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Research in the Classroom: Preserved Object Discrimination in the Morris Water Maze Following Lesions of the Fornix in Rats

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This project was part of a semester-long laboratory research course for undergraduates. The scientific content of this paper was prepared by students, and is presented to illustrate the potential outcomes of this model laboratory course.

Damage to the hippocampus usually results in a temporally-graded retrograde amnesia, suggesting that memories initially dependent on the hippocampus are ultimately consolidated in the neocortex. However, recent studies have found that remote spatial memories in the Morris Water Maze (MWM) are *not* preserved following hippocampal damage. In order to gain a better understanding of the role the hippocampus in retrograde memory, we trained rats with fornix lesions to discriminate between two beacons hung above the maze, one of which indicated the platform location. In probe trials we found that sham and fornix-lesioned rats both spent significantly more time in the quadrant with the correct beacon than the quadrant with the foil beacon and the two quadrants without any cues ($p<0.05$) and neither group spent significantly more time in the foil quadrant than the quadrants without cues ($p>0.05$). Our results indicate that rats with damage to the fornix are able to learn a simple object discrimination in the MWM, and can associate proximal cues with escape in the MWM. This supports Clark et al.'s (2007) interpretation of their finding that retrograde memory deficits in the MWM may be due to the inability of rats with hippocampal lesions to update their position (navigate) rather than to a deficit in remembering the platform location *per se.*

Keywords: *Object discrimination, water maze, memory retrograde amnesia, hippocampus*

Model Laboratory Course

Engaging undergraduate students in real research has been a goal of many colleges and universities, and is typified by the senior or honors research projects that are a part of many science curricula. However, we have proposed that this exposure to research can also happen in the classroom, and that this approach has distinct advantages for both students and faculty that outweigh the additional costs in time and resources that preparing such a laboratory would entail (Hauptman & Curtis, 2009; Yates, et al., 2006). First, students are exposed to desirable curriculum outcomes (Wiertelak, 2003) including 1) introducing students to experimental methodology, design, and data analysis, 2) advanced awareness of a particular field within neuroscience, 3) critical and independent thought, 4) effective communication skills, and 5) ethics (see Table 1 for a summary of how the course fulfills these goals). Second, faculty benefit from the opportunity to conduct research central to their own research program. While this may not often result in publishable data, the experiments could serve as pilot data that can later be pursued by other students or classes. As importantly, these research students have already been trained and are experienced in the basic techniques necessary to conduct independent research.

This project was carried out as a laboratory class exercise for the Laboratory in Behavioral Neuroscience: Learning and Memory at Bowdoin College, a small, residential liberal arts college with about 1600 students overall, and between 16-20

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Table 1: Desired Course Goals (Wieterlak, 2003) and Associated Classroom Activities

Goal	Classroom Activity
1) Introducing students to experimental methodology, design, and data analysis	Classroom discussions of design and analysis Perform experiment, including surgery, behavioral testing, perfusions, and histology and data analysis
2) Advanced awareness of a particular field within neuroscience	Overview lectures Journal Club discussion of relevant scientific articles
3) Critical and independent thought	Journal Club presentations Discussions data collection and analysis Preparation of Introduction exam and final journal-style article
4) Effective communication skills	Online posts (written) Journal Club presentations and other discussions (oral) Discussion of reading and writing scientific articles Introduction exam (written) Final journal-style article (written)
5) Ethics	Formal training in rules and regulations regarding animal care and use Classroom discussion of ethics of animal use in science

Neuroscience majors each year. This 14-week model laboratory course has been described previously (Yates, et al., 2006), including a complete syllabus. Briefly, students are engaged in a single, real scientific endeavor from conception of the experiment through "publication." In fact, the first two authors generated the scientific content of this manuscript as their final writing project for the semester. This course is often a student's first laboratory experience, since the prerequisites include only introductory Psychology, introductory Neuroscience (Physiological Psychology), and a course in statistical analysis (co-requisite. Twelve advanced undergraduate students (predominantly Juniors and Seniors) conducted this research project. This course has now been taught 8 times by a laboratory instructor (NC) and professor (SR).

