

Running Head: SPATIAL MEMORY AND THE HIPPOCAMPUS

THE EFFECTS OF DORSAL HIPPOCAMPAL OR CA3 LESIONS ON SPATIAL
REFERENCE AND WORKING MEMORY IN TWO SPATIAL TASKS

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Abstract

The rodent dorsal hippocampus is necessary for normal spatial memory, where dorsal lesions produce an impairment in this mnemonic function. It is not known which specific subregions within the dorsal hippocampus mediate spatial memory. In the present study, rats were trained in the radial maze before bilateral neurotoxic lesions of dorsal CA3 (dCA3) or dorsal hippocampus (dHPC). Post-operative testing showed that both groups were impaired on reference and working memory. The same rats were then tested for spatial acquisition in the Morris Water Maze (MWM) and later tested on retrieval. All groups acquired the task equally but only the dHPC, and not dCA3, group showed impaired retrieval. Upon subsequent testing of working memory ability in the MWM, dHPC rats, but not dCA3, showed deficits. Analysis of behavioral data followed histological confirmation of lesion placement showing selective dCA3 and dHPC lesions made by ibotenic acid and colchicine, respectively. These behavioral results suggest that encoding of a reference memory may only depend upon the tissue available within the hippocampus, where the ventral portion may compensate for the lost dorsal section. Upon retrieval of this information, however, the dHPC is required and may utilize a larger neural network for successful retrieval. As for spatial working memory, the dCA3 is differently involved in working memory where compensatory mechanisms can act depending on delay and/or task-specific demands that recruit other areas besides the dCA3.

THE EFFECTS OF DORSAL HIPPOCAMPAL OR CA3 LESIONS ON SPATIAL REFERENCE AND WORKING MEMORY IN TWO SPATIAL TASKS

It is known that the medial temporal lobe is involved in aspects of the spatial encoding, storage, and retrieval (Bird & Burgess, 2008; Moscovitch, et al., 2005). What is less well known are the underlying neural correlates involved in these types of memory processes and precisely how spatial mnemonic functions operate within the brain.

There are anatomical and functional differentiations within the hippocampus. With respect to anatomical connectivity, the hippocampus varies along its septo-temporal (dorso-ventral, respectively) axis. Most sensory information is sent to the dorsal two thirds of the HPC, via the entorhinal, perirhinal, and association cortices (Aggleton, Vann, Oswald, & Good, 2000; Burwell & Amaral, 1998; Furtak, Wei, Agster, & Burwell, 2007; Mohedano-Moriano, et al., 2007). This suggests that incoming sensory information is integrated into a spatial map in the hippocampus and updates continuously to provide the rat with an up-to-date spatial location. In contrast, the ventral hippocampus receives connections from the ventromedial lateral nucleus of the amygdala and projections from the hypothalamus (Bannerman, Yee, Good, Heupel, Iversen, & Rawlins, 1999; Petrovich, Canteras, & Swanson, 2001). These data, as established by behavioral studies, reveal a critical role of the ventral hippocampus in regulating internal physiology, such as fear and anxiety responses, and possibly playing a role in emotional-related learning.

With regard to functionality, behavioral studies that use hippocampal-dependent tasks demonstrate that rodents with hippocampal damage exhibit significant spatial memory deficits (Steele & Morris, 1999; Ramos, 2008). Complete and dorsal hippocampal lesions, but not ventral lesions, impair spatial navigation (Zhang, Pothuizen, Feldon, & Rawlins, 2004), which argues that dorsal lesions make complete lesions superfluous in spatial memory. Additional evidence for the role of the dorsal hippocampus comes from firing characteristics of cells in the CA subfields (Barnes, McNaughton, Mizumori, Leonard, & Lin, 1990). These place cells display a wider responsive spatial field as one goes towards the intermediate (splenial) and ventral portions of the hippocampus from the dorsal portion (Jung, Wiener, & McNaughton, 1994; Maurer, VanRhoads, Sutherland, Lipa, & McNaughton, 2005), also suggesting that more precise spatial navigation is processed by cells in the dorsal-most third of the hippocampus than the intermediate or ventral third. However, because these spatial fields exist in these latter two locations, animals may retain a rough representation of the environment in a familiar environment but not in a novel environment (Frank, Brown, & Stanley, 2006).

Functionality of the ventral portion of the hippocampus is mainly involved in regulation of anxiety-mediated behaviors, such as behaviors on the elevated-plus maze. Ventral hippocampal lesions, but not dorsal hippocampal lesions, produce a decrease in anxiety levels (McHugh, Deacon, Rawlins, & Bannerman, 2004) and are responsible for behavioral disinhibition (Bannerman et al., 2004). The ventral half of the hippocampus has direct effects upon fear, where these rats fail to avoid open arms in an elevated maze and show less predator-induced freezing responses. Interestingly, ventral lesions also produce a direct physiological effect resulting in a decrease in corticosterone levels in response to being

exposed to bright lights (Kjelstrup, Tuvnes, Steffenach, Murison, Moser, & Moser, 2002).

Others debate on this dissociation, citing evidence that if rats are trained in a certain way, the ventral hippocampus does become involved (de Hoz, Knox, & Morris, 2003). Rudy and Matus-Amat (2005) suggest that dorsal and ventral regions process the same information, such as context. The ventral hippocampus has been shown to be involved in spatial navigation in the Morris water maze, but this may reflect the nature of navigating water to be an inherently anxious task (Gray & McNaughton, 1983).

But, there needs to be a clear distinction between the types of spatial memory and navigation. Spatial memory (e.g. location of landmarks) is necessary for proper navigation in new and familiar environments. The term 'spatial memory' must be defined in a more precise manner, because it may refer to the short-term (spatial working memory) or the long-term (spatial reference memory) component of this process. Spatial reference memory acquisition is the establishment of mental representations of the environment, such as the encoding of landmark locations in relation to other landmarks (topological relationships), the relative distance between differing objects (metric relationships), and the encoding of landmark locations in reference to a goal. Reference acquisition needs the hippocampus, where lesions here produce a learning impairment (Pothuizen, Zhang, Jongen-Rêlo, Feldon, & Yee, 2004; Ramos, 2008). In terms of true reference memory, where acquisition of the task occurs prior to insult, this process is also disrupted by hippocampal lesions (Moser & Moser, 1998; Clark, Broadbent, & Squire, 2005a, 2005b; McHugh, Niewoehner, Rawlins, & Bannerman, 2008). There is probably a neural network difference between the acquisition/encoding and retrieval of the reference memory. Previous research has shown that acquisition only depends upon available tissue in the hippocampus but retrieval requires the entire hippocampus, which may

be an inherent characteristic about the process itself, where it requires a larger, more distributed network (Moser & Moser, 1998; Moser, Moser, Forrest, Andersen, & Morris, 1995).

Instead of the aforementioned spatial learning that occurs after insult, a model of retrograde amnesia is frequently used to measure retention. Animals that are trained to a memory criterion, then undergo complete hippocampal removal, and later tested on retention, have been shown by numerous studies to have a striking impairment on later recall of the previously acquired spatial information (Clark, Broadbent, & Squire, 2007; Winocur, Moscovitch, Caruana, & Binns, 2005). Additionally, training that started early in the rat's life is still impaired on retention of this previously acquired reference memory, meaning that this phenomenon is not a result of a consolidation issue (Clark et al., 2005b). Even after reminding partially lesioned (dorsal or ventral lesions) or completely lesioned hippocampal rats of their previously learned information, both groups show an impairment in retention (Martin, de Hoz, & Morris, 2005). This suggests there is a critical role for the hippocampus in spatial memory from early in life to late adulthood in rats.

The second distinction within spatial memory is working memory. Spatial working memory is the ability for one to hold, use, and manipulate spatial information to remember where one just came from a second or minute ago. Spatial working memory is easily thought about when one considers connections within the hippocampus. Connectivity of the hippocampus proper is thought to consist of a trisynaptic pathway, which is traditionally known as a one-way pathway. Recent electrophysiological and anatomical studies, however, have called this generalized pathway into question by showing additional direct pathways and recurrent systems (Bartasaghi R, Migliore M, Gessi T, 2006; Yeckel & Berger, 1990).

Anatomical research on the CA3 shows that this region has an extensive recurrent collateral system (Káli & Dayan, 2000). The CA3 recurrent connections probably serve to cycle information that is currently being manipulated, such as in spatial working memory. This function of the recurrent connections has been suggested by computational models (Durstewitz, Seamans, & Sejnowski, 2000; Rolls & Kesner, 2006) and experimental manipulations (Gilbert & Kesner, 2006; Lee & Kesner, 2002). In addition, there has been a debate on whether working memory can be divided into delay-dependent categories. When the dentate gyrus or CA3, but not CA1, are damaged, there is an impairment in working memory for short (10 seconds) delays, but damage to these two areas and to CA1 produces impairments in trials with long (five minutes) delays (Lee & Kesner, 2003a). Additionally, the prefrontal cortex may also be a critical structure in working memory, whereby this area may process this information in a parallel fashion but only become critical when compensatory mechanisms are needed in addition to spared hippocampal tissue (Laroche, Davis, & Jay, 2000; Lee & Kesner, 2003b).

With the unsettled debate of a functional dissociation for spatial memory along the dorso-ventral axis, the present study investigates whether the dorsal hippocampus is largely responsible for spatial learning and memory. Dissociations between reference and working memory is also tested on two different spatial tasks that report to use the same spatial cognitive processes but may engage different hippocampal structures depending on task-specific demands.

Method

Animals. Animals used for behavioral testing were 18 experimentally naïve, female Sprague-Dawley rats. At the start of the experiment, rats were 23-25 days old, weighing an average of 107.6 ± 6.7 g. At time of surgery, rats weighed an average of 255.1 ± 14.2 g. For Radial Arm Maze testing, rats were food deprived to no more than 85% their free-feeding body weight. Two extra weight control rats were used to monitor the natural developmental weight gain and 85% was calculated by averaging these two rats' weights. Water was available *ad libitum* throughout the experiment. Rats were housed in a 22-24°C room with 24-hour lighting. At various times, rats were transferred out and put into individual wire caging for weight control purposes.

After radial maze testing, they were tested on the Morris water maze, and food and water were available *ad libitum* throughout this part of the experiment. Housing conditions were the same as above.

Surgery. All surgical procedures adhered to and were accepted by the Animal Review Committee at Allegheny College. Before acquisition of the radial maze behavioral task (see below), rats were randomly assigned to one of three groups. Two of the groups received neurotoxic lesions of the dorsal hippocampus (dHPC) or dorsal CA3 subfield (dCA3) and the third served as controls. Rats were given free access to food and water for three days before their assigned surgery. After all surgeries, rats were given 10 days of post-operative recovery with full feeding and water for the first eight days and the last two were to ease their transition to their food deprivation weight. After post-operative recovery, rats were again group housed.

