indicates trained platform location with platform removed or relocated.**

After criterion was met, all rats were given a brief probe trial in which the platform and cue were removed from the pool (see Figure 1). Rats were released from the same release point used in the previous training trial. Comparisons were made within subjects on this probe trial and the last training trial to determine kinematic differences and heading error at various points along each swim path. Three additional days of training were administered after the probe trial, at which time rats were given a single test trial with the cued platform moved to a new location near the pool wall opposite the trained platform location (see Figure 1). All rats were released from the same far points as the last training trial in order to analyze within subject change in trajectory and heading error due to platform relocation.

Analysis. Video recordings were used to analyze swim paths, latency, and heading error using in-house designed software. Separate univariate analyses of variance (ANOVAs)

were used to analyze critical measures for the probe and test trials with lesion (HPC, DLS, and sham) as a between-subjects factor. Significant main effects were followed with Tukey *post hoc* tests. Where appropriate, planned comparisons or contrasts were used to test differences between lesion groups.

Results

Histology. Representative lesions for the HPC and DLS rats are presented in Figure 2. HPC rats exhibited near-complete damage to the entire hippocampal formation, including Ammon's Horn (CA1-3) and the denate gyrus (DG). Two rats (1 male, 1 female) were excluded from further analysis based on extreme scores for dependent measures (z-scores > 2) that coincided with incomplete or excessive lesions. DLS lesions were small and limited to the dorsolateral region of the striatum. Two rats (both female) were excluded based on the same criteria used for HPC rats. This resulted in the following group sizes: HPC (n = 7); DLS (n = 6); Sham (n = 8). Preliminary analyses found no significant main effects or interactions for sex or training platform location; therefore these factors were not included in the results reported below.

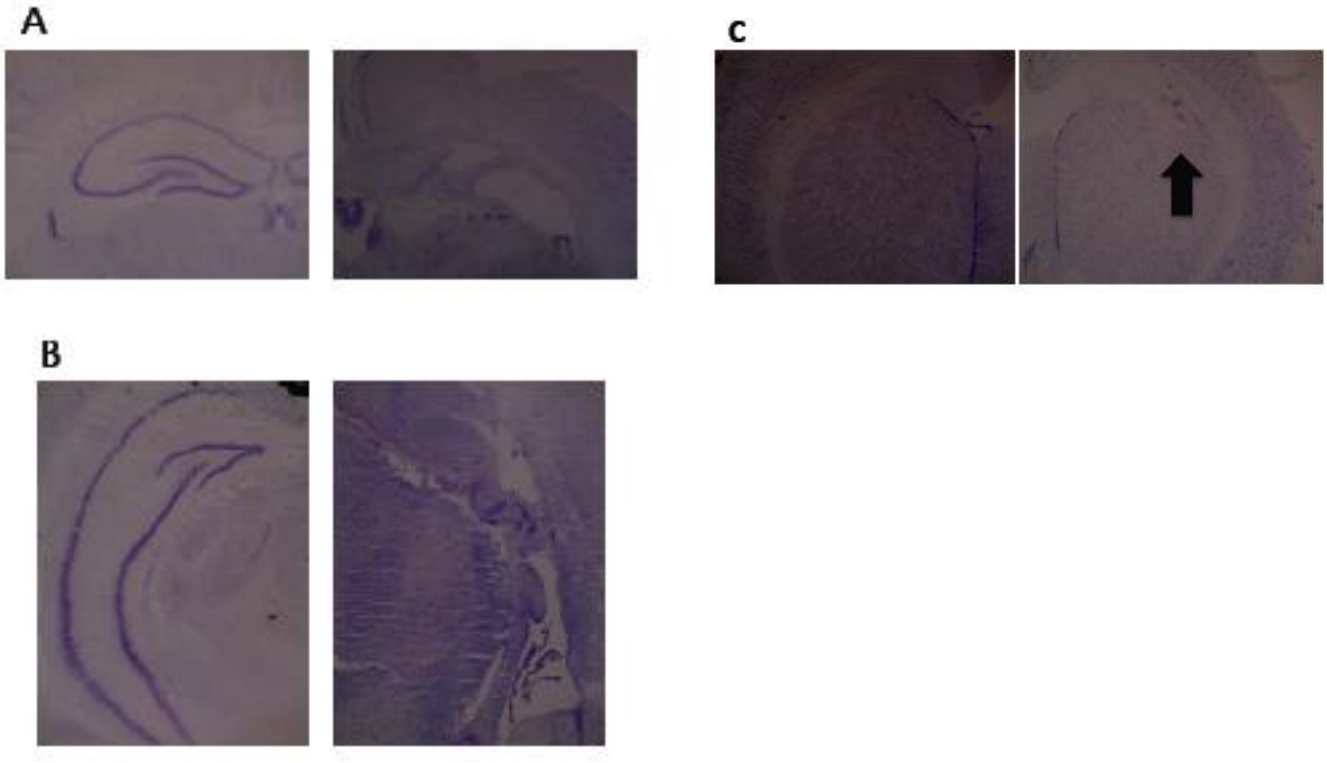

Figure 2. **Representative lesions for HPC and DLS rats. NMDA damage to the dorsal (A) and ventral (B) HPC is shown in the right panels, compared to shams on the left panels. Black arrow indicates damage to the DLS compared to shams (C).**

Training. Results for the number of trials to criterion are presented in Figure 3. HPC rats generally took longer to reach criterion compared to DLS and sham rats, while DLS and sham rats learned the task in a similar amount of time. The number of trials to reach a criterion of three consecutive direct swims from the long release points was recorded. A one-way ANOVA revealed a significant effect of lesion [$F(2,18) = 17.2$, $p < .001$]. Tukey *post hoc* tests revealed that HPC rats took longer to reach criterion than DLS ($p = .001$) and sham rats ($p < .001$), while the DLS and sham rats did not differ in number of trials to criterion ($p = .937$).

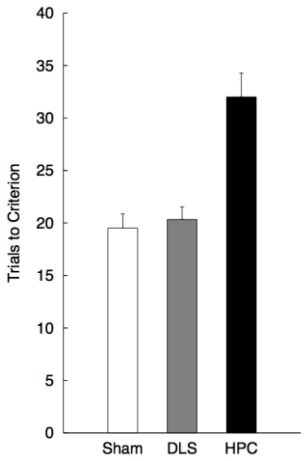

Figure 3. **Average number of trials to reach criterion for the three groups.**

No-platform probe trials. Immediately after reaching criterion, rats were given a brief probe trial from the last release point used during training with the platform and cue removed from the pool. HPC rats were impaired in the absence of the cued platform, while DLS and sham rats navigated toward the platform location despite the absence of the cue (see Figure 4). Latency, path length and the minimum distance to the trained platform location were analyzed using separate ANOVAs. A significant difference in latency was found [$F(2,18) = 4.32$, $p = .029$]; Tukey *post hoc* tests revealed that this difference was due to HPC rats having longer latencies than DLS rats ($p = .025$). It is important to note that no significant path length differences between groups were found [$F(2,18) = 2.31$, $p = .128$], indicating that HPC rats swam slower than the other groups but displayed similar distances traveled. There was a significant effect of lesion group for the minimum distance measure [$F(2,18) = 3.64$, $p = .047$]. Planned comparisons indicate that CPu and sham rats swam closer to the trained platform location than the HPC rats (Helmert Contrast, $p = .015$).

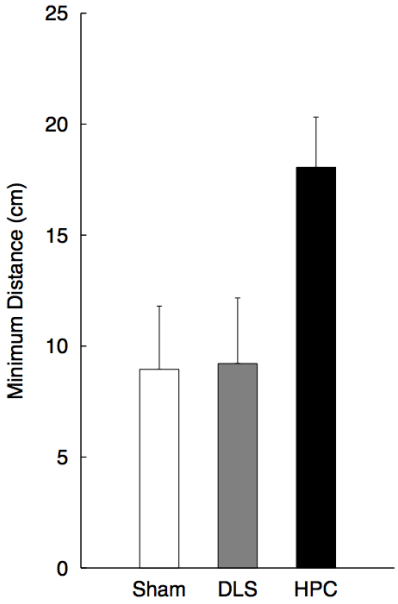

Figure 4. **Average minimum distance from the trained platform location for each group during the no-platform probe trials.**

Kinematic analysis. Kinematic analyses and representative swim paths for the no-platform probe trials are presented in Figure 5. Average velocity and heading error were computed for each of the lesion groups for the probe and test trials, as well as for the training trials that immediately preceded these trials. These measures were taken at 34 time points across each trial, with each no-platform probe trial being normalized to the previous training trial with respect to cumulative distance traveled. The specific aspects of heading error and velocity that are of interest to the present study are the early changes in velocity that are associated with the head scanning behavior described above, and how heading error between groups change beyond this point.

For the last training trial conducted before the probe trial, HPC rats, in general, displayed slower swim speeds than DLS or sham rats after the first three time points.

Interestingly, around the fifth time point, DLS rats were swimming faster than both the HPC and sham rats, a time point where a drop in velocity would be expected due to the head scanning behavior. Given that all groups initially displayed steady accelerations up to this point, it would appear that this serves as evidence for a shift point in the sham animals that was not evident in the DLS or HPC rats. In terms of heading error, the lesion groups did not differ early in the trial (up to the sixth time point). This was expected, given the fact that initial time points include turning away from the pool wall. From that point on, however, HPC rats displayed higher error rates compared to sham rats until the latter half of the trial, with DLS rats displaying similar error measures to controls. Based on these findings, it would appear that HPC rats are impaired in the early half of the trial, perhaps in an attempt to locate the cued platform, at which point their heading errors closely match the levels of sham and DLS rats. Interestingly, HPC rats appear to increase velocity after heading correctly in the direction of the cued-platform, but they do not reach the same velocity as the sham or DLS rats.