Course activities and goals are summarized in Table 1. In the first weeks of the course, students are introduced to the theoretical background of the project and procedures, discuss and receive training on the ethics of animals in research, and importantly discuss the process by which the experiment was designed. The professor and laboratory instructor design the project during the summer before the course so that the project is pertinent to the professor's research program, so that they can obtain approval from the college's IACUC, and so that the entirety of the project can fit within the 14 class weeks. However, the students are encouraged to think carefully about the project, and we often make adjustments to the experimental design based on student discussion.

Students are then divided into laboratory pairs or triads. The students work in these groups outside of class time to do surgeries, histology, and behavioral testing (approximately 3-5 hours per week). Two or three animals are assigned to each group. Class time is then devoted primarily to demonstrations of procedures, discussion of data and testing issues (with each student contributing to the discussion), and journal clubs to discuss the primary literature most relevant to the project. Each laboratory pair/triad presents the major findings from assigned journal articles thereby adding a public speaking component to the course. Finally, as a final project, students are expected to prepare their results in journal format "as if for publication." Issues of pedagogy are further considered in the Discussion.

The following scientific content was written by two students (NS and JS) from their final papers in the course, and is presented to illustrate the potential outcomes of such a model laboratory.

Introduction to the Scientific Content

Damage to the hippocampus in human patients produces both an anterograde amnesia and a temporally-graded retrograde amnesia (TGRA). In TGRA, memories learned just before brain damage are lost, while memories learned remotely are spared (Kapur & Brooks, 1999; Manns, Hopkins, Reed, Kitchener, & Squire, 2003; Squire & Bayley, 2007). This pattern of memory deficit is important because it suggests that while the hippocampus is initially important for the acquisition and storage of memories, memories are ultimately consolidated elsewhere in the brain, presumably in the neocortical areas that initially processed the to-be-remembered information (Alvarez & Squire, 1994; Squire, Haist,

& Shimamura, 1989; Zola-Morgan & Squire, 1990). Prospective studies in animals have generally supported this model (for a review, see Squire, Clark, & Knowlton, 2001). One notable exception to this pattern of results comes from studies of spatial retrograde memory that use the Morris Water Maze. In this task and its analogs, rats with hippocampal damage show no temporal gradient in their retrograde memory loss (Bolhuis, Stewart, & Forrest, 1994; Clark, Broadbent, & Squire, 2005; Clark, et al., 2007; Hollup, Kjelstrup, Hoff, Moser, & Moser, 2001; Martin, Hoz, & Morris, 2005; Mumby, Astur, Weisend, & Sutherland, 1999; Sutherland et al., 2001) that is, both recent and remote memories are impaired following hippocampal damage.

Two possible explanations have been developed to explain these results (Clark, et al., 2007): 1) the hippocampus is in fact the permanent storage place for spatial memories, or 2) remote spatial memory may be spared, but the ability to express the preserved memory might be impaired due to the role the hippocampus in tasks that require the subject to navigate through space using association between stimuli. By this second view, poor performance would be due to a navigational performance deficit rather than a memory deficit *per se*.

A series of studies suggest that the retrograde deficits following hippocampal lesions seen in the water maze can be explained by the rats' inability to express preserved memories. First, using an incremental training procedure (Whishaw, Cassel, & Jarrard, 1995) demonstrated that memory for the platform location ("knowing where") could be dissociated from spatial navigation ("getting there") in rats with fornix lesions. Second, a more recent study attempted to remove the navigational component of the water maze task by using proximal beacons to help rats locate the hidden platform (Clark, et al., 2007). In this retrograde memory task, four identical beacons were hung over each of the water maze quadrants, one beacon always hung over the platform. Thus, rats could use distal cues to identify which beacon indicated the location of the platform, and use that beacon to guide navigation to the hidden platform. However, they found that not only did rats with hippocampal lesions search indiscriminately in the four quadrants of the pool, the rats did not appear to use the beacons to help them locate the platform. While their results lead Clark et al. to the conclusion that rats with hippocampal lesions have more than a spatial memory deficit, one interpretation is that the rats with hippocampal lesions are not able to use the beacons to guide their performance.