At surgery, each rat was anaesthetized with 0.1 cc/100 g (i.p.) of a mixture of ketamine/xylazine (70 mg/6 mg per cc) and placed in a Kopf stereotaxic device (David Kopf Instruments). The incisor bar was adjusted to 3.7 mm below the interaural line. The skull area above the injection sites was removed in total. Injections into the dorsal hippocampal region were made with a 25- μ l Hamilton microsyringe (Hamilton Company) with a 22-gage needle attached to the end of the microsyringe that was held with a microinjector connected to the stereotaxic frame.

Three sites per hemisphere received injections into either the dorsal hippocampus or the dorsal CA3 subfield depending on the experimental group. Bilateral dorsal hippocampal lesions were produced by intradentate injections of 0.60 μ l/site of fiber-sparing colchicine (2.5 mg/ml; Sigma-Aldrich, St. Louis, MO) dissolved in phosphate-buffered saline at a rate of 20.0 μ l/hr (Table 1). The needle was left in place for two minutes after infusion and slowly removed in a step-wise manner. Bilateral CA3 subfields were lesioned with 0.25 μ l/site of fiber-sparing ibotenic acid (6.0 mg/ml; Sigma-Aldrich, St. Louis, MO) dissolved in phosphate-buffered saline at a rate of 15.00 μ l/hr (Table 1). The needle was left in place for four minutes after infusion and slowly retracted in a step-wise manner.

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Table 1.

Stereotaxic Coordinates for Lesioning Injections

Site	AP	ML	DV
dHPC			
(colchicine)	- 3.4	$\pm$ 1.6	- 3.7
	- 4.0	$\pm$ 2.0	- 3.5
	- 4.6	$\pm$ 2.6	- 3.2
dCA3			
(ibotenic acid)	- 3.0	$\pm$ 3.2	- 2.9
	- 3.6	$\pm$ 4.0	- 2.9
	- 4.2	$\pm$ 4.2	- 3.2

Note: AP, anteroposterior; ML, mediolateral; DV, dorsoventral (from top of dura mater); all measurements in millimeters (Paxinos and Watson, 2005).

Behavioral Apparatus. A radial arm maze (Olton & Samuelson, 1976) was used for behavioral testing of pre-operative memory encoding, working memory, and memory retention. The maze was surrounded by distinct extramaze visual cues (door, cabinets, posters, experimenter, signs). It consisted of eight arms radiating outwards from an octagonal center with a diameter of 32.5 cm. Each arm was 52.5 cm in length, with a height of 15.5 cm, and a width of 13.5 cm. Within each arm, a food well 3.0 cm from the distal end of each arm (measuring to food well's radius), measuring 2.5 cm in diameter, and 1.3 cm deep were used to contain the reward (Nestle's Swirled Milk Chocolate and Peanut Butter pieces, 45 mg/piece). Each arm was made out of wood and painted white. Transparent plexiglass was used on the top of each arm to allow visualization of the extramaze cues. The RAM was elevated 76.0 cm off the ground.

A Morris Water Maze (Morris, 1984) was used for testing post-operative spatial reference and working memory in all groups. In a new room, the water maze was surrounded

by distinct extramaze visual cues (housing shelves, posters, experimenter, door, etc...). For the second testing of working memory, the rats were tested in the same maze and same room. However, a different set of distinct extramaze cues were designed. The water maze used is 94.0 cm in length and width with a height of 40.5 cm. It was filled to a depth of 33.0 cm, approximately 2.0 cm above the platform, with room temperature water. The platform was hidden by opaque, plastic balls with a diameter of 1.0 cm. The radial arm maze testing was performed first and then water maze testing (Figure 1A).

Radial Arm Maze

Habituation. Before behavioral testing, rats were handled for five minutes each for four days. Rats were habituated to the maze for five days. A pair of cagemates were placed in the maze with rewards scattered in all eight arms and in the octagonal platform (days one and two) and then only in the eight arms and the food wells (day three). Next, single rats were placed in the maze with the rewards only in the food wells of all eight arms (day four). On days five and six, rewards were only located in the rat's assigned four arms. Rats were allowed to traverse the maze for 10 minutes except on days five and six where rats were removed from the maze after all four rewards were eaten.

Pre-Surgical Training. The four-baited/four-unbaited protocol (Jarrard, 1983) of the radial maze was used. The use of this protocol allows for a within-subjects, within-task quantification of spatial reference memory and working memory failures. Spatial reference memory is defined as the long-term memory component needed to remember information that remains constant throughout the task (e.g. which arms are never baited).

Spatial working memory is defined as the information that is critical for short periods of time (within the trial) storage and use of that information (e.g. what arms have already been visited and should avoid for within that trial).

Four arms were baited and randomly assigned to each rat with no more than one pair of arms being adjacent (e.g. arms 1, 2, 4, 7). The remaining arms were left empty. Each trial began with the rat being set on the maze in one of four directions (north, south, east, west) that changed for every trial. Once the rat entered a baited arm, a wooden stopper was placed in front of the entry way for approximately four seconds. A reference memory error (RME) was marked when a rat entered a never-baited arm. If the rat subsequently returned to that same never-baited arm in the same trial, it was considered a spatial working memory 'incorrect' error (SWMIE). When the rat entered a baited arm (correct) and subsequently returned to that same arm within the same trial, it was considered a spatial working memory 'correct' error (SWMCE). Time to complete the trial was recorded. Termination of a trial occurred when the rat ate all four baits or until 10 minutes had elapsed. Rats were allowed to commit as many errors as it took to retrieve all four food rewards or until time had expired. Testing occurred seven days per week with one trial per day for as long as it took them to reach criterion of three consecutive days with zero errors.

Post-Surgical Testing. Once rats recovered from surgery, they were assigned the same four arms that they learned in the training phase and the same protocol was used. The same four dependent variables (time to complete trial, RME, SWMIE, SWMCE) were measured in addition to time to reach the first arm; this was necessary as to obviate the

explanation in terms of a sensorimotor deficit. A behavioral timeline of radial arm maze testing is shown in Figure 1A.

Water Maze

Habituation. Thirty days after finishing radial maze testing, the same groups (dHPC, dCA3, control) swam the water maze to quantify spatial reference and working memory. Habituation to the maze and water occurred for two days prior to any testing. These habituation trials consisted of placing the rat in the water and having a visible escape platform in a standard location. Animals were allowed to swim up to 60 seconds before being guided to and resting on the visible platform for 10 seconds. Rats had three trials of habituation per day.

Spatial Reference Memory Acquisition. For testing spatial reference memory acquisition, the platform was submerged and the position was kept constant each trial and day. Rats were placed into the pool (pseudo-random position per trial) with their face pointed towards the maze wall and were allowed to swim up to 60 seconds. If this time expired without finding the platform, they were guided to the platform and remained there for 10 seconds. There was an inter-trial interval (ITI) of two minutes. Sketching of the rats swimming the maze was done to later examine swim paths. Latency to find the platform was also recorded. This procedure was repeated for six trials per day for four consecutive days. By that time, all control rats were swimming to the platform in eight seconds, which was the target time.

Spatial Reference Memory Retrieval. Retrieval of the acquired spatial reference memory was tested in two retrieval tests seven and 16 days after the acquisition phase ended. Rats were placed in the pool towards the wall from a fixed location for two retrieval trials per test. The platform was in the same location as acquisition and rats had been previously released from that starting position during acquisition. On the second retrieval test, rats were again released from the same location and the platform was located in the same position. In essence, the first trial of each retrieval test acted as a reminder trial for the second trial, and the first retrieval test acted as another reminder for the second retrieval test.

Spatial Working Memory. Ten days of resting after the reference memory acquisition phase, rats' spatial working memory ability was tested. Before testing occurred, rats were placed on the platform for 10 seconds in order to know where to find the hidden platform. They then swam four trials per day for four days, with an ITI of 10 seconds. The working memory procedure consisted of placing the rat in the same starting location each trial. Rats always entered the water with their face towards the maze wall. The platform was in the same position each trial for the day but varied day to day. Sketching of the swim path was done to later look at swim path. In addition, latency to find the platform was recorded. Rats were allowed to swim up to 60 seconds, and if they did not find the platform in that time, they were guided to the platform and placed on it for two seconds. The same procedure was used every day except with varying platform location each day. Behavioral timeline of water maze testing is shown in Figure 1B.

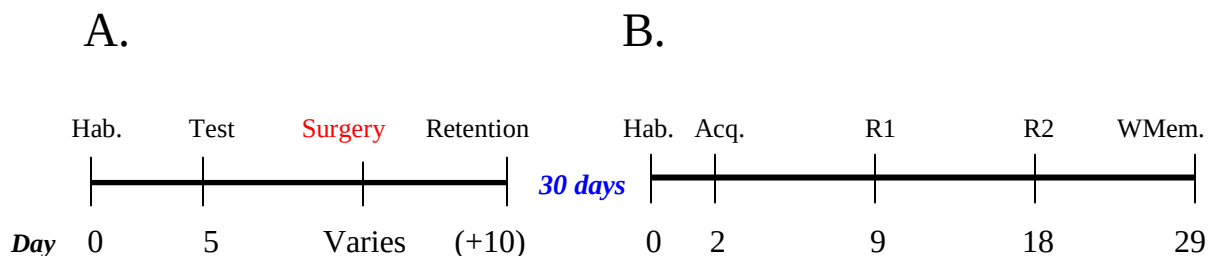


Figure 1. Behavioral and surgical timeline. **(A)** Timeline of four-baited/four-unbaited radial arm maze within-testing of reference and working memory. **(B)** Timeline of water maze reference acquisition, retrieval, and working memory tests. Note: Acq. = acquisition; Hab. = habituation; R1/2 = retrieval test 1/2; WMem. = working memory test.

Histology.

Rats were overdosed with euthasol (sodium pentobarbital and sodium phenytoin) intraperitoneally at 1 cc/kg and perfused transcardially with formol-saline (10% formaldehyde). After brain extraction, brains were post-fixed for at least 24 hours in formol-saline. Coronal sections of 50 μ m through the entire hippocampus using a CO₂ cryostat were taken and every fourth section mounted. Tissue was dried at room temperature and Nissl stained with buffered thionin.

Data Analysis.

All behavioral data analysis took place after histological confirmation of selective lesions. Upon examining the slices, caution was taken to make sure the fimbria-fornix and/or subiculum were not damaged. When either area was severely damaged, the data from these rats was not analyzed. Behavioral data were analyzed by running an analysis of variance

(ANOVA). A repeated measures ANOVA was performed for Block/Day x Lesion for all dependent variables listed for the radial maze testing and for water maze acquisition. A one-way ANOVA of Retrieval Test x Lesion was run for water maze retrieval testing. All post-hoc analyses were run using the Sidak Correction. Data in results section are expressed as means $\pm$ standard deviations. For the water maze testing, qualitative sketches of the rats' swim paths were averaged together by lesion group. Adobe Photoshop was used to average and draw swim paths and stitch coronal hippocampal images together.