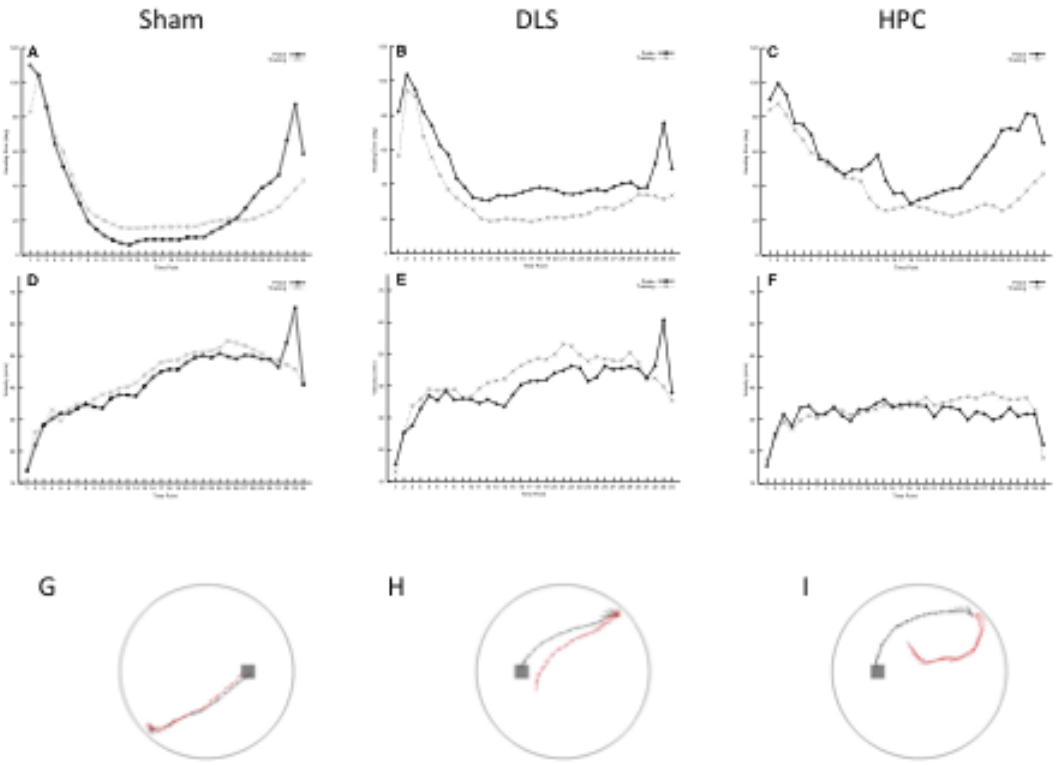

Figure 5. **Kinematic data for lesion groups during no-platform probe trials. Heading error for the last training trial and probe trial for each group (A-C), Velocity for the last training trial and probe trial (D-F), and representative trials for each group (G-H). Grey lines in graphs indicate training trials and black lines indicate probe trials. Black line indicates path for the last training trial, red indicates path for the probe trial.**

For the no-platform probe trials, the interesting differences between lesion conditions were evident only during the first half of the trial with regard to heading error. From the fifth time point on, HPC rats had higher heading errors compared to controls, with DLS rats somewhere in-between. DLS rats also displayed higher heading errors than shams for time points seven through eleven, indicating a disruption in the absence of the cue. Velocity measures again showed an overall decrease in swim speed for HPC rats, but this difference became more apparent in the latter half of the probe trial, as DLS and sham rats gradually increased their swim speed, while HPC rats swam at more or less the same speed throughout

the trial. Shift point head scans were not as clearly evident in shams, but there is a point mid-way through the trial where shams showed a distinct decrease in velocity. Specific changes in velocity that coincided with distinct changes in heading error were not evident in the DLS or HPC rats, and could be taken as evidence for a shift point head scan, or at least a disruption due to the removal of the cued platform. The general disruption in HPC rats would seem to support the idea that the removal of the cued platform disrupted these rats throughout the trial while errors were initially low in shams, but increased about halfway through the trial.

Platform relocation trials. After the no-platform probe trials, three additional days of training were carried out before rats received a platform relocation test trial. Data for the lesion groups for latency and path length to the new platform location are presented in Figure 6. DLS rats were impaired compared to HPC and sham rats at navigating to the new platform location. While HPC and sham rats swam more or less directly to the relocated platform, DLS rats spent more time and navigated closer to the old platform location. No significant differences were found between lesion conditions for latency [$F(2,18) = 1.70$, $p = .21$] or path length [$F(2,18) = 1.85$, $p = .186$] to the new platform location. For a more specific analysis of swim behavior, the number of times each rat entered a circular region (30 cm in diameter) immediately surrounding the old platform location was recorded, as well as the total time spent within this region during the test trial. There was no overall lesion effects for either the regional cross measure [$F(2,18) = 3.01$, $p = .075$] or the time in region [$F(2,18) = 2.58$, $p = .103$], but planned contrasts revealed that DLS rats made more entries into the old platform region than HPC or sham rats (Helmert contrast, $p = .042$) and spent more time in the old platform region as well (Helmert contrast, $p = .038$).

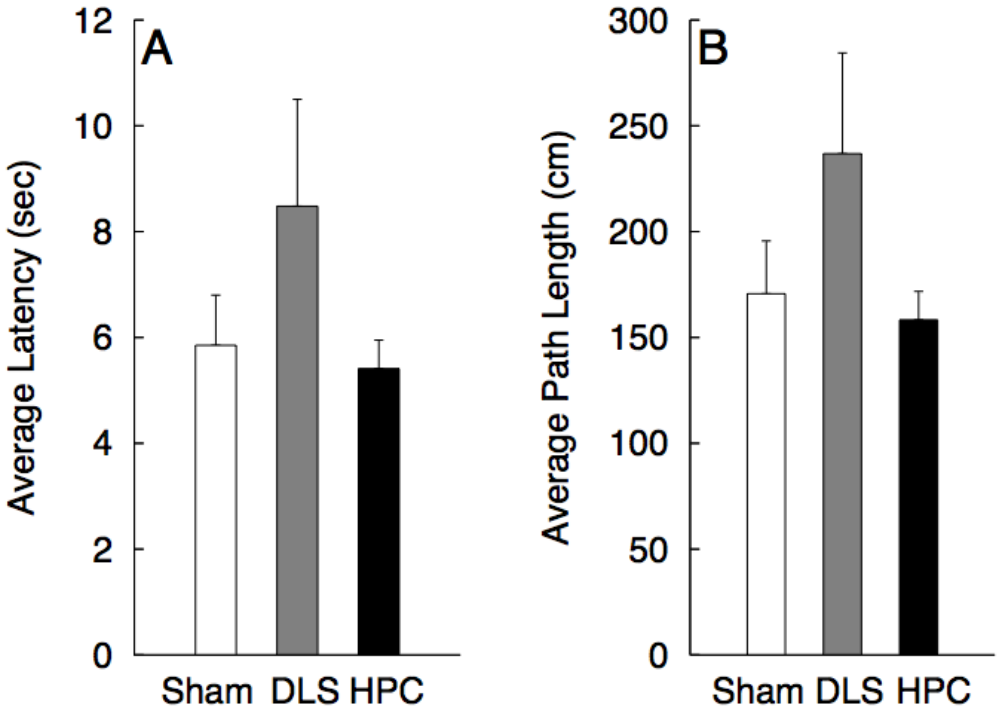

Figure 6. **Average latency (A) and path length (B) to the new platform location for the three groups on the platform relocation test trials.**

Kinematic analysis. Similar kinematic analyses were carried out in the platform relocation trials, with separate heading error measures taken for the old (trained) platform location and the new (test) platform location for the last training trial and the test trial. Data for these test trials, as well as representative swim paths for each group are presented in Figure 7. For the velocity measure taken during the training trial, all groups had similar swim speeds until about the fourteenth time point, at which time the sham and DLS rats swam consistently faster than the HPC rats until the end of the trial. This finding was consistent with the results from the training trial preceding the no-platform probe trial, indicating that HPC rats, overall, swam slower during training than the other groups.

Heading to the old trained platform location, as was expected, was direct for all groups, with a slight increase from points nine through eighteen for the HPC group. This

could be due to a more circuitous path taken by several HPC rats, but it is important to note that HPC rats had similar heading errors compared to DLS and sham rats for the remainder of the trial. All groups also displayed similar heading errors to the test platform location. This was expected, as this had never been a reinforced location during training.

During the platform relocation test trial, measures of swim speed were almost identical to the results for the training trial, with HPC rats having slower speeds after the twelfth time point. Interestingly, around the fifth time point, DLS rats were swimming much faster than HPC and sham rats, which could reflect a lack of attention to the location of the cue. Heading error to the trained platform location was greatest for the HPC rats during the first thirteen time points, with DLS and sham rats having similar low error rates. At about the mid-point of the trial, however, DLS rats had the highest error rates, which could indicate a circling persistence at the old platform location. During this time, sham and HPC rats had similar heading errors, possibly a reflection of locating the new location and heading towards it. By the latter third of the trial, all groups displayed similar heading errors. This would indicate that all rats had found the new location, and were swimming in that direction, as opposed to the trained location.

For the new platform location, all groups showed an initial decrease in heading error to the relocated platform until about the eleventh time point. From this point on, HPC rats displayed steady decreases in heading error until the end of the trial, an indication that the cue was guiding behavior throughout the trial. Sham and DLS rats had steady decreases in heading error until the end of the trial, with DLS rats displaying the greatest errors until the end of the trial. This would appear to support the hypothesis that sham rats initially navigated towards the old platform location, but then correctly navigated to the new location. DLS rats,

on the other hand, showed more persistence at the old location later in the trial before navigating to the new location.