Because this study was conducted as part of a laboratory exercise, we elected to use fornix lesions as a proxy for hippocampal damage (see *Pedagogical Issues* in Discussion). The fornix is the primary subcortical connection of the hippocampus, and fornix lesions have been used extensively to disrupt hippocampal function (e.g., Wible, Shiber & Olton, 1992; Whishaw, Cassel, & Jarrard, 1995; Ferbinteanu & McDonald, 2001), possibly due to disruption of hippocampal theta EEG (Hasselmo, Bodelon &Wible, 1992). Studies directly comparing lesion techniques have at most found modest differences in the performance of animals with hippocampal or fornix damage (e.g., Ferbinteanu & McDonald, 2001; Sziklas, Lebel & Petrides, 1998; Sziklas & Petrides, 2002), and then only in tasks that test complex conditional associations.

To determine whether rats with fornix damage can in fact use beacons to locate a hidden platform, we developed a version of the water maze task in which rats needed to discriminate between distinctive beacons in order to locate a hidden platform. This version of the task should not require spatial memory retention or spatial navigation. Instead, the task only requires simple object discrimination and line-of-sight navigation. Consistent with the findings that object discrimination in the rat relies primarily on the dorsal striatum (Broadbent, Squire, & Clark, 2007) and a line-of-sight navigation should be independent of the hippocampus (Morris, Garrud, Rawlins, & O'Keefe, 1982; Save & Poucet, 2000), we found that sham rats and rats with fornix lesions performed the discrimination task equally well, demonstrating that rats with fornix lesions can discriminate between and use beacons as cues to locate a hidden platform in the water maze.

Method

Subjects

Subjects were 15 male Long-Evans rats (Charles River Laboratories, Raleigh, NC) weighing between 200-250g at the beginning of the study. Rats were pair-housed in standard laboratory caging and kept on a 14:10h light:dark cycle. Food and water were available *ad libitum*. Rats were pseudo-randomly assigned to sham (SH; n=7) and fornix lesion (FX; n=8) groups. All procedures were approved by Bowdoin College Research Oversight Committee and were conducted by twelve undergraduate students in the Laboratory in Behavioral Neuroscience: Learning & Memory

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course during the fall semester of 2007 at Bowdoin College (Brunswick, ME).

Surgery

Anesthesia was maintained throughout the surgery using isoflurane (1-3% in O_2 at 1 L/min). Rats were given 0.1ml atropine intramuscularly and 0.01 ml/100g body weight Butorphanol subcutaneously (Torbugesic® [10mg/ml] Fort Dodge Animal Health, Fort Dodge, IA). FX rats were placed into a stereotaxic headholder (Kopf Instruments, Tujunga, CA), and on top of a heating pad to maintain body temperature during surgery. A midline incision was made and the skull was leveled along the bregma-lambda axis. Two craniotomies were drilled over the coordinates of the fornix lesions. Custom electrodes (Model UEK, FHC Bowdoin, ME) were lowered into the fornix (AP: 1.3, ML: ±1.5, DV: 3.6 from pial surface) and held in place for 60s before passing a 1.4mA DC current for 40s using a digital stimulus generator (Model 6bp, FHC, Bowdoin, ME). Electrodes were kept in place for an additional 60s before removal and the craniotomies packed with gel-foam , the wound was closed with staples and a topical antibiotic applied to the wound. After surgery, rats were given 5ml lactated ringer solution subcutanously and acetaminophen was introduced into their drinking water for one week. SH rats underwent procedures identical to the FX rats except that electrodes were not lowered into the brain and no current was passed. All rats were given one to two weeks to recover before the start of behavioral testing.

Apparatus

Rats were tested in a circular pool 180cm in diameter with 20cm of opaque water (nontoxic latex paint) maintained at 18˚C. A 20cm diameter clear Plexiglas platform was used. In addition to distinct distal visual cues in the room, two proximal beacons were hung 22cm above the water surface for discrimination and probe trials. The beacons were spheres 20cm in diameter and visually distinct, one painted completely beige and the other striped black and white horizontally. Rats were always placed in the water maze facing the pool wall. Swim paths were tracked using a digital video camera and the WaterMaze data acquisition program (Actimetrics, Wilmette, IL). Testing occurred 5 days/week.