Results

Histology

Rats were only accepted for behavioral analysis if they received selective bilateral lesions of the intended area of the hippocampus. There was minimal extra-hippocampal damage in any of the brain slices (Figures 2). In both cases of the fimbria-fornix and subicular complex, damage to these structures was minimal. Two control rats were perfused and their brains displayed no damage (Figure 2a, 3a) ($n = 5$). In addition to these animals, one control rat died prematurely.

In the case of rats with dCA3 lesions that were made with ibotenic acid (Figures 2b, 3b), as is common in long survival times after surgery, the boundary between lesioned and healthy tissue was clear. Area CA2 in the dorsal half was considered part of the CA3 region and there was thinning of the CA4 regions but cell bodies were still present (Figure 3b). In some cases, there was sparing of the dorsal CA3 region in the most rostral (Figure 2b, top) and caudal part (Figure 2b, bottom) of the hippocampus. Two rats were removed from

behavioral analysis because there appeared to be very minimal, if any, damage to the dorsal CA3 region and the other tissue was unilaterally damaged ($n = 4$).

For the dHPC lesions with colchicine (Figures 2c, 3c), there was a visible boundary between the healthy and lesioned tissue. It was fairly common in this group to have visible needle tract marks on the cerebrum. In some cases, there was minimal damage to the dorsal hippocampus in the most caudal sections (Figure 2c, bottom). The tissue displayed drastic re-organizational attempts by the spared cell bodies, typically in the most rostral sections, signifying the spread of the neurotoxin. Two rat brains were eliminated in the dHPC group because of damage that extended beyond the septal half of the hippocampus into the temporal part, and, in one case, lesioned the entire hippocampus with damage to the fimbria-fornix and dorsal subiculum ($n = 4$).

The maximal and minimal damage was represented on brain schematics that extended from nearly the most rostral to caudal areas of the hippocampus (Figure 2). Computer-stitched brain slices were taken to display typical damage to each group under 40x magnification (Figure 3).

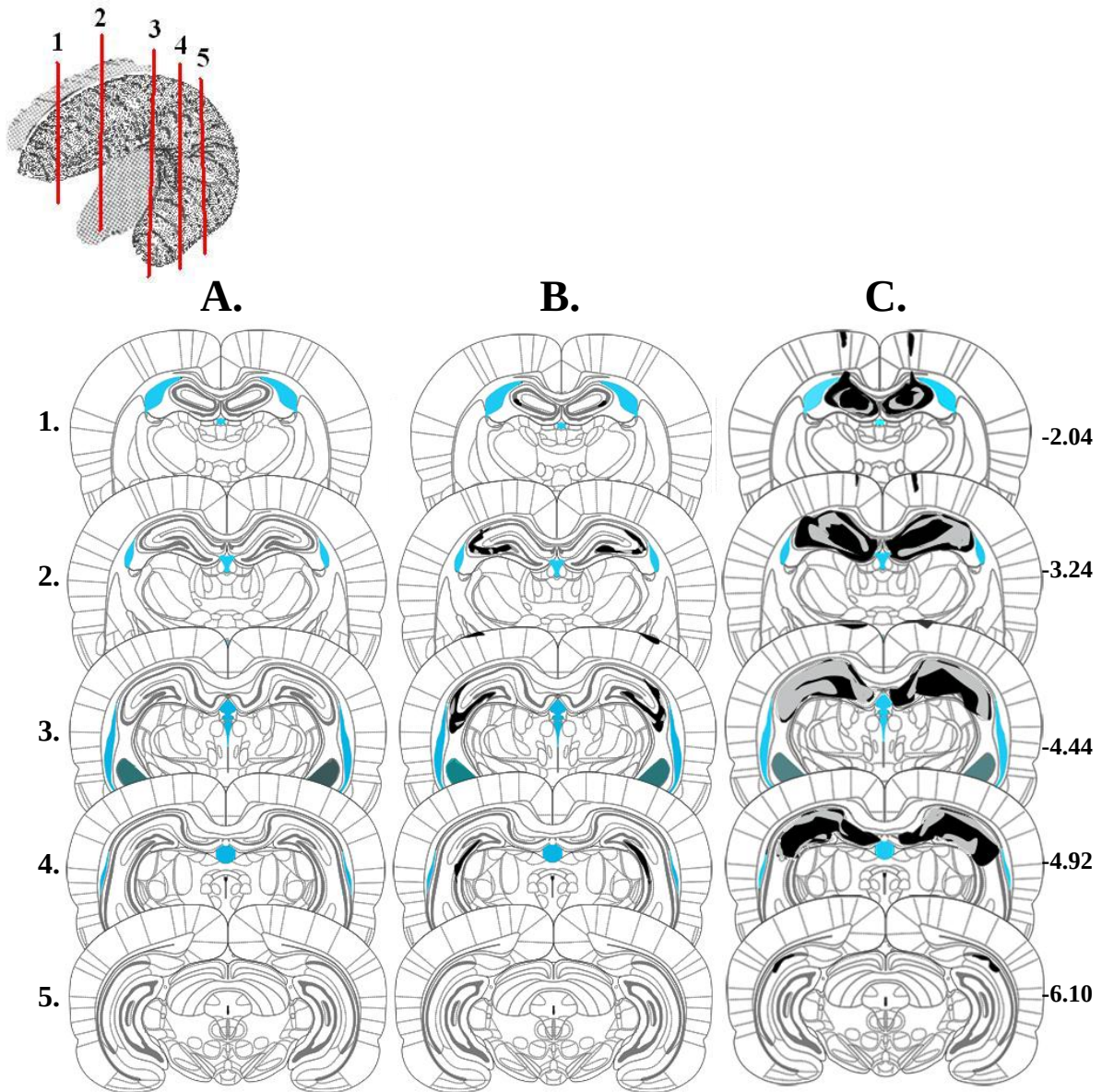


Figure 2. Brain schematics that represent maximal (black + grey) and minimal (grey) damage to the intended area; light blue are ventricles. **(A)** Control animals ($n = 5$) sustained no damage, **(B)** dCA3 group ($n = 4$) sustained damage to dorsal CA2 and CA3 regions with slight damage to CA1, CA4, or dentate gyrus, and **(C)** dHPC lesions ($n = 4$) destroyed the septal half with minimal damage to the fimbria. Top picture is a dissected hippocampus displaying location of coronal sections corresponding to numbered rows of actual schematics. Brain schematics are from Paxinos & Watson (2004) and measurements on right of schematics are in millimeters posterior from bregma.

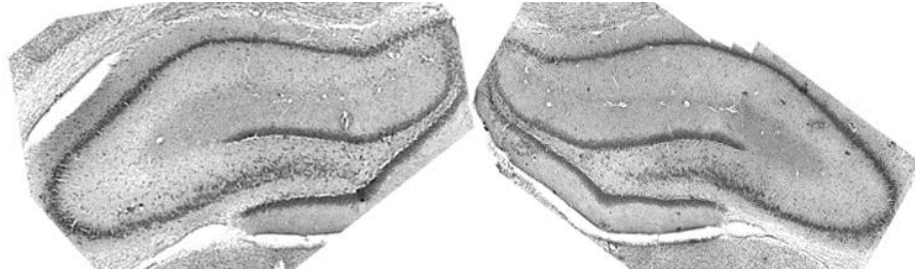
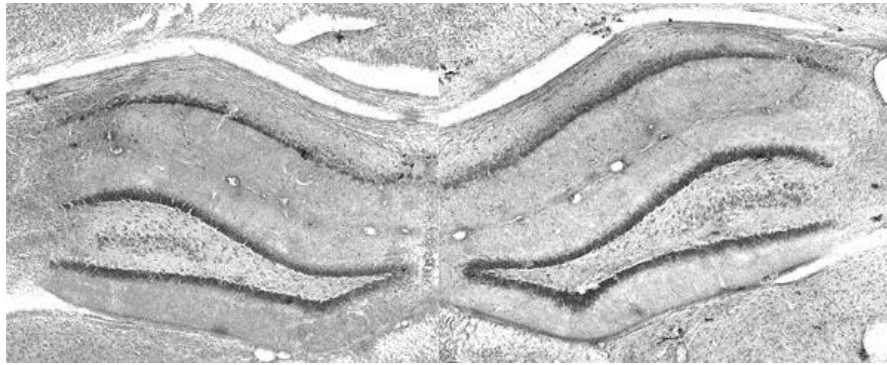
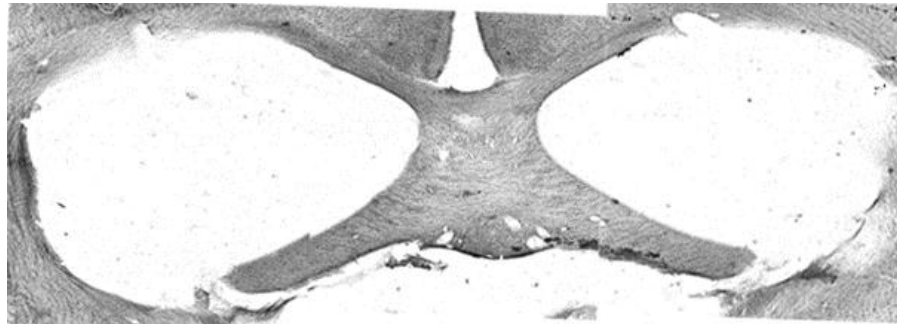
A.**B.****C.**

Figure 3. Computer-stitched, representative coronal sections through the dorsal hippocampus. **(A)** There was no damage done to control ($n = 5$) brains. **(B)** The dorsal CA3 lesion ($n = 4$) made by ibotenic acid was selective to these pyramidal neurons; damage frequently included dorsal area CA2. **(C)** Lesions made with colchicine damaged the dorsal hippocampus ($n = 4$), showing negligible damage to adjacent tissue.

Radial Arm Maze: Pre-surgical Training

Data. The data that are graphed and had analyses run on them were from the last 25 days of training. Data prior to the last 25 days were higher than the ones that had statistical analyses run on them.

Habituation. At the end of the habituation trials, all rats were moving around freely, retrieving food rewards, and showing no signs of anxiety.

Trial Time. As pre-surgical training progressed, there was a reduction in trial time ($M = 61.08 \pm 11.35$ s) (Figure 4A). This was supported by the fact that there was a significant main effect of block, $F(4, 52) = 18.322$, $p < 0.001$, $\eta^2 = 0.36$. Post-hocs revealed significant progressions between every block except the first two and the last two blocks. There was no difference between intended lesion groups, $F(2, 13) = 1.993$, $p > 0.05$, $\eta^2 = 0.08$, or an interaction, $F(8, 52) = 1.474$, $p > 0.05$, $\eta^2 = 0.06$.