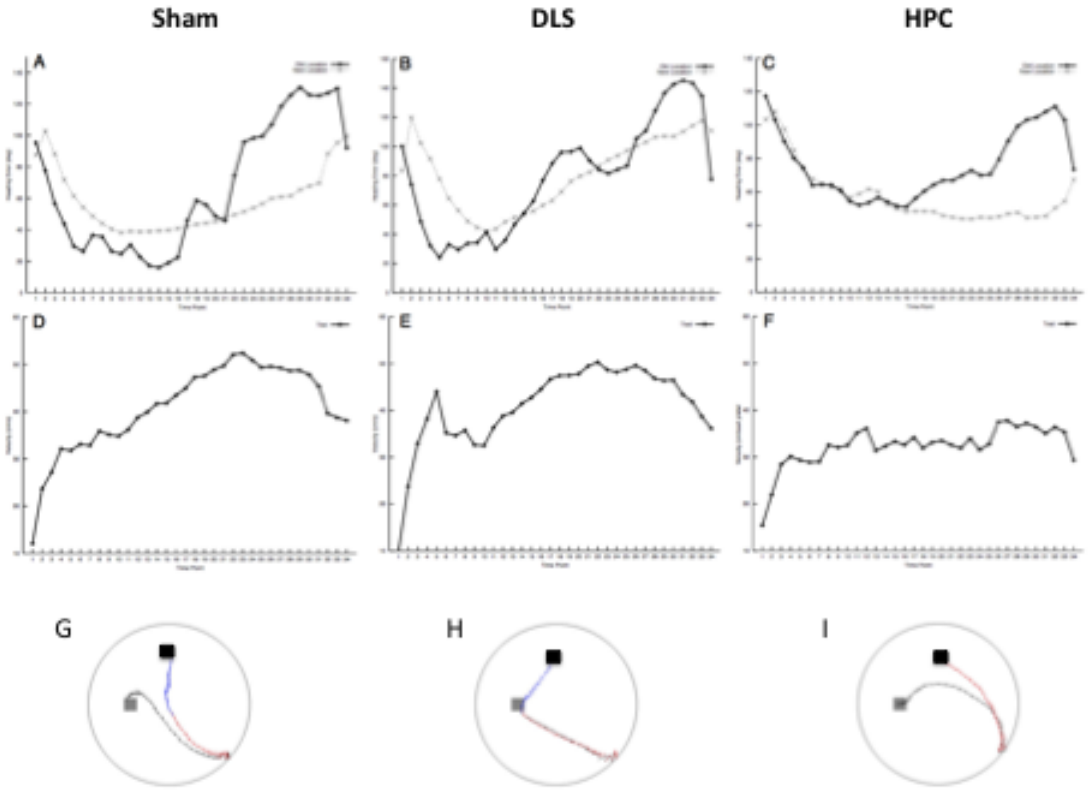

Figure 7. **Results for the kinematic analysis of the platform shift test trials. Average heading error to the trained platform location (gray lines) and the new platform location (black lines) during the trial (A-C). Average velocity during the trial (D-F). Representative trials for each condition (G-I). Black path indicates last training trial to old location (gray platform) and red/blue lines indicate test trial swim paths to new location (black box). Red path indicates equal distance compared to last training trial, blue path indicates swimming beyond this distance.**

Discussion

The results of Experiment 1 lend support to the hypothesis that the HPC is involved in establishing initial trajectories based on distal room cues, while the DLS is involved in terminal swim trajectories based on proximal cues, and that these learning systems interact in a cooperative manner in the MWT. Specifically, in the absence of the cued platform in the

no-platform probe trials, HPC rats were disrupted compared to sham and DLS rats. While DLS and sham rats swam closely to the platform location, HPC rats displayed greater minimum distances to the location. On the cued platform relocation trials, DLS rats were disrupted relative to HPC and sham rats. HPC rats overwhelmingly navigated directly to the new platform location, while sham rats displayed initial trajectories to the old location that were quickly corrected to the new location. There were a few sham rats that navigated near the old location first, but their persistence and location crosses were less than the DLS rats. DLS rats spent more time persisting at the old platform location, as evidenced by the number of crosses of the region surrounding the old platform location and the amount of time spent in this region.

These findings lend support to the idea that initial trajectories in the MWT are dependent on the distal room cues, while terminal swim paths are dependant on more proximal intra-pool cues, in this case the cued platform (Hamilton et al., 2009). Given the findings that an intact HPC is necessary for navigation based on a constellation of distal cues (Morris et al., 1982, Eichenbaum et al., 1990) and the role of the DLS in navigating to a cued or visible platform (Devan & White, 1999), it would appear that these two memory systems interact in a cooperative manner within trials in the MWT.

Results from the kinematic behavior analysis provide some evidence for the shift point head scanning that was first reported by Hamilton and colleagues (2004). In the present study, sham rats did appear to show a deceleration spike early during training trials that was followed by a subsequent increase in swim speed and direct heading to the platform. This specific behavior was not evident in rats with HPC or DLS lesions. This is taken as evidence that sham rats shift between hippocampal- and striatal-based strategies while navigating to

the platform, while HPC rats rely primarily on the cued platform and DLS rats rely primarily on the distal room cues.

Specific kinematic analysis of the no-platform probe trials indicate that groups were reliably navigating to the platform on the last training trial, but that HPC rats were impaired during the probe trial in the absence of the cued platform. DLS rats also appeared to be impaired compared to sham rats, albeit to a lesser degree than HPC rats. This impairment was largely due to a single DLS rat that had significantly higher heading errors compared to other DLS rats. When this rat's data were excluded DLS rats had similar error rates as shams, and even had lower error rates than shams by the end of the probe trial. These kinematic results lend additional support with the minimum-distance measures results showing a disruption in HPC rats compared to DLS or sham rats when the cued platform is removed from the pool.

Similar swim speed measures were found in the analysis of test trial behavior. This would indicate that the velocity and head scanning behavior (Hamilton et al., 2004) is consistent across training, presumably when the animal has reached asymptotic performance levels. All groups were swimming directly to the trained location on the last training trial, and not near the new location, as expected. On the relocation test trial, sham and DLS rats displayed direct initial headings to the old location, while HPC rats had increased initial heading errors to the old location. With regard to the new location, on the other hand, HPC rats demonstrated a steady decline in heading errors as the trial progressed, while sham and DLS rats had higher errors. By the end of the trial, DLS rats had the highest errors, with shams displaying errors between the HPC and DLS rats. This would seem to support the idea that the cue was guiding the behavior of the HPC rats throughout the trial, and for the latter

aspect of the trial for the shams; while the distal cues were guiding the behavior of the DLS rats throughout the entire trial.

It is important to note that DLS rats were able to locate the new platform location, often rather quickly, after searching for the platform at the trained location. This may indicate that DLS rats did not have neglect for the cue that marked the platform location, but that the cued navigation strategy was not the preferred method for solving the task. This would fit with previous work indicating that rats with damage to the dorsal striatum are able to use proximal cues to guide behavior, but only when forced to do so (Whishaw et al., 1987).

HPC rats took longer to reach criterion than DLS and sham rats, this an unexpected result given the fact that other studies found no deficits in acquisition for HPC rats in a visible or cued platform task (Devan et al., 1996). Additionally, HPC rats displayed slower swim speeds as well throughout training and on the probe and test trials. One possible explanation is that the hippocampal lesions achieved in the current study involved almost complete damage to the hippocampus, while other studies have focused on the dorsal hippocampus (i.e., Oliveria, Bueno, Pomarico, & Gugliano, 1997), or the fimbria/fornix (i.e., McDonald & White, 1994). Alternatively, the training protocol employed here (two trials per day) could also explain the increased trials to criterion for HPC rats, given the finding that cued learning is incremental. Thus, giving rats reduced training across sessions could exasperate the effects of HPC lesions. Likewise, cued training might be enhanced in sham and DLS rats, thus driving the significant effect of trials to criterion. With the results of this experiment indicating that sham and DLS rats use distal cues to learn the platform location, this could also explain the superior training performance of these groups. Given the fact that HPC rats in the current study were able to eventually reach criterion, and were able to

navigate directly to the new location during platform relocation trials, there is no reason to suspect that these rats had deficiencies in motivation or sensory processes involved in solving the MWT. Although HPC rats received more training than DLS or sham rats, the results of the test and probe trials were in line with the expected outcomes based on prior research (e.g. Devan et al., 1999).

The conclusion from Experiment 1 is that HPC lesions disrupt initial trajectories based on distal cues, but not subsequent navigation based on the platform cue. DLS lesions, on the other hand, do not disrupt initial trajectories based on distal cues, but do result in the disruption in the use of proximal cues to guide subsequent navigation to the cued platform.

Experiment 2

Previous research has shown that when a single cue marks the platform location in the Morris water task, this cue comes to overshadow the distal room cues (Redhead et al., 1997). However, other studies indicate that rats might still learn to navigate to the platform based on its location with respect to the distal cue set in the environment, even when a single cue constantly marks the platform location (Whishaw et al., 1987). Previous work in our lab indicates that even in the presence of a cued platform, distal cues may still contribute to the directionality of the swim trajectory to the platform (Hamilton et al, 2007). Rats were given 36 trials across three days of training with the cued platform in the same location within the pool and room. On test trials in which the pool was translated (shifted laterally) in the room, the platform either moved with the pool such that it remained in the same relative location in the pool or remained in the same absolute location within the room. A majority of the rats in both conditions showed a directional response to the previously trained platform location within the pool, even though the cued platform was still present. For rats tested with the cued platform in the absolute location in the room, this means that they swam to the relative location in the pool prior to swimming to the cued platform. If initial trajectories taken by rats are based on the distal room cues, then navigation to a cued platform should be sensitive to changes in the pool location within the testing environment. The purpose of this experiment was to investigate the effects of these manipulations on rats with HPC, DLS, or sham lesions.