Shaping

Rats were shaped to a visual platform (clear Plexiglas platform covered with blue material 1cm above water) 3 trials/day for 5 days. The platform had a fixed location (N) while start positions were variable (NE, SE, SW, NW). Rats were given 60s to find the platform and allowed to stay on it for 15s. Rats that could not locate the platform within 60s were still placed on it for 15s.

Discrimination

For discrimination learning, the platform was lowered 1.5cm below the water surface and had a variable location. The invisible platform was always under the beige beacon. Both the beige beacon (and platform) and the striped foil beacon were pseudo-randomly assigned to the centers of either the N, E, S, or W quadrant for each trial. Rats were pseudo-randomly released from NE, SE, SW or NW and rats were given 3 trials/day for 20 days. Probe trials were given on the third trial of every third day of testing as well as the last day of testing. During probe trials, both beacons were present, but the platform was removed from the pool and rats' swim patterns were tracked for 60s before the rats were removed from the pool.

Histology

Following behavioral testing, rats were administered a lethal dose of sodium pentobarbital (0.7ml) and perfused transcardially with saline 0.9% NaCl solution and then 10% buffered formalin. Fixed brains were stored in 30% glucose/PBS solution at 4˚C until sectioning. Coronal sections (50µm) were made using a cryostat and every fourth section was mounted on gelatin-coated slides and stained with a 0.25% thionin stain to visualize the extent of lesions.

Results

Histology

The histological sections from two rats in the FX group were lost due to an error during staining. However, the data from these two rats were included in the behavioral analysis below, as they did not change the outcome of the statistical tests performed. As a group, the SH rats sustained no damage to the fornix, and only minor cortical damage associated with craniotomies (Figure 1A), The extent of the cortical damage was similar to the cortical damaged observed in the FX group (Figure 1B). This damage was primarily confined to primary motor

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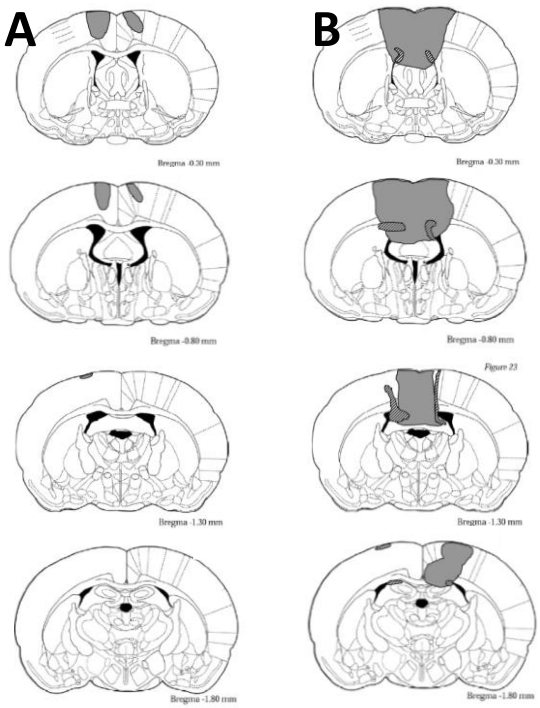

Figure 1: Coronal sections showing largest (gray) and smallest (striped) areas of damage to SH (A) and FX (B) brains. Sections progress from anterior (top) to posterior (bottom) and numbers represent the distance posterior to bregma in millimeters. SH rats did not have any fornix damage and minimal cortical damage (fornix damage: 0%, cortical damage: ~0-5%) while FX rats sustained extensive fornix damage and dorsal cortical damage (fornix damage: ~5-75%, cortical damage: ~0-15%).

cortex, and the damage did not affect performance during shaping to the visible platform (see below). By contrast, all rats in the FX group sustained damage to the fornix (range 5-75% transection)