RME. Learning of the maze task was evidenced by the fact that total number of reference memory errors decreased as training went on ($M = 0.89 \pm 1.06$), suggesting that rats learned to avoid the never-baited arms. There was a main effect of block, $F(4, 40) = 5.365$, $p = 0.001$, $\eta^2 = 0.17$. Post-hocs showed a significant difference between block two and five (Figure 4B). There was no difference between the lesion groups, $F(2, 10) = 1.335$, $p > 0.05$, $\eta^2 = 0.06$, or a block by lesion interaction, indicated by $F < 1.000$.

SWMCE. There was a general decrease in the number of spatial working memory 'correct' errors made by the rats ($M = 0.57 \pm 3.56$), signifying that they were learning not to return to the previously baited arm (Figure 4C). ANOVA revealed a main effect of block, $F(4, 52) = 6.323$, $p < 0.001$, $\eta^2 = 0.20$. As shown by post-hocs, the first two blocks were significantly different from the last. There was no effect of lesion group, $F(2, 13) = 1.424$, $p > 0.05$, $\eta^2 = 0.06$ and no significant interaction between lesion and block, $F < 1.000$.

SWMIE. The number of spatial working memory 'incorrect' errors committed decreased as training continued ($M = 0.05 \pm 0.25$), suggesting that rats learned to avoid re-entering never-baited arms. There was a large effect of block, $F(4, 52) = 16.021$, $p < 0.001$, $\eta^2 = 0.45$, where the first two blocks were statistically different from the last two blocks and the third from the fifth (Figure 4D). There was no main effect of intended lesion group, $F(2, 13) = 1.384$, $p > 0.05$, $\eta^2 = 0.03$, supporting the fact that all rats were making equal strides in learning abilities. There was no interaction between block by lesion, $F < 1.000$.

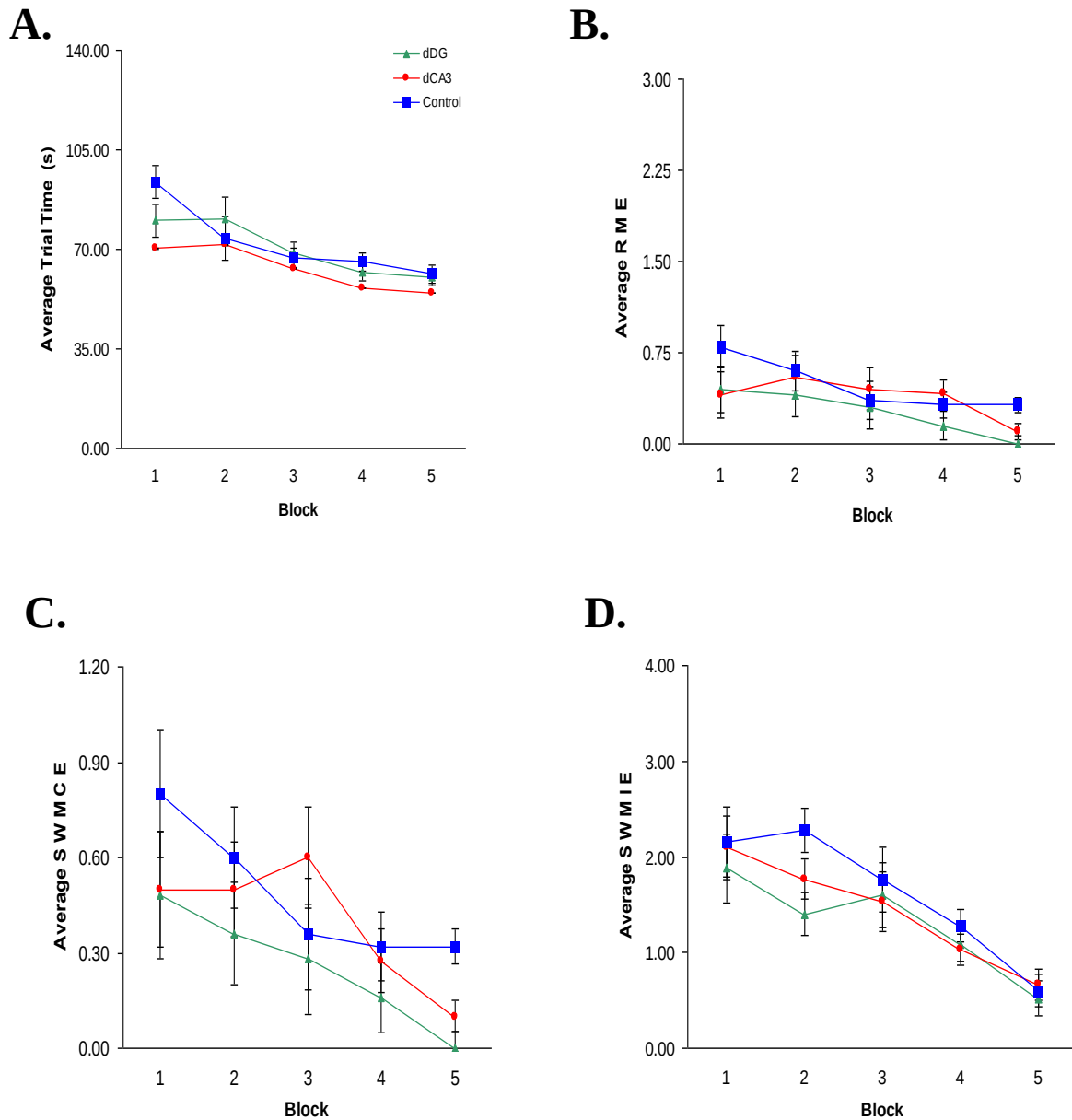


Figure 4. Pre-surgical testing on the radial arm maze. **(A)** As training went on, there are reduced trial times, **(B)** reduction in the number of committed reference memory errors, **(C)** reduced spatial working memory 'correct' errors, and **(D)** reduction in the number of spatial working memory 'incorrect' errors committed as the behavioral testing progressed over four blocks of 5 trials per block. First and last block were significantly different in all graphs, showing proper learning of the maze ($p < 0.05$). There was no difference between any of the lesion groups in any graphs. Data graphed are from the last 25 days of training and are expressed for each lesion as mean $\pm$ SEM.

Radial Arm Maze: Post-surgical Testing

Trial Time. Post-operative times to complete a trial ($M = 55.9 \pm 19.75$ s) were comparable to those in the pre-surgical condition. Rats continued to decrease their trial time as testing progressed (Figure 5A). There was a main effect of block, $F(3, 30) = 9.081$, $p < 0.001$, $\eta^2 = 0.31$, and post-hocs revealed that block one was different from blocks three and four. There was no main effect of lesion, indicated by $F < 1.000$ and also no interaction between the two variables, $F(6, 30) = 2.215$, $p > 0.05$, $\eta^2 = 0.15$, suggesting that lesion had no effect on trial time.

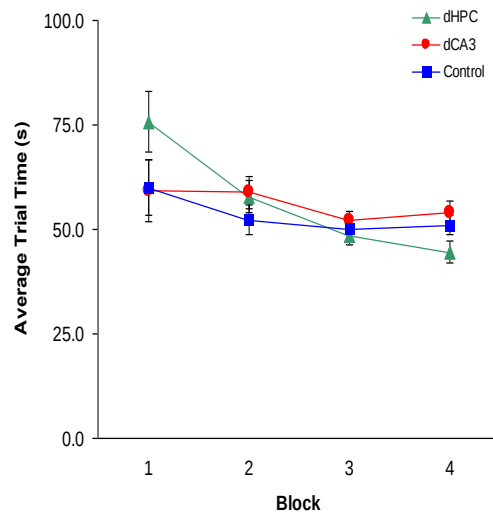
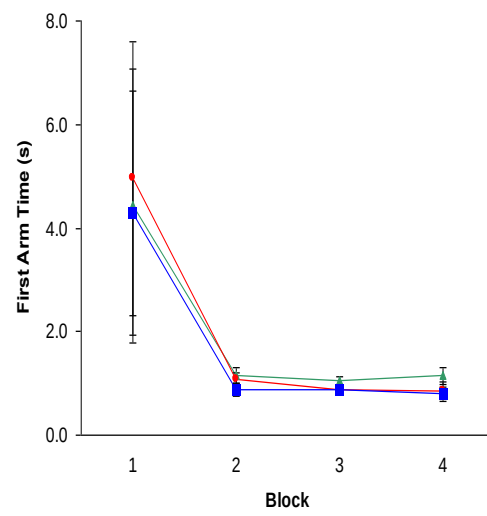
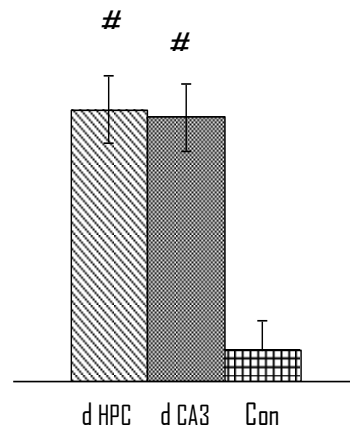
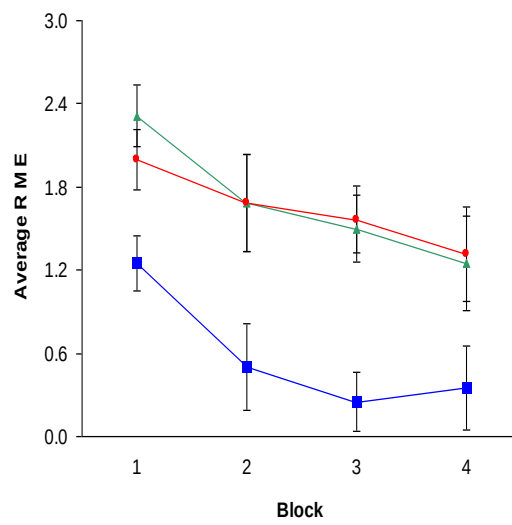
First Arm Time. All groups decreased the time it took them to make their first choice ($M = 1.81 \pm 5.58$ s) (Figure 5B). This was necessary to eliminate the possibility of a sensori-motor impairment. ANOVA revealed a main effect of block, $F(3, 30) = 6.045$, $p = 0.002$, $\eta^2 = 0.31$, where the first was statistically different from the third and fourth. It was apparent that all groups, including controls, took much longer in the first block as compared to the rest. There was no effect of lesion, $F < 1.000$, eliminating the possibility of a sensori-motor deficit. There was no interaction between lesion and block, $F < 1.000$.

RME. Rats decrease the number of reference memory errors committed as testing progressed, where there was a significant decrease in blocks three and four from the first block, $F(3, 30) = 8.893$, $p < 0.001$, $\eta^2 = 0.42$, with a large effect (Figure 5C, graph). In addition, there was a significant main effect of lesion (Figure 5C, histogram), where both dHPC ($M = 1.69 \pm 0.21$) and dCA3 ($M = 1.64 \pm 0.21$) were significantly impaired as

compared to controls ($M = 0.59 \pm 0.18$), $F(2, 10) = 10.021$, $p = 0.004$, $\eta^2 = 0.4167$, with a large effect. There was no such interaction between lesion and block, $F < 1.000$.