In Experiment 1, the pool and the cues in the room were static. If the effects of HPC and DLS lesions are to be further characterized, it is important to investigate how a shift in the pool location relative to the distal room cues might disrupt rats on their initial trajectory,

which is hypothesized to be controlled by the available distal cues. To that end, rats were given massed training followed by test and probe trials that involved pool shifts and/or cued platform removal. On test trials, the pool was shifted laterally so that the trained platform location with respect to the distal room cues (absolute location) was now in the opposite quadrant within the pool. The cued platform was either in the absolute location, or in the same quadrant within the pool used during training (relative location). All rats received test trials for both platform locations in a within-subjects, counterbalanced manner. It was expected that Sham and DLS rats would show a preference for the relative location based on the expectation that distal cues will contribute to navigation in these groups and prior research indicating that normal rats display a preference for directional responding in the MWT (Hamilton et al., 2007). In contrast, hippocampal rats should not be sensitive to the pool shift manipulation and navigate directly to the cued platform regardless of its location. These expectations are based on findings that an intact hippocampus is necessary for directional responding in open field tasks (Stringer et al., 2005) and the MWT (Rice et al., 2008) and the observations of Experiment 1 in which hippocampal rats navigated directly to the platform location regardless of absolute or relative position.

After additional training, probe trials were administered in two conditions: with the cued platform removed and the pool in the same training position, or with the pool shifted in the same manner used during the test trials. These tests were undertaken to address control by the distal cues in the absence of the cue located at the platform. Again, all rats received both conditions in a within-subjects, counterbalanced manner. Sham and DLS rats were expected to show preferences for the absolute platform location in the no-shift condition and a preference for the relative platform location in the shift condition. HPC rats were

hypothesized to be disrupted in the absence of the cued platform, showing no preference for either the relative or absolute locations.

Methods

Subjects. Subjects were 50 naïve male and female hooded Long-Evans rats. The origin, age, feeding, and housing conditions are identical to those of the rats used in Experiment 1.

Surgery and histology. Rats were given either HPC lesions (n = 17), DLS lesions (n = 17), or sham surgeries (n = 16). All surgical and histological procedures used in Experiment 2 are identical to those used in Experiment 1.

Apparatus. All materials and apparatus used in Experiment 2 are exactly the same as those used in Experiment 1 with the following exception: The wooden frame on which the pool was set was mounted on appliance rollers, allowing the pool to be moved laterally while full of water.

Design and procedure. In Experiment 2 rats received cued-platform training in three blocks of four trials per day for four days. Two pool positions were used and the platform was always located in the same overlap position of these pool positions (location B in Figure 8 below). Half of the rats from each lesion condition were trained with the pool in position 1 and the other half trained with the pool in position 2 (see Figure 8). At the end of the eighth block of training trials on day three, a competition test trial was administered. In this test trial the pool was shifted 75 cm (the radius of the pool) laterally from the trained position for all rats, so that the rats trained in pool position 1 were tested with the pool in position 2, and vice versa. Half of the rats in each lesion condition were assigned to the *absolute* shift condition where the cued platform was in the same location relative to the

distal room cues (location B), but in the opposite quadrant of the pool than where training occurred. The other half of the rats from each lesion condition were assigned to the *relative* condition where the cued platform shifted along with the pool so that it is in the same quadrant of the pool as it was during training (location C for rats trained in pool position 1, location A for rats trained in pool position 2), but shifted away from the absolute location relative to the distal room cues. Rats were released from novel release points for these test trials (N or S, counterbalanced). At the end of the ninth block of trials on day 3, a 30 s probe trial was administered with the cued platform removed from the pool. The pool remained in the same location within the room for half of the rats in each condition, and for the other half the pool was shifted as in the test trial. Again, rats were released from the N or S so that each rat experienced each release point in the test and probe trials.

On day four rats received the same training, test, and probe trials as on day three with each rat receiving the opposite conditions. Thus, rats that were given the pool shift test trial with the platform in the absolute location on day three received the test trial with the platform in the relative location on day four. Likewise, rats that received the no shift probe trials on day three were given the shift probe trial on day four. Again, release points (either N or S) were counterbalanced so that each rat experienced each release point across the test and probe trials. A summary of the combinations of pool positions and platform locations used for the test and probe trials is presented in Table 1.

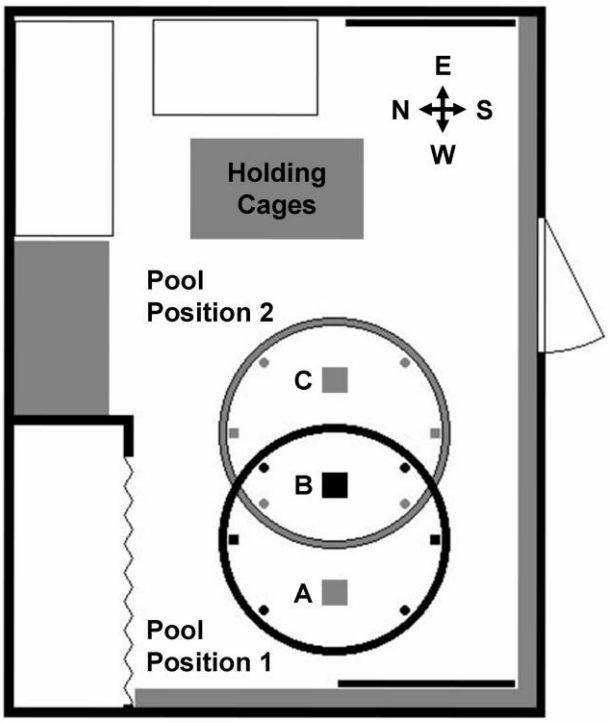

Figure 8. **Layout of the MWT environment used in Experiment 2. Two pool positions were used during training with the platform always in location B. During Test trials, locations A and C served as relative platform locations. Small circles indicate release points used during training, small squares represent release points used during test and probe trials.**

Table 1. ***Pool positions and platform locations for groups of rats in each lesion condition in Experiment 2. The release points were counterbalanced across days 3 and 4 for the test and probe trials.***

Rats (n)	Training Pool Postion	Training Platform Location	Test Pool Position	Day 3 Test Platform Location	Day 3 Probe Pool Position	Day 4 Test Platform Location	Day 4 Probe Pool Position
Sham (3)	1	B	2	B	1	C	2
Sham (5)	2	B	1	B	1	A	2
Sham (4)	1	B	2	C	2	B	1
Sham (4)	2	B	1	A	2	B	1
DLS (4)	1	B	2	B	1	C	2
DLS (3)	2	B	1	B	1	A	2
DLS (4)	1	B	2	C	2	B	1
DLS (4)	2	B	1	A	2	B	1
HPC (3)	1	B	2	B	1	C	2
HPC (4)	2	B	1	B	1	A	2
HPC (3)	1	B	2	C	2	B	1
HPC (4)	2	B	1	A	2	B	2

Analysis. Latency to the platform for all training trials was averaged across blocks of four trials, with three trial blocks per day. These data were analyzed with a two-way repeated measures ANOVA with lesion condition as a between-subjects factor and trial block as a within-subjects factor. The only significant effect that was expected was the main effect of trial block as all rats' performance was expected to improve as training progressed. For the absolute vs. relative test trial, latency to the platform and path length were analyzed using two-way repeated measures ANOVA with lesion group as a between-subjects factor and absolute/relative platform location as a within-subjects factor.

For the platform removal probe trials, measures were taken to compare preferences for two regions within the pool. In the no shift condition where the pool is in the same location as it was during training, the absolute platform location is considered the location where the platform was during training. The comparison location is in the opposite quadrant, where the rats had never been reinforced for navigating to. In the shift condition, again the absolute location is the same location with respect to the distal room cues that the rats experienced during training, while the comparison region is the relative platform location in the opposite quadrant. The regions analyzed included a 30 cm area around the possible platform locations (see Figure 6).

Four measures were taken for each of these regions in each probe trial. Latency to region, time spent in region, number of platform crosses, and proximity to region (average distance from the locations of interest). Again, two-way ANOVAs were conducted with lesion as a between-subjects factor and absolute/relative locations as within subjects factors. Where appropriate, planned comparisons and contrasts were used to assess specific hypothesis, and Tukey *post hoc* tests were used to follow up all other significant differences.

Results

Histology. As in Experiment 1, lesions resulted in nearly complete damage to the hippocampal formation in HPC rats, and small, but reliably detectable damage to the dorsolateral striatum in DLS rats (see Figure 2). Using the same criteria for exclusion in Experiment 1, three HPC rats (two females, one male) and two DLS rats (one female, one male) were excluded from further analysis. This resulted in the following group sizes: HPC (n = 14), DLS (n = 15), and sham (n = 16). Preliminary analyses did not reveal significant main effects or interactions for the factors of sex or trained pool position, so these factors were not included in the analyses reported below.