Shaping

The latencies to reach the visible escape platform across 5 days of shaping are shown in Figure 2A. The shaping data from two rats in the SH group and one rat in the FX group were lost, and so are not included in the analysis of the visible platform task. These three rats, however, learned to find the visible platform. A 2-way, mixed design ANOVA revealed a significant decrease in latency to escape across the 5 days of training, $F(4,40)=28.05$, $p<0.05$). There was no main effect for the performance of the SH group or the FX group ($F(1,40)=1.74$, $p>0.05$) and no group X training day interaction $F(4,40)<1$). These findings indicate that the FX group did not have any gross locomotor or motivational problems affecting their ability to navigate in the water maze.

Discrimination Learning

The latencies to reach the cued (submerged) platform during the 20 days of discrimination training are shown in Figure 2B. A 2-way repeated-measures design ANOVA revealed a significant decrease in latency to escape across the 20 days of training for both groups ($F(19,247)=13.09$, $p<0.05$). There was no main effect of the performance of the SH group or the FX group ($F(1,247)<1$) and group by training day interaction ($F(19,247)<1$). These results indicate that the FX group was able to learn the discrimination as quickly as the SH group.

Probe Trials

Additionally, on every third day of training, the rats' memory for the location of the platform was probed for a single trial. A repeated-measures 2-way ANOVA revealed that during discrimination probe trials where no platform was present, SH and FX rats did not spend significantly different amounts of time in the target quadrant (effect of group, $F(1,78)<1$). Furthermore, there was no effect of probe trial number ($F(6,78)=1.02$, $p>0.05$) and no interaction between group and trial number ($F(6,78)<1$; data not shown). Thus, the FX group spent the same amount of time in the target quadrant as the SH group, indicating that they were able to learn the task. Rats did not improve performance across trials, suggesting they had already learned the task quickly, as indicated by the significant effect of discrimination day seen in Figure 2B.

Similarly, SH and FX rats did not spent significantly different amounts of time in the foil quadrant on discrimination probe trials (a repeated-measures 2-way ANOVA revealed no effect of group, $F(1,78)<1$), and no interaction between group and probe trial number ($F(6,78)<1$; data not shown). There was, however, a significant effect of probe trial number ($F(6,78)=4.03$, $p<0.05$), indicating that both groups of rats spent less time in the foil quadrant as training progressed.

Post-hoc ANOVAs further emphasized that there were no differences in the performance of the FX and SH groups on the probe trials. Repeated-measures 2-way ANOVAs revealed that that both the SH rats ($F(1,72)=12.33$, $p<0.05$ and FX group

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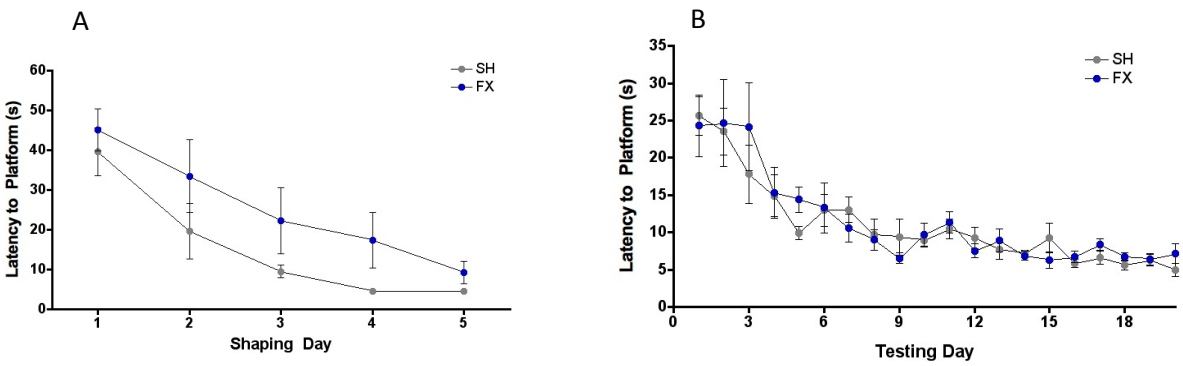