SWMCE. Rats' SWMCE were varied by lesion group. ANOVA revealed that there was a main effect of lesion group, $F(2, 10) = 12.282$, $p = 0.002$, $\eta^2 = 0.29$, where both dHPC ($M = 0.69 \pm 0.14$) and dCA3 ($M = 1.05 \pm 0.14$) groups were impaired overall compared to controls ($M = 0.11 \pm 0.13$) (Figure 5D, histogram). There was no main effect of block overall, $F < 1.000$. However, as testing progressed, an interaction between the variables emerged, $F(6, 30) = 3.105$, $p = 0.017$, $\eta^2 = 0.22$, where the dHPC group returned to the level of controls and dCA3 group worsened their performance significantly by block four and differed from control and dHPC groups by the end of testing (Figure 5D, graph).

SWMIE. There was no change in spatial working memory 'incorrect' errors as post-operative testing progressed (Figure 5E) by block, $F(3, 30) = 2.542$, $p > 0.05$, $\eta^2 = 0.08$. In addition, there was no difference between the three lesion groups ($M = 0.15 \pm 0.53$), $F(2, 13) = 1.384$, $p > 0.05$, $\eta^2 = 0.19$, and no interaction between the variables, indicated by $F(6, 30) = 1.053$, $p > 0.05$, $\eta^2 = 0.07$.

A.

B.

C.


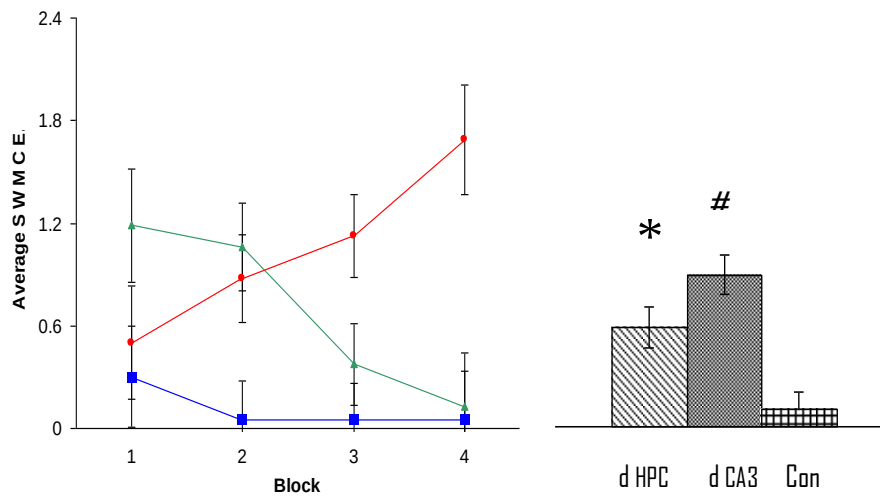
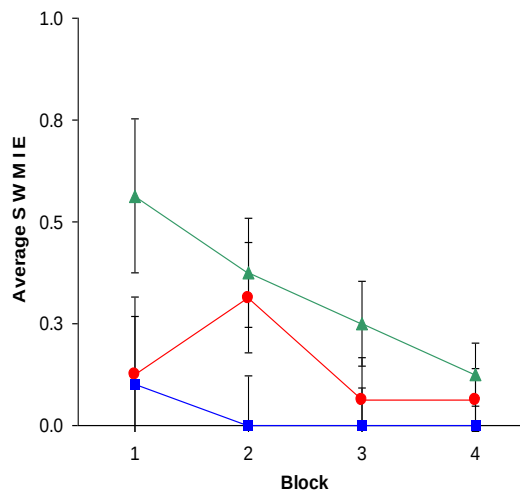
D.**E.**

Figure 5. Post-surgical retention graphs representing times and errors. **(A)** The average time to complete a trial on the radial maze per lesion group was not different for any group. **(B)** Average time to make their first choice of arm to enter did not differ between lesion groups. **(C)** Less reference memory errors were committed by blocks three and four for dCA3 and dHPC groups. Histogram in (C) shows that dHPC and dCA3 were impaired equally. **(D)** Spatial working memory 'correct' errors for dCA3 group worsened over time while dHPC group improved to the level of the controls. The histogram in (D) shows that dHPC and dCA3 were significantly impaired on spatial working memory 'correct' errors overall when compared to controls. **(E)** There was no difference between lesion groups or block for spatial working memory 'incorrect' errors. Data are expressed as mean $\pm$ SEM. Testing took place over 16 days, where each block represents four trials. Note: * $p < 0.05$; # $p < 0.01$ as compared to controls (Con).

Morris Water Maze: New Learning, Retrieval, and Working Memory

Habituation. At the end of the habituation, the rats were swimming around without showing significant anxiety-like behaviors, such as floating, defecation, or constantly swimming against the wall. In addition, upon setting rats in the pool, they appeared to not be afraid of going in the water, indicated by repeatedly trying to climb up the sleeve of the experimenter and attempting to jump out of the pool.

Reference Memory Acquisition. In the reference memory acquisition phase, the platform stayed constant throughout the whole phase, only varying starting points. A Mixed ANOVA with day as a repeated measure reported that all groups acquired the task to the same degree, showing no main effect of lesion, $F(2, 9) = 1.011$, $p = 0.402$, $\eta^2 = 0.04$. There was a significant main effect of day, $F(3, 27) = 27.854$, $p < 0.001$, $\eta^2 = 0.57$, showing a large effect, where post-hocs showed that rats were significantly faster on days three and four as compared to both the first and second day (Figure 6A). There was no interaction between lesion and day, $F < 1.000$. As evidenced by the average of all swim paths to the platform, in their respective lesion groups (Figure 7, row A), there was no real difference in length of path taken.

Reference Memory Retrieval. The platform was in the same location as the acquisition phase for the first and second retrieval trials. For the first and second retrieval trials (Figure 6B), seven and 16 days after acquisition, respectively, the same general finding appeared where the dHPC was the only group impaired. On the first retrieval test, there was a significant main effect of lesion group, $F(2, 14) = 7.354$, $p = 0.008$, $\eta^2 = 0.55$, with a large effect, as shown by ANOVA with retrieval test as a repeated measure. Post-hocs revealed

that dHPC ($M = 26.29 \pm 21.17$) group was significantly impaired compared to control ($M = 8.25 \pm 3.85$) and dCA3 ($M = 11.92 \pm 6.56$) groups. On the second retrieval test, there was a main effect of lesion, $F(2, 11) = 5.046$, $p = 0.34$, $\eta^2 = 0.53$, with a large effect, where the dHPC group ($M = 32.31 \pm 23.89$) was still impaired as compared to control ($M = 9.61 \pm 3.24$) and dCA3 group ($M = 10.50 \pm 5.57$)f. When both retrieval tests were compared directly against the last day of acquisition, ANOVA revealed an overall main effect of lesion, $F(2, 9) = 22.781$, $p < 0.001$, $\eta^2 = 0.46$, with a large effect (Figure 6B, histogram). However, the last day of acquisition and retrieval days did not differ, $F < 1.000$, and there was not an interaction between lesion and test type, $F < 1.000$, showing that dHPC was the only group impaired on both retrieval tests. In terms of length of swim path during the first retrieval test, the dHPC group showed a longer swim path to the platform, whereas controls and dCA3 swam about the same length (Figure 7, row B). The dHPC group also had a longer swim path in the second retrieval test as compared to controls and dCA3, whereas the latter two did not differ (Figure 7, row C). Qualitatively from the swim paths of the retrieval trials (Figure 7, row B and C) and from the retrieval test graphs (Figure 6B), dHPC group appears to forget more readily as compared to dCA3 and control, where the forgetting curve from the first to the second retrieval test appears to be steeper for the dHPC group than either for the control or dCA3.

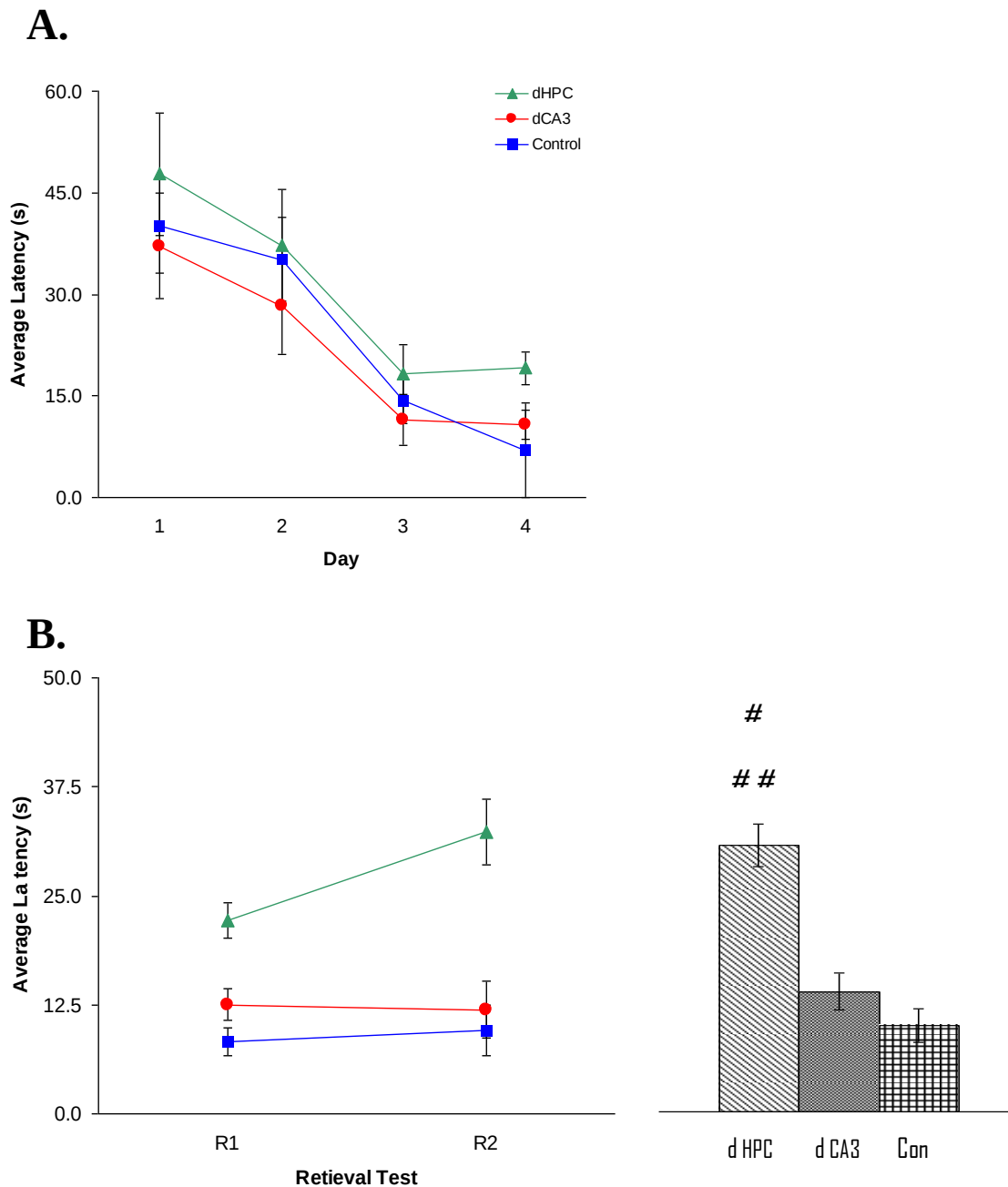


Figure 6. Reference memory acquisition and retrieval trials. **(A)** During reference memory acquisition, all group acquired the task equally within the same amount of time. **(B)** Seven and 16 days after the acquisition, all groups swam two retrieval trials. The dHPC was significantly impaired in both retrieval tests. The histogram in (B) shows that the dHPC group was significantly impaired compared to the control and dCA3 group. Data are expressed as mean $\pm$ SEM. Note: # $p < 0.01$; # # $p < 0.001$ as compared to dCA3 and controls (Con), respectively. Each rat swam two trials per retrieval test. Note: R1 = first retrieval test; R2 = second retrieval test.