Training trials. Group performance across training trial blocks is presented in Figure 9. Sham and DLS rats improved performance rapidly across trial blocks. HPC rats also improved across days of training, but never reached the levels of the other groups. A repeated measures ANOVA was conducted with lesion as a between-subjects factor and the 12 trial blocks as a within-subjects factor. Significant main effects were found for lesion [$F(2,42) = 18.33$, $p < .001$] and trial block [$F(11,462) = 75.78$, $p < .001$], as well as a significant lesion X trial block interaction [$F(22,462) = 3.91$, $p < .001$]. *Post hoc* analyses of the significant interaction (Fisher's LSD) revealed that HPC rats had significantly higher latencies on all trial blocks compared to DLS and sham rats (all p's < .05), while no differences were found between sham and DLS rats on any trial blocks (all p's > .05).

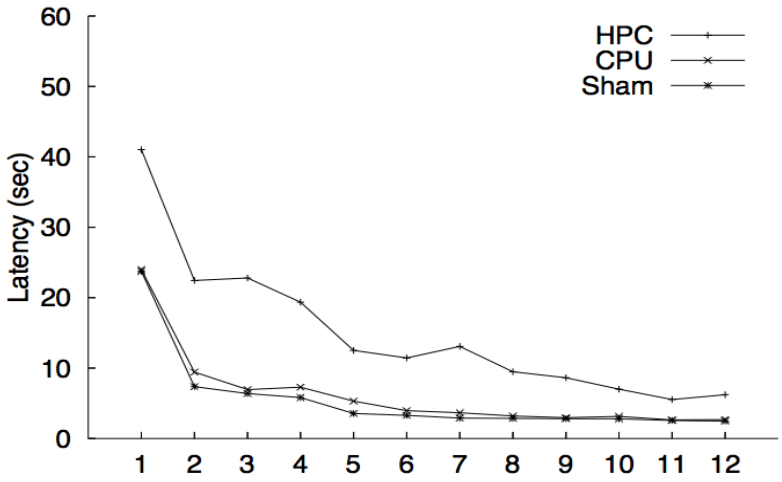

Figure 9. **Mean latencies for the lesion groups across training trial blocks.**

Pool shift test trials. Results for the lesion groups, as well as representative trials for each condition, are presented in Figure 10. Sham and DLS rats swam quickly to the cued platform when it was in the relative location, but typically navigated in the direction of the relative location in the pool when the platform was in the absolute location. HPC rats, on the other hand, navigated to the cued platform regardless of whether it was in the absolute or relative location. A repeated measures ANOVA with lesion as a between-subjects factor and latencies to the absolute and relative platform location as a within-subjects factor revealed a significant main effect of platform location [$F(1,42) = 13.89$, $p = .001$], indicating that there were in general longer latencies for the absolute location compared to the relative location. The main effect of lesion [$F(2,42) = 0.41$, $p = .668$] and the lesion X location interaction [$F(2,42) = 1.63$, $p = .208$] were not significant. Planned comparisons among the lesion groups indicated that sham [$t(15) = 2.14$, $p = .049$] and DLS [$t(14) = 4.14$, $p = .001$] rats had longer latencies to the absolute location than to the relative location, HPC rats demonstrated no preference for either location [$t(13) = 1.87$, $p = .084$].

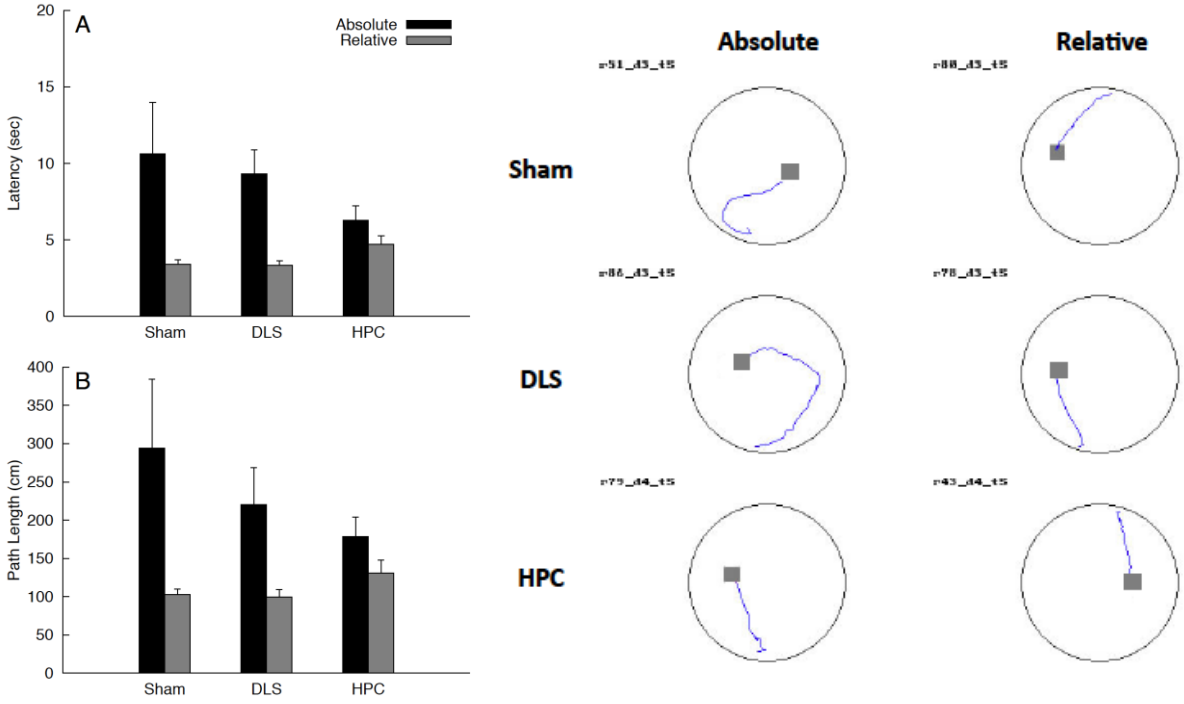

Figure 10. **Results for the lesion groups on the cued-platform pool shift test trials. Latency (A) and path length data (B) are on the left, representative trials for each group in each condition are presented on the right.**

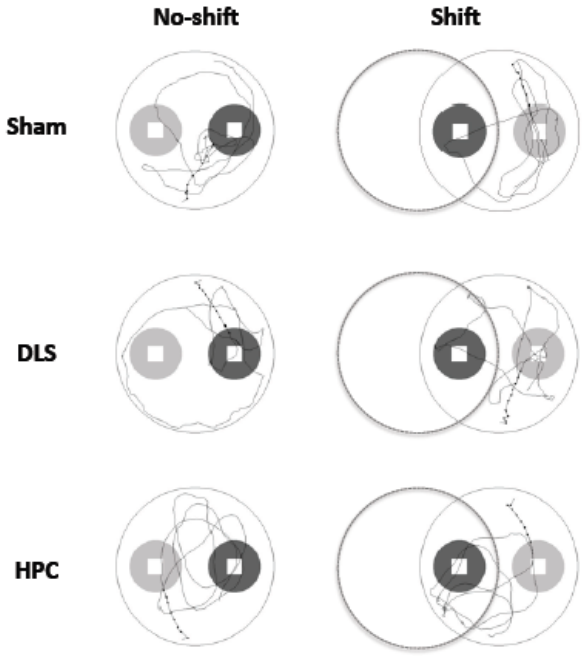

Figure 11. **Representative swim paths for the no-shift (right) and shift (left) conditions for each lesion condition.**

Shift and No-Shift Probe Trials. Results for the probe trial measures for the lesion groups are presented in Figure 12. Representative swim paths for each lesion group in both the no-shift and shift probe trials are presented in Figure 11. For the no-platform probe trials, a repeated measures ANOVA with lesion as a between-subjects factor and with pool (shift and no-shift) and location of interest (absolute vs. other/relative) as within-subjects factors was conducted with latency to region, time spent in region, number of region crosses, and proximity to region as dependent variables. Significant lesion X shift X location interactions were found for latency [$F(2,42) = 6.77$, $p = .003$], time in region [$F(2,42) = 8.54$, $p = .001$], region crosses [$F(2,42) = 7.81$, $p = .001$], and proximity to region [$F(2,42) = 7.15$, $p = .002$]. To further evaluate the pool shift X location interactions, the data were further analyzed separately for each lesion group.

Sham rats. Sham rats showed preferences for the absolute location during the no-shift probe trials and for the relative location during the shift probe trials. Within the sham group, there were no significant main effects of pool shift for latency to region [$F(1,15) = 0.20$, $p = .661$], time in region [$F(1,15) = 0.65$, $p = .433$], region crosses [$F(1,15) = 0.0$, $p = 1.0$], or proximity to region [$F(1,15) = 0.202$, $p = .659$]. Similarly, the main effect of location was also not significant for latency to region [$F(1,15) = 1.0$, $p = .333$], time in region [$F(1,15) = 0.02$, $p = .883$], region crosses [$F(1,15) = 0.04$, $p = .841$], or proximity to region[$F(1,15) = 1.0$, $p = .332$]. There were significant pool shift X location interactions for latency to region [$F(1,15) = 38.91$, $p < .001$], time in region [$F(1,15) = 68.57$, $p < .001$], region crosses [$F(1,15) = 17,44$, $p = .001$], and proximity to region [$F(1,15) = 32.05$, $p < .001$].