Figure 2: **A.** Both SH (n=5) and FX (n= 7) rats are able to escape using a visible platform during shaping and both reduce latencies over days (effect of shaping day, $p<0.05$). There was no group effect on latency ($p>0.05$) or an interaction between shaping day and group ($p>0.05$). **B.** Both SH (n=7) and FX (n=8) rats were able to learn to escape from the water maze in the discrimination testing and improved performance throughout testing (effect of testing day, $p<0.05$). As in shaping, there was no group effect ($p>0.05$) or an interaction between testing day and group ($p>0.05$). These results suggest both SH and FX rats are capable of learning to use beacons to escape. Data points and error bars represent the mean ± SEM.

($F(1,84)=14.57$, $p<0.05$) spent significantly more time in the quadrant over the foil quadrant. Finally, a repeated-measures 2-way ANOVA was performed to observe any differences between preference for the target quadrant over the foil quadrant or the quadrants without cues (Figure 3). To find the percent time rats spend in the quadrants without beacons during probe trials, the average time spent in the two quadrants was calculated. Since there was no significant effects of probe trial number in previous comparisons, averages were taken for each of the three quadrant types over all seven probe trials and a repeated-measures 2-way ANOVA (2 groups x 3 quadrant types) was performed to observe any quadrant preferences. While there was no significant effect of group ($F(1,26)<1$) or interaction ($F(2,26)<1$), there was a significant effect of quadrant on percent time ($F(2,26)=25.18$, $p<0.05$). Bonferroni post-tests revealed that rats spent significantly more time in the target quadrant (34.88 ± 4.30%) than the foil quadrant (24.38 ± 6.25%; $p<0.05$) and the quadrant without cues (20.37 ± 2.71%; $p<0.05$). Furthermore, the percent time spent in the foil quadrant and the average quadrant without beacons were not significantly different from one another for either group ($p>0.05$). These results indicate that rats not only learned to discriminate between two beacons in order to find the platform, but also treated the foil beacon the same as the quadrants without beacons. These results also indicate there were no differences in performance between SH and FX rats on this task.

Discussion

We found that rats with lesions of the fornix were able to learn a simple object discrimination task in the water maze. This was true whether we examined the latency to find the platform (Figure 2B), or compared the time spent in the target quadrant vs. the foil quadrant vs. quadrants without cues (Figure 3). In fact, both groups of rats learned the discrimination quickly, reaching asymptotic performance within approximately 9 days (27 trials).

Since the location of the hidden platform and the start location were pseudorandomly assigned, rats adopting a place strategy would not be expected to perform well in this task. Instead, rats needed to learn to discriminate between two distinct beacons in order to correctly associate the target beacon with the location of the hidden escape platform. We found that both SH and FX rats acquired the task quickly, as was seen in Figure 2B as a decrease in the latency to platform across testing days. We also found that both SH and FX lesion rats spend significantly more time in the quadrant containing the target beacon than any other quadrant during probe trials (Figure 3), indicating SH and FX rats were able to correctly discriminate between two distinct beacons and use the correct beacon as a cue to guide their search for the hidden platform. This suggests that the hippocampus is not required for completing a simple object discrimination and cued guidance to a hidden platform in the water maze.

These results support previous findings showing that rats with hippocampal damage can use cue navigation to locate a hidden platform in the