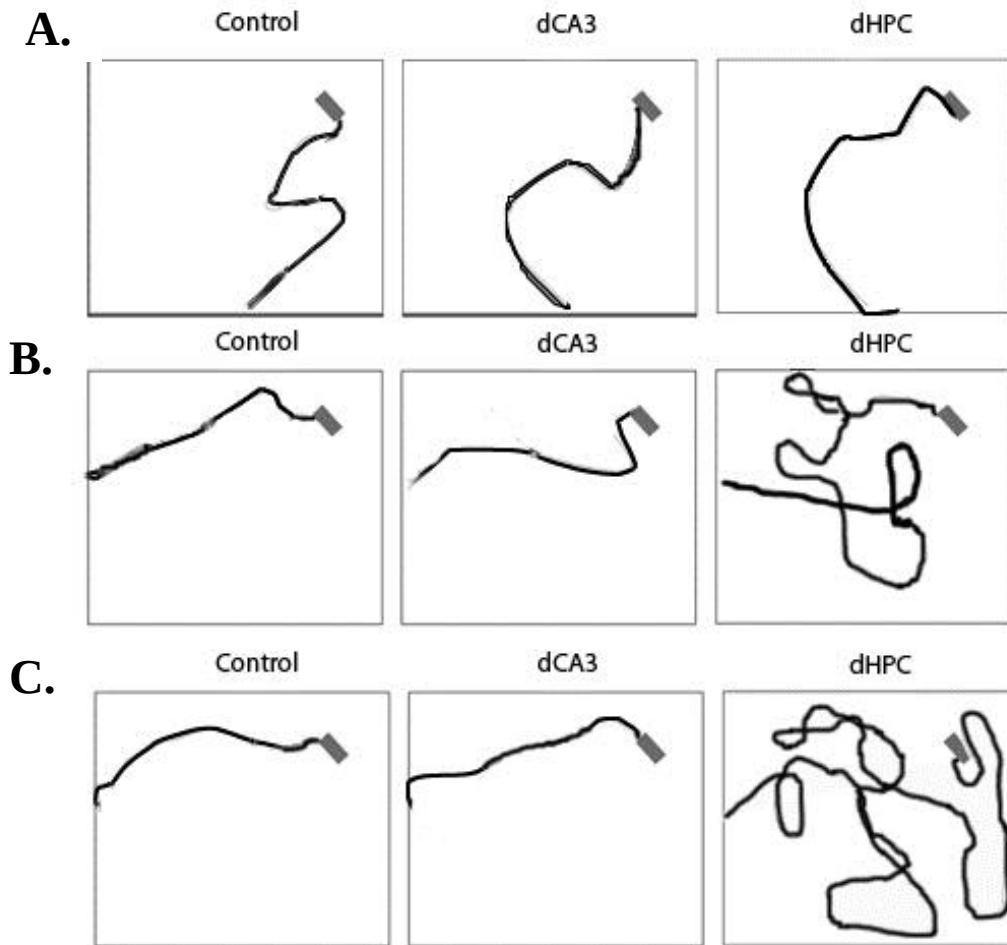


Figure 7. Representative sketched swim paths for the reference memory acquisition and retrieval phases. (Row **A**) All groups acquired the reference memory portion of the water maze without difficulty. (Row **B**) On retrieval test one, seven days after acquisition, the dHPC group was impaired as compared to control and dCA3 rats. (Row **C**) On retrieval test two, 16 days after acquisition, dHPC rats were still impaired relative to control and dCA3 rats.

Working Memory. In the working memory phase after acquisition and retrieval, the platform stayed constant between same-day trials but differed between days. Mixed ANOVA with day as a repeated measure revealed a main effect of day, $F(3, 27) = 18.654$, $p < 0.001$, $\eta^2 = 0.40$, with a large effect, such that there was a difference between the first day and all other days (Figure 8). There was also an effect of lesion, $F(2, 9) = 8.789$, $p = 0.008$, $\eta^2 = 0.25$, where the dHPC group ($M = 25.96 \pm 20.57$) was impaired as compared to controls ($M = 11.26 \pm 13.1$) but not different from dCA3 ($M = 16.57 \pm 14.48$), with dCA3 being at control level (Figure 8, histogram). There was no interaction between the two variables of lesion by day, $F < 1.000$. Qualitatively, starting with the first day testing of working memory (Figure 9, row A), the swim path of the dHPC was longer than as compared to controls and dCA3. In subsequent two days (Figure 9, rows B and C), dHPC group was still impaired, but all groups showed a slight decrease in swim path. By the fourth day of testing, the controls and dCA3 rats showed a decrease in path length; however, the dHPC rats appeared to increase the length of their path (Figure 9, D), though this effect in latency was not significant, as established by ANOVA.

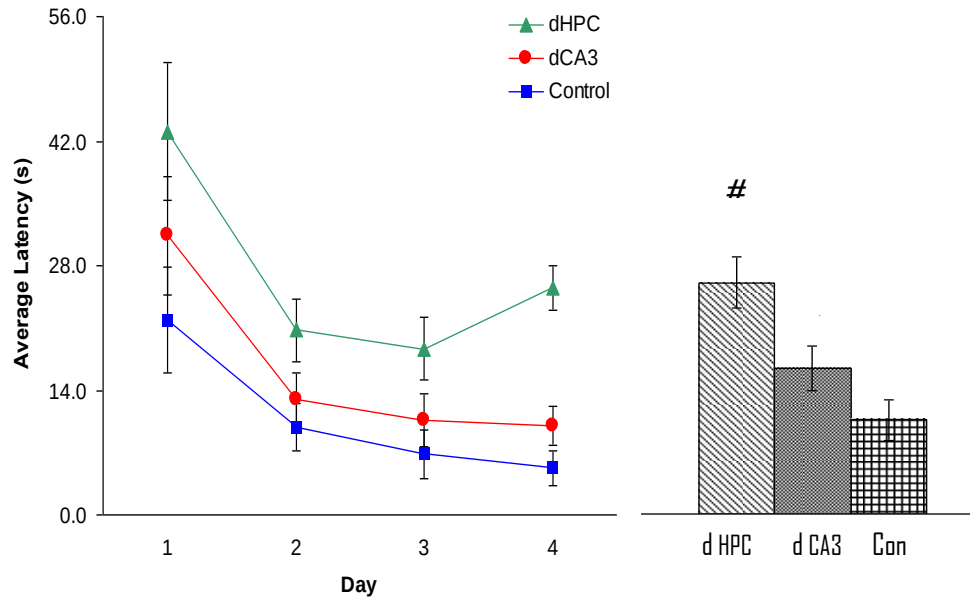


Figure 8. Rats working memory abilities. The graph shows that rats tended to decrease the escape latency to the platform and that the dHPC group was impaired as compared to controls in their abilities (histogram). Rats swam four trials per day for four days. Data are expressed as mean $\pm$ SEM. Note: # $p < 0.01$ as compared to controls (Con).

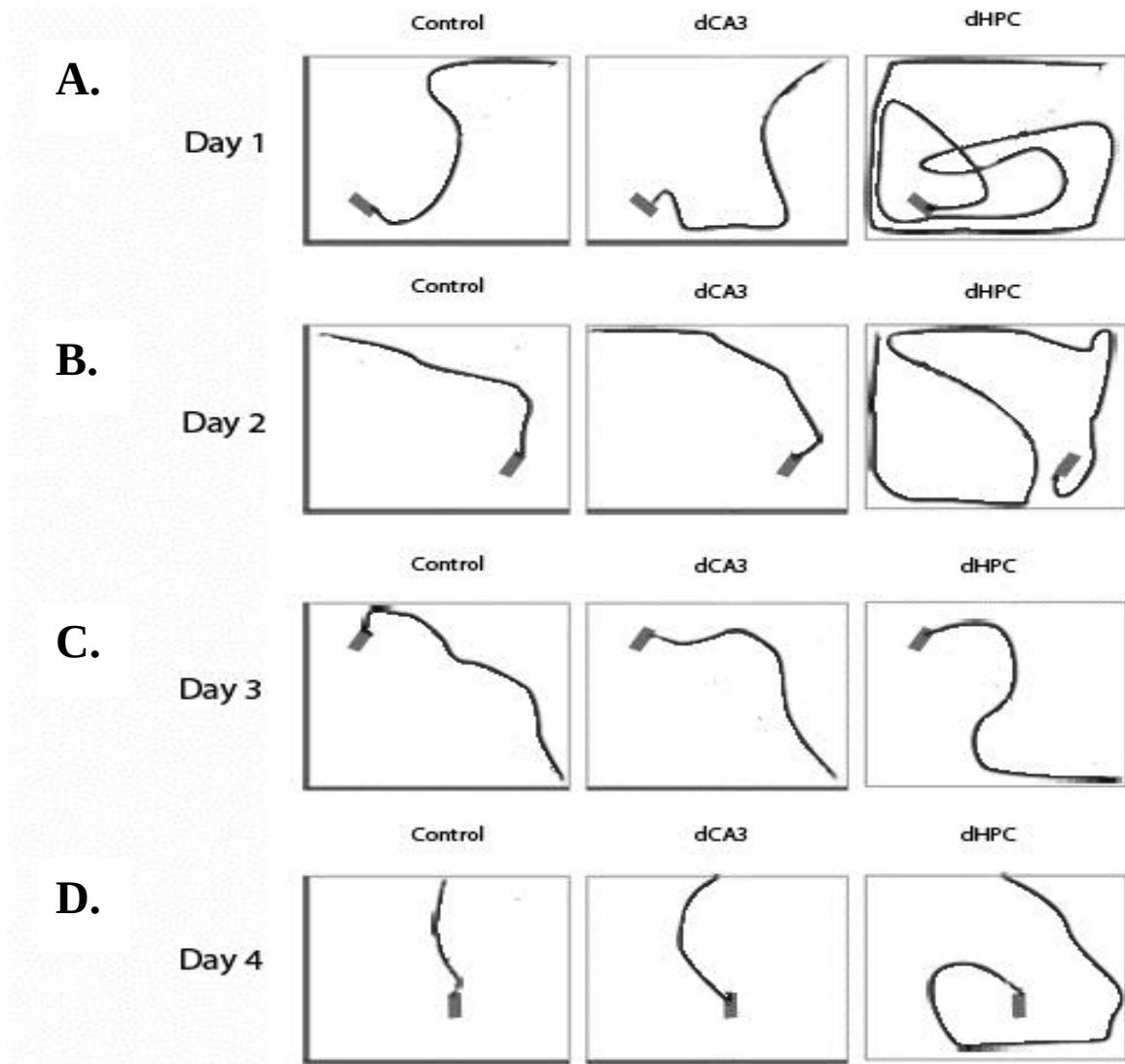


Figure 9. Swim paths taken during the working memory portion of water maze testing. (Row **A**) The dHPC group took a longer path as compared to both control and dCA3 on the first day. (Rows **B**, **C**) On the subsequent two days, all groups tended to slightly decrease the length of the swim path taken, thereby decreasing the escape latency to the platform. (Row **D**) On the fourth day, dCA3 and controls rats swam directly to the platform, but the dHPC group seemed to take a longer path as compared to their path on the third day.