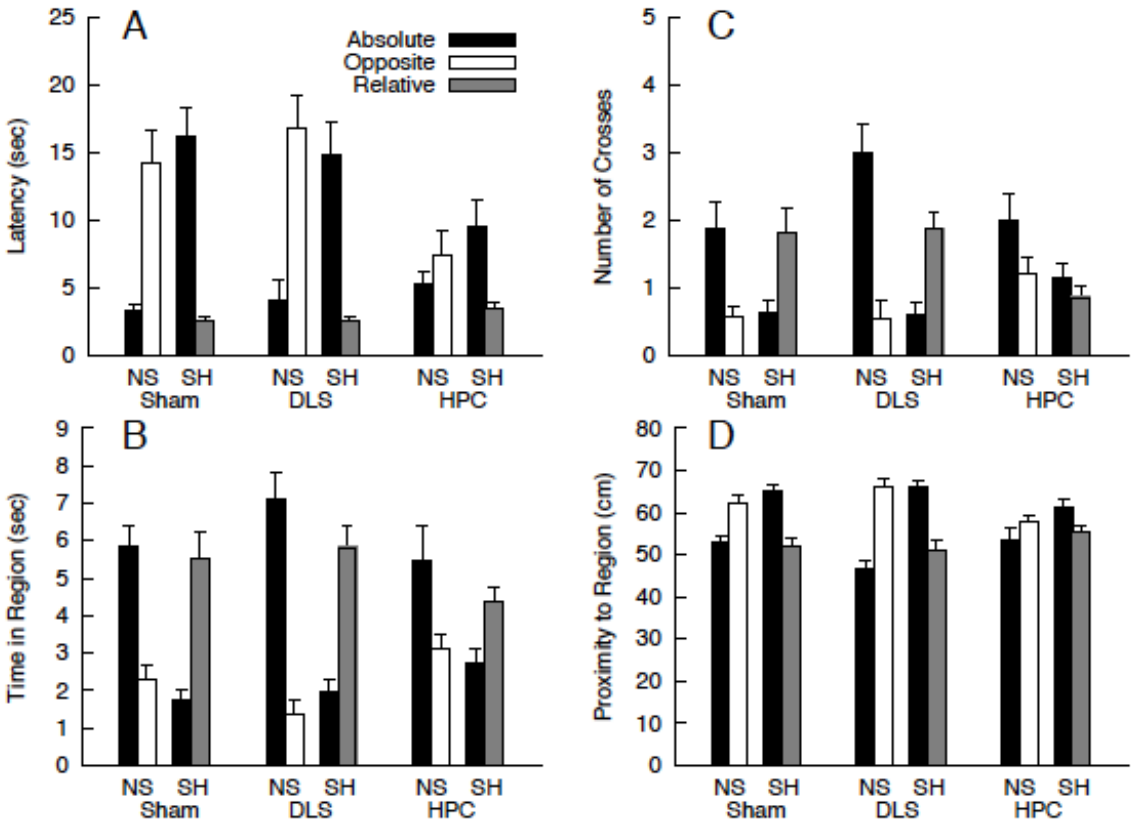

Figure 12. **Results for the no-shift (NS) and shift (SH) probe trials for the lesion groups. The measures taken include latency to region (A), time in region (B), region crosses (C), and proximity to region (D).**

Simple effects tests indicate that during the no-shift probe trials, sham rats navigated to the absolute location faster than to the comparison location in the opposite quadrant [$t(15) = 4.23$, $p = .001$], spent more time in the absolute region [$t(15) = 4.73$, $p < .001$], crossed the absolute region more often [$t(15) = 2.78$, $p = .014$], and navigated closer to the absolute location [$t(15) = 3.59$, $p = .003$]. During the no-shift probe trial, sham rats navigated to the relative location faster than the absolute location [$t(15) = 6.39$, $p < .001$], spent more time in the relative region [$t(15) = 4.76$, $p < .001$], crossed the relative location more often [$t(15) = 3.14$, $p = .007$], and swam closer to the relative location [$t(15) = 5.03$, $p < .001$].

DLS rats. DLS rats displayed preferences similar to shams for the absolute location during the no-shift probe trials and for the relative location during the shift probe trials. No significant main effects of pool shift were found for latency to region [$F(1,14) = 0.66$, $p = .43$], time in region [$F(1,14) = 0.37$, $p = .553$], region crosses [$F(1,14) = 3.8$, $p = .07$], or proximity to region [$F(1,14) = 1.73$, $p = .209$]. Main effects of location were also not found for latency to region [$F(1,14) = 0.01$, $p = .992$], time in region [$F(1,14) = 1.82$, $p = .199$], region crosses [$F(1,14) = 2.74$, $p = .12$], or proximity to region [$F(1,14) = 0.70$, $p = .418$. Significant pool shift X location interactions were found for latency to region [$F(1,14) = 5.36$, $p < .001$], time in region [$F(1,14) = 133.26$, $p < .001$], region crosses [$F(1,14) = 32.19$, $p < .001$], and proximity to region [$F(1,14) = 53.01$, $p < .001$].

Simple effects tests show that, during the no-platform probe trials, DLS rats swam to the absolute location faster than to the comparison location [$t(14) = 4.03$, $p = .001$], spent more time in the absolute region [$t(14) = 6.39$, $p < .001$], crossed the absolute location more often [$t(14) = 4.05$, $p = .001$], and swam closer to the absolute location [$t(14) = 4.74$, $p < .001$]. During the shift probe trials, DLS rats swam to the relative location faster than to the absolute location [$t(14) = 4.96$, $p < .001$], spent more time in the relative location [$t(14) = 5.47$, $p < .001$], crossed the relative location more often [$t(14) = 3.83$, $p = .002$], and navigated closer to the relative location [$t(14) = 4.86$, $p < .001$].

HPC rats. In contrast to the sham and DLS rats, HPC rats did not show any preferences for either the absolute or comparison location in the no-shift probe trial. On the shift probe trials, HPC rats displayed similar gross preferences for the relative location as sham and DLS rats, but these effects were blunted in the HPC rats. For the main effect of pool shift, a significant effect was found for region crosses [$F(1,13) = 6.65$, $p = .023$]

indicating more crosses of the absolute location across both the no shift and shift probe trials. The effects of latency to region [F(1,13) = 0.01 p = .922], time in region [F(1,13) = 2.23, p = .159], and proximity to region [F(1,13) = 3.15, p = .099] were not significant. For the main effect of location, no significant effects were found for latency to region [F(1,13) = 2.02, p = .179], time in region [F(1,13) = 0.34, p = .571], region crosses [F(1,13) = 2.16, p = .166, and proximity to region [F(1,13) = 0.08, p = .784]. Analysis of the pool shift X location interaction revealed significant main effects for latency to region [F(1,13) = 5.14, p = .041] and time in region [F(1,13) = 11.83, p = .004]. The proximity to region measure approached significance [F(1,13) = 4.31, p = .058], and the region crosses measure was not significant [F(1,13) = 1.54, p = .236].

Simple effects analysis of the interactions reveal that, during the no-shift probe trials, HPC rats did not show any preference for either the absolute or comparison location based on latency to region [t(13) = 0.87, p = .398], region crosses [t(13) = 1.52, p = .151], and proximity to region [t(13) = 1.19, p = .254] measures. The time in region measure approached significance [t(13) = 2.14, .052], with HPC rats showing a trend for favoring the absolute location. For the shift probe trial, HPC rats demonstrated a preference for the relative location based on latency to location [t(13) = 2.78, p = .016] and time in region [t(13) = 3.62, p = .003]measures. No significant effects were found for region crosses [t(13) = 1.0, p = .336] or proximity to region [t(13) = 1.85, p = .087].

Discussion

The purpose of Experiment 2 was to describe how a translation of the pool within the testing environment altered navigational strategies in rats with HPC or DLS lesions. When the pool was shifted and the cued platform remained in the relative location within the pool,

HPC, DLS, and sham rats readily navigated directly to the platform. When the cued platform remained in the absolute location with respect to the distal cues, however, only the HPC rats swam to the platform. Sham and DLS rats initially swam towards the relative location, displaying longer latencies and path lengths. This preference for the relative location, even in the presence of a cued platform, in shams has been described previously (Hamilton et al., 2007). As was expected, HPC rats showed no specific preference for either location, using the cued platform to guide behavior. From this finding, and the results of Experiment 1, it would appear that the cued platform is controlling the behavior of HPC rats, even when the pool is translated within the testing environment. The finding that DLS lesions do not disrupt the preference for the relative location observed in normal rats is intriguing for two reasons. First, the fact that the directional responding reported by Hamilton and colleagues (2007; 2008; 2009) is intact despite disruption of the DLS rules out habit formation as a possible explanation for this result. Second, based on the results from Experiment 1, it would be possible to theorize that DLS rats became hypersensitive to changes with respect to the distal room cues. The fact that DLS rats displayed similar preferences for the relative location compared to sham rats would suggest that this is not the case. This effect in DLS rats could only have been revealed by manipulation that put the absolute and relative locations into competition with each other.

When the cued platform was removed from the pool for the no-shift and shift probe trials, sham and DLS rats showed preferences for the absolute location in the no-shift condition and for the relative location in the shift condition. HPC rats showed no such preferences for the absolute location in the no-shift condition, instead searching randomly in the absence of the platform cue. This was to be expected, given previous work showing a

disruption in rats with HPC lesions when the cued platform is removed from the pool (Devan et al., 1996). In the shift condition, HPC rats displayed slight general preferences for the relative platform location based on measures of latency and time spent in the relative location. More specific measures of persistence (region crosses, proximity to region), however, were not significant for either the absolute or relative locations in HPC rats. It is also worth pointing out that the preferences for the relative location demonstrated by HPC rats were not as robust as the preferences exhibited by sham and DLS rats.

The finding that HPC lesions disrupt directional responding, in this case the ability to navigate to the relative location, confirms previous reports in the MWT (Rice et al., 2008) in open field and T-maze tasks (Stringer et al., 2005). One issue that needs to be addressed is the fact that HPC rats were impaired across all trial blocks compared to DLS and sham rats during acquisition. It is important to note that, although HPC rats did demonstrate a slower rate of learning, they did improve across trial blocks, and by the final day of training had latencies < 5 seconds on average. The significant lesion effects on these blocks were due to the fact that DLS and sham rats had averages in the 2 - 2.5-second range, with very little variability. Given that no lesion effects were found for the first training trial (data not shown), it would not appear that pre-existing differences could account for the differences found throughout the training trial blocks. Taking this into account, combined with the performance of HPC rats on the test and probe trials, would seem to indicate that these rats had learned some strategy for locating the platform, and this is what was meant to be tested in the current experiment.

Previous studies in our lab have found that, using a similar pool shift paradigm where the pool was filled nearly to the top to reduce the influence of the pool wall, rats showed a

preference for the absolute platform location during no-platform probe trials after 12 trials (1 day), no specific preference for either the absolute or relative location after 24 trials (2 days), and a preference for the relative location after 36 trials (3 days) (Hamilton et al., 2009). One possible interpretation for this shift in strategies is that there may be a corresponding shift in the neurobiology governing such behavior, in this case from the HPC to the DLS. This shift has been noted in other studies using place versus response discriminations (Packard & McGaugh, 1996). The results of the present study would seem to discount this assertion, given that rats with DLS lesions showed the same directional preferences as sham rats on all test and probe trial measures. We can conclude from this that directional responding is not dependent on the DLS, but both directional and place navigation strategies do appear to be dependent on an intact hippocampus.

Of particular interest are the results from the pool shift test trials and the no-platform pool shift probe trials. In the case of the test trials, the cued platform remained in the pool; this was, of course, not the case for the probe trials. Although the performance of the DLS and sham rats were similar in both cases, with both groups showing a preference for the relative over the absolute location, this effect seemed to be enhanced with the cued platform removed from the pool. In an unexpected result, HPC rats demonstrated similar preferences in the no-platform pool shift probe trials based on latency and time in region measures, but not for platform crossed and proximity measures. One possible explanation is that the measures for latency to region and time in region might reflect a preference for the general location in question, while regional crosses and proximity to the region reveals a more specific knowledge of where the platform should be. A recent meta-analysis in mice found that the proximity to region measure is the most sensitive measure of MWT probe

performance compared to latency to region, time spent in region, and number of regional crosses measures (Maei, Zaslavsky, Teixeira, & Frankland, 2009). Perhaps more sophisticated measures of platform preference (i.e. the "H" measure developed by Maei and colleagues (2009)) would lead to different results in HPC rats.

Given the research that has shown that rats with damage to the hippocampal formation can learn to navigate to a specific location in a general manner (Whishaw & Jarrard, 1996; Day et al., 1999), this would seem to fit with our observations here. Another possibility is that HPC rats had learned to navigate to a specific quadrant in the pool, and then learned to use the cue to navigate to the platform. This behavior would result in the probe trial results reported here. In observing the specific behavior of HPC rats that navigated to the relative location far more quickly than to the absolute location, four of the six rats were trained in pool position 1, which means that, during the pool-shift probe trials, the pool was translated in the direction of the holding cages. Thus, the relative location was closer to the place where the rats were stationed between trials. It might be possible that HPC rats (and rats from the other groups) could at least partially be using path integration or "dead reckoning" to navigate in the direction of the cages. Alyan and McNaughton (1999) found that damage to the HPC did not disrupt this type of navigation (but see Wallace & Whishaw, 2003). Finally, amount of damage to the HPC might also be a factor. Although rats were excluded from lesion groups based on extreme scores for behavioral measures combined with incomplete or excessive lesions, a more specific analysis of extent of spared tissue in the HPC could help explain the the unexpected results for the HPC rats.

Another issue to be addressed is the role of the rat striatum in spatial navigation. In the current set of experiments, the DLS was targeted due to this regions well-documented

role in S-R learning (Yin & Knowlton, 2006) and cued navigation (Whishaw et al, 1987). Several studies have also investigated the role of the dorsomedial striatum (DMS) in spatial navigation tasks (i.e., Devan et al., 1999) with at least one study dissociating the posterior and anterior aspects of the DMS, with the posterior DMS showing a role in spatial navigation in the plus-maze (Yin & Knowlton, 2004). It is still possible that the DMS might be involved in the spatial tasks described in the current set of experiments, further research using the pool shift paradigms described here and elsewhere (Hamilton et al., 2008; Rice et al., 2008) could offer a more sensitive way to elucidate the behavioral and neurobiological functions of the striatum.

General Discussion

The results presented here provide strong evidence for the existence of cooperative, sequential interactions between the HPC and DLS during navigation involving distal and proximal cues in the MWT. Results for Experiment 1 and 2 are summarized in Table 2. A double-dissociation between the HPC and DLS was demonstrated in Experiment 1, where rats with HPC lesions were impaired on probe trials where the cued-platform was removed from the pool, while this impairment was not evident in shams or rats with DLS lesions. On the other hand, during a trial where the cued-platform was relocated within the pool DLS rats were impaired at navigating to the new platform location while sham and HPC rats were not. These results support the hypothesis that initial trajectories to a cued platform in the MWT are dependent on the hippocampus, while subsequent trajectories are dependent on the dorsolateral striatum. Results from Experiment 2 indicate that such interactions occur even when the apparatus is translated within the environment in order to put the distal cues in the training environment into competition with the proximal cues within the pool.

These results are important when considering much of the previous literature has been taken as evidence for competition between learning and memory systems (for a review see Poldrack & Packard, 2003). The evidence from the current experiments suggests that lesions of the HPC or DLS selectively disrupt specific aspects of swim behavior within trials in the MWT. Comparisons between lesion groups and controls during swim trials could only be made when swim trials were analyzed on a moment-to-moment basis, as in Experiment 1 and elsewhere (Hamilton et al., 2004). Evidence for competition between learning and memory systems has been based on evidence from between-trial comparisons over the course of training (i.e. Packard & McGaugh, 1996). This would suggest that large scale (molar)

Table 2. ***Summary of results for Experiments 1 and 2. Bold statements indicate differences compared to sham rats.***

	Lesion Condition		
Manipulation	**Sham**	**DLS**	**HPC**
Exp.1			
Training	Rapid Acquisition	Rapid Acquisition	**Disrupted**
Probe trials	Navigated close to the platform location	Navigated close to the platform location	**Disrupted**
Probe Heading	Initial heading to platform location	Initial heading to platform location**, disrupted later in trial**	**Disrupted heading to platform location**
Relocation Trials	Qucik correction to new location	**Disrupted**	Quick correction to new location
Relocation Heading	Intial heading to old location, then to new location	Initial heading to old location, **disrupted to new location**	**Disrupted to old location,** less error new location
Probe/Relocation Kinematics	Steady increase in velocity, slowing at trained platform location	Steady increase in velocity, slowing at trained platform location	**Consistent slower velocity throughout the trial**
Exp. 2			
Training	Rapid Acquisition	Rapid Acquisition	**Disrupted**
Cued-Platform Pool Shift	Relative > Absolute	Relative > Absolute	**Relative = Absolute**
No-shift Probe Trials	Absolute > Opposite	Absolute > Opposite	**Absolute = Opposite**
Shift Probe Trials	Relative > Absolute	Relative > Absolute	**Relative ~ Absolute**

analysis leads to the conclusion that HPC and DLS based learning and memory systems interact in a competitive manner, while small scale (micro) analysis reveals that these systems may, in fact, be operating in a cooperative and sequential manner within a trial. Although several prior studies have dissociated HPC- and DLS-based navigational strategies in the MWT (Devan et al., 1996) and land based tasks (Packard et al., 1989), the method of analysis was limited to describing the expression of one of the strategies or the other, thus limiting the possible explanations of behavioral outcomes.

In Experiment 1, consistent with prior research, HPC lesioned rats were disrupted when the cued platform was removed from the pool, while DLS lesioned rats were disrupted when the cued platform was relocated within the pool (Devan et al., 1996). Specifically, when the cued platform was removed during probe trials, HPC rats were impaired based on a measure of minimum distance to the trained platform location. Sham and DLS rats were not disrupted and navigated close to the trained platform location. On the other hand, DLS rats were impaired on test trials where the cued platform was relocated within the pool. While HPC and sham rats swam more or less directly to the cued-platform in a new location, DLS rats searched for the platform at the trained location before navigating to the new location. Since the same release point was used for the last training trial and the subsequent probe and test trials, and the platform was the same physical distance from this release point, these effects can only be attributed to disruptions of the learning systems engaged by the HPC and DLS, respectively.

Kinematic Analysis

The critical novel outcome of this study was the analysis of moment-to-moment kinematics while the rats navigated during the training trials that preceded the probe and test

trials, as well as the probe and test trials themselves. This allowed for a more specific description of the behaviors exhibited by rats with lesions to the HPC or DLS. Since these analyses have been described previously in normal rats (Hamilton et al., 2004), it was interesting to note the behaviors observed in HPC and DLS lesioned rats. On training trials preceding no-platform probe trials, all groups displayed similar heading errors, indicating that all rats had learned to readily navigate to the cued platform. When the platform was removed for the probe trial, sham rats had the lowest errors across the trial, but also had sharp increases later in the trial, presumably due to the absence of the cued platform. Heading errors were initially highest in HPC rats. This was expected, given that HPC rats were hypothesized to be navigating based on the platform cue. Interestingly, the DLS rats also had higher heading errors during the middle of the trial despite the fact that they swam close to the platform location. This could be an indication that DLS rats swam past where they expected the platform to be. Another possibility is that the cue had become part of the overall constellation of cues in the testing room and the removal of this cue disrupted behavior.