Discussion

The present study found three main results. First, spatial memory can be acquired with only ventral hippocampal tissue available, as demonstrated by the reference memory acquisition phase. Second, retrieval of spatial memory depends upon at least the dorsal hippocampus, evidenced by water and radial maze retrieval phases of reference memory. Third, the dorsal hippocampus contains a critical component for proper working memory abilities and working memory circuitry appears to shift depending on task-specific demands.

Spatial Acquisition

The fact that dorsal hippocampal lesioned rats could learn a new spatial task was unexpected, given the proposed functional differentiation along the septo-temporal axis. This was evidenced by the fact that days three and four of water maze acquisition were statistically different from the first two days. This finding eliminates the possibility that rats were solely relying on their working memory abilities day-to-day, but, rather, the memory was successfully encoded. This has been shown before when ventral or dorsal lesioned rats could learn a spatial task comparable to that of controls but complete lesions to the hippocampus resulted in a significant deficit (Zhang et al, 2004). These results from previous studies suggest that normal spatial encoding is done through a distributed network. When muscimol is infused into the dorsal pole of the hippocampus, rats are shown to be able to learn a new water maze spatial reference memory task and another set of rats are able to learn the same task when they have lesions to the ventral pole of the hippocampus (Moser & Moser, 1998). The data from Moser and Moser (1998) and the current data from this study

showing dorsal hippocampus-lesioned rats can learn implies that acquisition of spatial memory can be encoded in a localized manner with available tissue only. A more extreme example of localization comes from studies that show that rats can acquire the water maze reference memory task with a 'minislab' ($\leq 25\%$) of dorsal tissue (Moser, Moser, Andersen, 1993; Moser et al., 1995).

In contrast, Pothuizen and colleagues (2004) found that the ventral portion plays no role in spatial learning and the dorsal hippocampus is solely responsible. They have shown that dorsal, but not ventral, hippocampal neurotoxic lesions impair acquisition on a radial arm maze using the four-baited/four-unbaited protocol and with an exact training regimen as this study (one trial per day, every day). This group only tested the rats' acquisition for 25 days on the radial maze. Compared to the pre-surgical acquisition of this study's radial maze results, it took normal rats on average 40 days to acquire the task to a sufficient degree to which there was not a question as to whether a 'memory trace' was established. Upon examination of the Pothuizen data (Figure 3C of their data), control rats' reference memory errors were approximately 1.5 errors at the end of their 25-day testing phase, where, in this study, it took 33 days to reach an equal level of reference memory errors, which is not a large discrepancy. The author of the present study is confident that a memory was established because of the strict criterion, where Pothuizen and colleagues had no set 'memory criterion', even for its control group, leaving open the possibility the rats may have not properly or not as strongly acquired the task, and this results in their lesion groups' data being called in to question. Moreover, their dHPC group data shows that significance was only reached by the last two blocks (5 trials per block), and not the first three blocks, as compared to controls. This is only showing that dHPC group did not reduce their RME as quickly as controls, but

they did start to show a trend towards reduction by the last block. A study by Ramos (2001) shows that rats with dorsal hippocampal lesions can acquire a place response task in the plus maze but they only learn it slower. Therefore, more thorough testing of the Pothuizen's rats, like the testing done in this study, should be done to conclude that the dHPC group is truly impaired on acquisition.

Other commonly cited studies that suggest there is a clear dissociation among the dorsal and ventral portion in spatial encoding are studies of lidocaine injections, which temporarily inactivates certain tissue, as to see if later functional restoration of the tissue results in recovery of mnemonic processes. When lidocaine is injected into ventral CA1 and ventral subiculum, rats are impaired on a spatially-cued radial maze task with and without delays (Floresco, Seamans, & Phillips, 1997). The problem with temporary inactivation is that the tissue is still available in the physical sense and these injections of lidocaine have been shown to spread through the fibers to other areas (Levy et al., 2001), thereby suggesting that in all actuality, rats may not be having selective, temporary tissue inactivation as these studies claim.

As for the rats with dorsal CA3 lesions that are unimpaired on acquisition of reference memory in this study, these results are similar to studies that have found an impairment in synaptic plasticity, either through NR1-subunit knockout mice selective to area CA3 (Nakazawa et al., 2002) or injections of CREB antisense into dorsal CA3 (Florian, Mons, & Roullet, 2006) can still acquire a reference memory task in the water maze and can successfully retrieve the information. In general, these results suggest that dCA3 may not be involved in acquisition or that it is not involved to a critical degree in which compensatory mechanisms cannot take over, like entire ventral or dorsal hippocampal lesions.

In addition to ventral engagement, adult neurogenesis may play a critical role in cognitive processes, especially in this study. Proliferation and maturation of new neurons increases at younger ages. It has been shown that there is a 93.5% difference in proliferation and successful maturation of newborn neurons in the dentate gyrus between juvenile, 35-day old, and middle-aged, 12-month old, Sprague-Dawley rats (McDonalds & Wojtowicz, 2005). Because rats tested in this study were only approximately 30-days old when spatial training began, it is quite possible that these new neurons were more readily recruited for spatial learning. When activation and integration of new-born neurons into existing circuitry is examined in 10-week old rats after water maze training, there is a significant increase in *c-fos* activation of new neurons in the ventral hippocampus, showing that young neurons do integrate and participate in spatial learning (Snyder, Radik, Wojtowicz, & Cameron, in press). This suggests a need to look closely at age of rats' spatial training, seeing how the ventral portion may be more important when rats are trained at younger ages.

Reference Memory

In pre-operatively trained rats that all acquired the task to the same high degree, dCA3- and dHPC-lesioned rats were both impaired on the reference memory retention over the surgical period. These results are consistent with other findings that dorsal lesions made after training impairs retention in spatial tasks (Clark et al., 2005b; Vago, Bevan, & Kesner, 2007). When an AMPA GluR1-5 antagonist was injected into the dorsal hippocampus for inactivation, Riedel and colleagues (1999) found that these lesions caused an impairment in retention and trace consolidation of the spatial memory, presumably reflecting the inability for subsequent LTP needed for consolidation. Furthermore, rats pre-trained on a reference

memory task in the water maze and given lesions to either the dorsal or ventral hippocampus show a striking impairment at both retention intervals of two days and six weeks (Martin et al., 2005), further supporting the fact that retention/retrieval depends on a distributed network throughout the hippocampus that depends on both the dorsal and ventral poles.

There seems to be a more specific role for the dorsal CA3, however. In this study, the dCA3 group was as impaired on reference memory retention as well as the dHPC group. Apparently, the dorsal CA3 may be the critical component in the retention process and not the entire dorsal hippocampus for pre-operatively trained rats. As for post-operative acquisition, in contextual fear conditioning where the rats learn a context of where they would receive a foot shock, lesions of the dCA3 impair learning of the task but not the retrieval of the contextual memory (Lee & Kesner, 2004). The inability of the dorsal CA3-lesioned rats to learn, while learning occurs in this study, may reflect specific processes inherent to contextual fear conditioning rather than overall learning of spatial memory. However, retrieval of information from post-operatively lesioned animals that is context-dependent, such as spatial memories or the case of this fear conditioning paradigm, is presumably coming from the same source in the dorsal hippocampus. The likely component in dorsal CA3 retrieval lies in its distinctive recurrent collateral system. Because this system is gone after lesions, it is likely that an attractor network cannot be established, thereby leaving the memory to be retrieved in fragments rather than a coherent whole (see 'Working Memory' below for more details).

In contrast, new learning in the water maze was acquired by all groups but only the dHPC showed impairments on both retrieval days. The dCA3 group was not impaired on retrieval of post-surgically acquired information but impaired on the retention of the pre-

surgically acquired information on the radial maze. This dissociation reflects differences between pre- and post-operatively acquired information and the amount to which this information was consolidated. The newly learned information was likely in a more labile state and failure of the dHPC group to retrieve this reflects that a possible interruption in the consolidation process itself. However, if post-surgically acquired information relies more heavily on the hippocampus because it was not consolidated out into the cortex (see Shimamura, 2002), then dorsal hippocampal lesions will impair the context needed to successfully retrieve the highly context-dependent spatial memories. In contrast, because the dCA3 was not impaired and only a fraction of the total dorsal hippocampus was gone, information was still able to acquire the context needed for successful retrieval either because dCA3 is not involved in post-surgically acquired information or sufficient tissue was available for compensatory mechanisms to act.

To be certain as to which is more responsible, future research will need to investigate different dorsal subregions to see which area(s) are critical and see if the dorsal hippocampus is homogenous or heterogeneous. Further research needs to be done concerning pre- versus post-operatively learned information, which is not explicitly studied, and further research will need to include study of the consolidation process in these two stages, letting the same high degree of consolidation to occur, whatever that time interval may be, eliminating a consolidation argument. Additionally, one cannot expect the same processes to occur in the same way when a lesion comes before or after learning. Of course the rats are going to use different tissue, or at least a different neural network; this may require additional cognitive resources to be used for the additional spatial acquisition after surgery.

One could, however, make a processing argument for the results found here. Previous work has found a subregion dissociations within the dorsal hippocampus. It is thought that the dorsal dentate gyrus is for pattern separation, whereby information about incoming stimuli is not all merged together but rather the orthogonal relationships between the patterns are kept constant (Gilbert, Kesner, & DeCoteau, 1998; Gilbert, Kesner & Lee, 2001). The dorsal CA3, on the other hand, receives this separated information and is responsible for calculating the metric relationships between the objects and the parietal cortex provides information about the topological relationships between the objects and the goal (Goodrich-Hunsaker, Hunsaker, & Kesner, 2008).