Analysis of swim speed showed that sham and DLS rats had similar velocities, increasing steadily throughout the trial, until reaching the point where the platform was expected to be located. HPC rats consistently swam slower during training and probe trials. It has been suggested that the intermediate HPC could be critical for the flow of spatial behavior from memory to actual motor output (Bast, Wilson, Witter, & Morris, 2009), thus, disrupting hippocampal output may be expected to influence the quality of movements involved in navigation. While this effect has not been reported in other studies involving damage to the HPC (i.e. Devan et al., 1996), most studies involved selective lesions of the

dorsal HPC or the fimbria/fornix. It is possible that velocity deficits might only be revealed by complete damage to the HPC, as was achieved in the present set of experiments. Because these rats did not receive any pretraining, the slower swim speeds could also be an indication that the HPC is important for learning motor behaviors involved in swimming to some degree.

During platform relocation test trials, sham and DLS rats had low errors to the old platform location while heading errors to the old location were highest in the HPC rats. With respect to the new platform location, shams initially had high errors that were quickly corrected as these rats identified the platform cue. DLS rats had the highest average heading errors to the new location, an indication that these rats were persisting at searching near the trained platform location. This could also be evidence of the importance of the DLS in adaptive navigation (Mizumori et al., 2009), in that these rats were not able to adjust to the new platform location as quickly as shams. HPC rats had the lowest errors to the new location, again supporting the hypothesis that the cue was guiding behavior in these rats. In summary, the kinematic analyses provide evidence for the sequential nature of the interactions between HPC and DLS, given that HPC rats were impaired early in trials and DLS rats were impaired in the latter aspects of the trial, particularly when the platform was relocated.

Pool Translation

In Experiment 2, the pool was shifted within the training environment for test and probe trials. Our lab has used this method frequently to describe the importance of the proximal reference frame (i.e. the pool wall) in spatial navigation (Hamilton et al., 2007; 2008; 2009). The results of Experiment 2 are the first known reports of how lesions to the

HPC or DLS might affect preferences for directional responding using these pool translation manipulations.

Sham rats navigated to the relative location during probe and test trials, even when the cued platform was located in the absolute location. Even though normal rats use the platform cue to guide navigation as shown in Experiment 1 and elsewhere (Hamilton et al., 2004), when the pool was shifted, sham rats still navigated to the relative location first. This preference for the relative location was evident even in probe trials where the cued-platform was removed from the pool, which suggests that the distal room cues are guiding this directional response. This could be due to a shift in place cell firing as a result of the pool translation. Knierim and Rao (2003) have shown that the translation of a local apparatus can alter place cell firing, with some cells showing coherence with the distal reference frame, while others shifted with the proximal reference frame. Thus, shifting the pool may have caused a re-mapping of place cells in normal rats, which could explain the initial disregard for the cued platform. This could also explain the fact that sham rats in Experiment 1 were equally split between navigating directly to the new location (four rats) and navigating to the old location first (four rats) during the cued platform test. Since some place cells shift with the distal frame and some with the local frame (Knierim, 2002), this could be expressed behaviorally in different rats.

In cued-platform and no-platform pool shift trials, DLS rats had similar preferences for the relative location. Although head-direction cells have been previously described in the dorsal striatum (Ragozzino et al., 2001), lesions of the DLS did not impair directional responding in this experiment. It could be that these head direction cells might be localized in the DMS, as this region has been found to be critical for spatial navigation (Yin & Knowlton,

2004), or that head direction cells located in other brain regions might be critical for this navigation strategy (Taube, Muller, & Ranck, 1990). It is important to note that lesions of the dorsal tegmental nucleus that disrupt head direction cell activity throughout the brain impair initial acquisition in the Morris water task, however, animals ultimately learn to solve this task (Hamilton, Clark, Rice, Johnson, Akers, & Taube, 2009).

In the presence of the cued-platform, HPC rats navigated directly towards the platform regardless of whether it was in the relative or absolute location. This was not the case when the platform was removed for probe trials. When the pool was shifted for these probe trials, HPC rats navigated faster to, and spent more time in, the relative location compared to the absolute location. However, more precise measures of platform preference, including the number of platform crosses and proximity to platform location, were not significantly different for the relative or absolute location. Although several HPC rats navigated to the relative location before the absolute location, there was no evidence for strong persistence in this region. Perhaps measures of quadrant preference might be more appropriate for HPC rats, given that the measures used in the current experiments are often applied to detect slight specific differences between normal rats. Gross measures, like quadrant preference, might be better for capturing the random swimming behavior displayed by HPC rats. More importantly, though, is the fact that compared to DLS and Sham rats in which consistent preferences for the relative location were observed for all measures that were utilized, the effects observed in HPC rats were either weak or not observed.

In conclusion, the data suggest that navigational strategies based on the distal reference frame appear to control behavior in a similar fashion in sham and DLS rats, while HPC rats appear to base their navigation solely on the proximal reference frame. These

results support the findings in humans that boundary learning involves the hippocampus, while specific cue learning within the boundary involves the dorsal striatum (Doeller, King, & Burgess, 2008). That sham and DLS rats navigated to the relative location in the pool shift test and probe trials more readily than the absolute location while HPC rats did not (to the same extent) supports the idea that the HPC is critical for learning about boundary locations. Similarly, that sham and HPC rats were able to flexibly navigate to the cued platform regardless of location with respect to the pool (Experiment 1) and the distal cue array (Experiment 2) while DLS rats were impaired in both cases supports the idea that the DLS is critical for learning about local cues.

Unexpected Outcomes/Alternative Explanations

The finding that HPC rats demonstrated slight preferences for the relative location during pool-shift probe trials in Experiment 2 was unexpected based on the hypothesis that the HPC is necessary for navigating based on a constellation of distal cues (Eichenbaum et al., 1990). In addition to the possible explanations for this result discussed earlier, it could also be that a polarizing cue, such as the door to the testing room could have driven this effect. Devan and coworkers (2002) have shown that such polarizing cues can guide behavior even in the face of dramatic changes to the rest of the distal cue environment. Another unexpected outcome was the similar performance of sham and DLS rats on the test and probe trials in Experiment 2. Based on the results of Experiment 1, it would be plausible to predict that DLS rats would be hypersensitive to the distal cue array, and thus be effected to a greater degree than the sham rats during the pool translations. Thus, these rats might be expected to navigate to the absolute platform location. Again, this might be the case for damage to other

regions of the striatum, but not the DLS. Further studies are needed to explore this possibility.

Another issue that is raised by the results of the current experiments is the role of the platform cue. In previous experiments investigating control of distal and proximal cues on navigation strategies, the platform was visible, but just above the surface of the water. In The experiments described here, the cue was more prominent, and elevated above the submerged platform (~11 cm). This leads to the possibility that the platform cue could have become part of the distal cue array, and this could explain the similar performance of sham and DLS rats. It could also explain the deficits in acquisition demonstrated by HPC rats in Experiments 1 and 2.

Limitations

There are limitations related to the experimental design that need to be addressed. In both Experiments 1 and 2, naïve rats with lesions were trained in the MWT. Although the case can be made that pre-training might have reduced some of the differences between lesion groups with regard to training in the task, we were interested in describing the emergence of learning in rats with HPC or DLS lesions. Another limitation was the sample size in Experiment 1, and the within-subjects design utilized in Experiment 2. Although the trends for the platform relocation test trials were in the expected direction for latency and path length, these effects were not significant. More subjects could have helped to reduce the error terms and increase the power to detect DLS disruptions. The within-subjects design was useful for Experiment 2, but the fact that each rat experienced two cued-platform test trials and two pool-shift probe trials could introduce more variability. Probe trials are unreinforced, which could influence future behavior. Also, the pool-shift experience is not novel after the

first cued platform test trial, and could change behavior in the probe trials. Inactivation studies would be useful for within-subjects designs, or more subjects per group in a between-subjects design could lead to more powerful results related to the data presented here.

Future Directions

In order to solidify the sequential nature of the interactions between HPC and DLS, studies involving inactivations of these brain regions (e.g. Packard & McGaugh, 1991) could yield more convincing results using rats that are well trained in the MWT. For example, if these interactions are operating in normal rats trained in a similar manner as in Experiment 1, then immediate inactivation of the HPC should disrupt initial trajectories, while subsequent navigation remained intact. The opposite effect would be expected for DLS inactivated rats. Since no delay between training and test trials would be necessary for infusion studies like this, a more specific analysis of the nature of these interactions could be possible. Along the same lines, studies using functional neuroimaging (fMRI, MEG, or EEG) in humans and/or single-unit recordings in rodents offer the temporal resolution to observe activity in the HPC and DLS while the subject is actively navigating in an environment. Although studies using these techniques have been previously reported in humans (Doeller et al., 2008) and animals (Eschenko & Mizumori, 2007), questions regarding cooperative interactions within a single trip have not been addressed.

Another consideration to be addressed in future studies is an expansion of the nature of the "shift" from HPC- to DLS- based navigation strategies. The nature of this shift has been described behaviorally in Experiment 1 and using eye tracking in humans (Hamilton et al., 2009). The neural basis of such a shift remains to be described. Recent findings using single-unit recordings in rats indicate that this could be an attentional process mediated by

the medial prefrontal cortex (Rich & Shapiro, 2009). Again, inactivation studies targeting this brain region in rats, or imaging studies in humans could examine this hypothesis. The pool shift paradigm described in Experiment 2 could also be useful for dissociating sub-regions of the HPC, as well as the striatum, to further describe the specific neuronal underpinnings of spatial learning and memory.

In demonstrating the cooperative nature of interactions between HPC and DLS, the experiments presented here present significant opportunities to further the understanding systems-level cooperation between brain regions involved in learning and memory, and how these interactions shape behavior.

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