The impairment in both groups does not reflect a pattern separation deficit because in later testing in the water maze, if pattern separation were really impaired, they would not have been able to acquire the information in the first place and dCA3 group would not have been able to successfully swim the retrieval trials (this also rules out pattern completion). The deficit could be a metric representation problem. Metric relations between objects is more critical when testing on the radial maze than it is in the water maze, because radial maze is more conducive to using non-spatial cues, such as subtle intra-maze cues, where water maze is almost exclusively forcing the rat to use extramaze cues for allocentric memory. In the water maze, rats have an open arena to swim to the relative, familiar area using spatial cues. However, on the radial maze, rats are confined to this small octagonal center facing as many as four different arms at a single time and have a more limited view of environmental cues. Rats are able to discriminate these arms and environmental cues adequately but are not able to judge the metric distance between the limited cues, thereby

more likely confusing the distances between the arms and distance between the environment and having a harder time integrating relationships between the arms and the cues.

If metric relationships were compromised in the radial maze, why would they not be compromised in the water maze? This is a difficult question but the water maze is a more open environment to the rat and allows the rat to move around more. The only objective here is to find a platform by swimming around and using spatial cues. This more open arena allows for relying on familiarity-based memory more readily. In the radial maze, the rats were forced to make a decision to enter an arm that was directly adjacent to two other arms, thereby limiting their familiarity-based memory and taxing their reference, metric, and topographical relationship memory more.

Working Memory

Spatial working memory deficits were expected for the dCA3 group and, ultimately the dHPC group, which include this area. This was expected because the CA3 contains the recurrent collateral system which serves to cycle the new information throughout the fibers. One may expect this system to be overloaded as time continues, with it cycling more environmental and mental representations as time continues within a trial. However, this system acts as an attractor network, whereby an input can activate the rest of the network at once and then keep information reverberating, thus keeping the working memory 'active' (Rolls & Kesner, 2006) and not overload the system by cueing up associations made earlier. If this cycling is damaged, even partially, as done by dorsal CA3 lesions, a cue from the cycling information cannot properly cue up the working memory, resulting in deficits in spatial working memory. In addition to the CA3 subregion, it has also been shown that there

are neurons in the prefrontal cortex (PFC) that continually fire during a delay, thereby representing working memory in this area, too (see Fuster, 2001). As such, these two systems work in concert to hold on to visuo-spatial information.

In the post-operative radial maze retention test, the dCA3 tended to increase the number of working memory errors while the dHPC group returned to the level of controls by the end of testing (Figure 5D). The working memory deficit that lessened in the dHPC rats may be due to compensatory mechanisms, where a critical level of tissue needs to be damaged for these mechanisms to start operating. After all re-organization attempts made by the dorsal hippocampus have been exhausted and turn out to be fruitless, recruitment or compensation by other brain areas may ensue. Kesner, Hunt, Williams, and Long (1996) and Lee and Kesner (2003b) found that rats with dorsal hippocampal lesions produced an initial deficit in working memory but improved over time, which are similar to the results found here. The same deficit-improvement result was also seen with medial PFC lesions in the Lee & Kesner study, following that the medial PFC and dorsal hippocampus process this spatial information at shorter delays in parallel, either competitively or redundantly, acting as compensatory networks for each other. Here, when working memory was observed for dCA3-lesioned rats in this same phase, essentially the opposite result (normal to deficient working memory) was seen (Figure 5D). This may be due to the fact that there was not enough tissue damaged to warrant compensation by the PFC this early after surgery. Additionally, this gradual impairment may be due to the fact that this occurred 10 days after surgery, possibly suggesting that gliosis and/or surrounding tissue re-organization occurred and these factors may have encroached on the spared hippocampal networks. This invasion of tissue may have interfered with compensatory mechanisms within the dorsal hippocampal

tissue itself that may have taken over this mnemonic function. Such tissue that was spared in this area was the dorsal CA1 and dentate gyrus and the encroachment would have interfered with proper neural transmission. However, after complete re-organization of the tissue of these spared areas, they may have picked up this mnemonic function and compensated for the dCA3 later on in the water maze.

Working memory testing in the water maze resulted in the dHPC group being impaired, with the dCA3 rats being at control level. This is curious because working memory on the radial maze showed the exact opposite result at the end of testing. One explanation is that the ITI delay between picking arms in the radial maze was the amount of time the rat took to eat the reward, which was at most five seconds. The delay in the water maze working memory was 10 seconds. This increase in five seconds may be enough time to have working memory abilities switch to a different subregion within the (dorsal) hippocampus or extra-hippocampal areas. It has been shown that the CA1 region does become more active in working memory when the delay becomes longer than a few minutes. When rats are tested on a short (10 seconds) or an intermediate (5 minutes) delay on a spatial task, dorsal CA3-lesioned rats are impaired at the 10-second delay but not the dorsal CA1 group. In contrast, when tested at a 5-minute delay, the dorsal CA1 group is impaired, suggesting that this area becomes more critical to working memory at longer delays (Lee & Kesner, 2003a). The crucial point is, the dorsal CA1 region is also able to handle working memory along with the dorsal CA3. Because our rats were impaired after surgery, this may have enlisted the dorsal CA1 region, along with possibly the prefrontal cortex, which was suggested above, to compensate for the lost dorsal CA3, resulting in spared working memory in the water maze even though there was not a long delay.

Because the dHPC-lesioned group was not impaired, this demands another interpretation, such as task demands and reinforcement that may dictate working memory abilities. The radial maze working memory is a task which ends in a food reward for a correct choice. Failure to make a correct choice results in no immediate threat, and thereby may allow the rat to develop an alternative spatial or a non-spatial strategy. The water maze is a negative task in the sense that rats escape a negative stimulus (water). Failure to find a platform in water does result in an immediate life-threatening situation, resulting in fast acquisition of the task through pure allocentric cues (see Hodges, 1996). Lee and Kesner (2003b) used a delayed nonmatching-to-place task in the radial maze with a delay of 10 seconds and found that lesions to either the dorsal CA3 and dentate gyrus impaired rats' working memory performance. In the water maze task of this study, we used a delayed matching-to-place protocol with a 10-second delay and found no such impairment with the dCA3 group. This is probably a direct result of inherent factors in the tasks. In all, the dHPC is important in short-term working memory tasks and different pathways for working memory information may be engaged depending on the type of task employed.

In the water maze protocol used here, which includes more motor responses and requires more physical activity and a different strategy on the rat's behalf, it may be the case that the dorsal hippocampus communicates with the medial caudate nucleus in this case. Electrophysiological studies have shown that there exist connections between these two regions. Neurons in the medial caudate nucleus and dorsal hippocampus show location- and reward-specific firing patterns and correlated firing in these areas is affected by strategy, visual spatial, and reward context change (Mizumori, Yeshenko, Gill, & Davis, 2004; Yeshenko & Mizumori, 2007). In addition, when rats were trained on a delayed matching-

to-sample, which is similar to the protocol use for water maze testing, for direction task on the radial maze, the rats learned the task to asymptotic performance. Upon lesioning the medial caudate, these rats showed a striking impairment upon retest but were able to learn a new direction discrimination task, which suggests that the caudate is a critical structure involved in direction learning (DeCoteau, Hoang, Huff, Stone, & Kesner, 2004). With regard to the prefrontal cortex and working memory, it has also been shown that there are direct projections from the ventral hippocampus to the medial prefrontal cortex (Ishikawa & Nakamura, 2006). It is believed that the dorsal hippocampus and medial prefrontal cortex process spatial working memory information possibly in parallel. Lee and Kesner (2003) showed that these two areas can compensate for the lost function in the other area using temporary inaction techniques.

Taking these different spatial working memory pathways into consideration of the recent results found in the present study, one can see different 'weighted pathways' emerging in spatial working memory depending on task. Because the water maze probably engages the medial caudate to a higher degree than the prefrontal cortex, it is expected that the dHPC would be impaired on retrieval in the water maze because the total dorsal hippocampus is lesioned and thus has no connection with the caudate, leaving working memory in the water maze impossible. However, because the radial maze preferentially engages the prefrontal areas instead of the caudate (less of a motor response required), the medial prefrontal cortex is able to compensate for the lesioned dorsal hippocampus because the connections exist between the ventral hippocampus, which was spared, and the medial prefrontal cortex, and this compensation engagement was observable by the gradual improvement in working memory on the radial maze by dHPC rats. There was a deficit, however, with the dCA3 rats

on the radial maze but not in the water maze. In light of weighted pathways explanation, it is possible that there was not enough tissue damaged for the prefrontal cortex to compensate on the radial maze portion of testing. However, because the water maze engages the caudate to a higher degree, there was still enough tissue left in the dorsal hippocampus for direct connections between the medial caudate and the dorsal hippocampus to still exist, thus allowing working memory to communicate with both areas.

Conclusion

These results suggest that, when a spatial memory task is learned pre-operatively with an intact hippocampus, the memory is encoded in a distributed manner throughout the hippocampus. When the hippocampus is damaged, however, information is able to be learned in a more localized fashion with only the tissue available. Retrieval, on the other hand, is not possible with a partial hippocampus, because the retrieval process relies on the dorsal hippocampus or, more likely, needs the entire hippocampus for successful retrieval, as previous studies have shown. Additionally, spared tissue is able to compensate for not only reference memory (such as during encoding), but also working memory, suggestive of the dissociation between working memory in the radial and water maze for the dCA3 group and weighted pathways for working memory between the dorsal hippocampus, prefrontal cortex, and the caudate nucleus.

In general, the main point is that the hippocampal area is a dynamic and highly plastic structure that connects with multiple brain areas in order to subserve the same basic mnemonic processes, such as working memory. This structure has a long axis that connects the septo-temporal pole areas, horizontal connections that wire dorsal with dorsal and ventral

with ventral subregions, large recurrent collateral systems in the dentate gyrus and CA3 that may act as attractor networks, and continuous replenishment of adult neurons that are highly proliferative in younger rats and enhanced by learning and memory. This study has done its best to demonstrate the basic neural underpinnings of working and reference memory, but there are a plethora of questions that need to be addressed before any clear picture of the hippocampus reveals itself. Future research questions that need to be considered include, (1) how is working memory affected by delays and task demands, (2) what are the structural changes that can occur to allow for compensation of specific functions, (3) what effects on memory consolidation does pre-training serve as compared to training after lesioning, and (4) how big of an impact does age matter, especially with the discovery of adult neurogenesis, when we are trying to uncover the neural correlates of spatial memory. Once these questions have a clearer answer, we can then begin to read the literature on spatial memory in a new light and begin to ask more probing questions that start to directly target spatial memory in isolation of other cognitive and behavioral processes.

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