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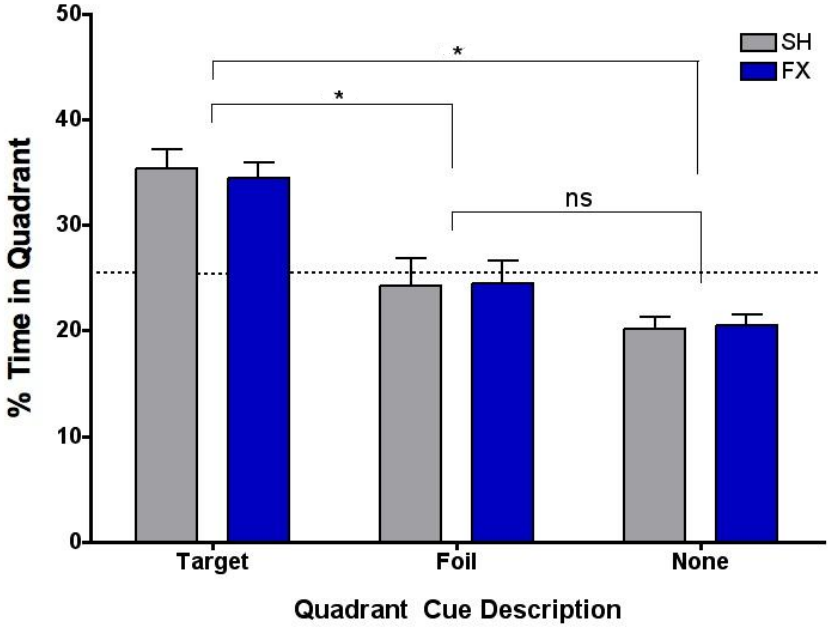

Figure 3: There were no differences between the SH (n=7) and FX (n=8) groups for the averages across the seven probe trials for time spent in the target quadrant, foil quadrant and the quadrants without beacons (group effect, $p>0.05$). Both groups spent significantly more time in the target quadrant than the foil quadrant and the quadrants without beacons ($p<0.05$ for both). Furthermore, neither group spent significantly more time in the foil quadrant than the quadrants without beacons ($p>0.05$), suggesting that both groups not only learned to use the target beacon to escape, but also learned that the foil beacon was never rewarded with escape. Bars and error bars represent the mean ± SEM, dashed line represents the chance percent time (25%) in any quadrant.

water maze (Morris, et al., 1982; Save & Poucet, 2000). Further, other studies have found intact object discrimination following hippocampal lesions outside of the water maze (Broadbent, et al., 2007; Wible, Shiber, & Olton, 1992). Thus, it is likely that rats in our study relied on the dorsal striatum memory system to learn the discrimination (Broadbent, et al., 2007). It is also possible that the intact discrimination was supported by the cortex surrounding the hippocampus (parahippocampal region, including the perirhinal and entorhinal cortices) although these cortical areas seem to support higher-order discrimination between objects with ambiguous features (Clark, Reinagel, Broadbent, Flister & Squire, 2011).

However, there is evidence suggesting the hippocampus is involved in any task in the water maze. For example, Teixeira, Pomedli, Maei, Kee, & Frankland, (2006) found that the hippocampus showed similar *c-fos* expression when performing both spatial tasks and nonspatial tasks in the water maze. While the Teixeira results imply the hippocampus is *involved* in nonspatial tasks in the water maze, our results suggest that the hippocampus is not *necessary* for nonspatial object discrimination in the water maze. A model proposed by Broadbent, et al. (2007) can explain this divergence. They assert that while the hippocampus is involved in object discrimination in intact animals, the dorsal striatum is sufficient to support learning and memory of an object discrimination task when the hippocampus is damaged.

In addition, our results are consistent with studies that found the hippocampus is necessary for place discriminations but not for nonspatial discriminations (Hollup, et al., 2001; Mumby, et al., 1999). It has been previously shown that the performance of rats with hippocampal lesions is impaired when the task requires the recognition of a place (annular water maze) but they are able to perform a nonspatial delayed non-matching to sample task in the water maze (Hollup, et al., 2001). Like the delayed non-matching to sample task, our object discrimination task showed no evidence of hippocampal dependence.

One additional possibility is that the lack of deficit seen in this study is due to the fact that we used fornix lesions as a proxy for damage to the hippocampus itself. This decision was made based on pedagogical constraints (see *Pedagogical Issues*

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below). While studies directly comparing lesion techniques have at most found modest differences in the performance of animals with hippocampal or fornix damage (e.g., Ferbinteanu & McDonald, 2001; Sziklas, Lebel & Petrides, 1998; Sziklas & Petrides, 2002), and then only in tasks that test complex conditional associations, it remains possible that our findings would have been different if we had used complete hippocampal lesions.

The purpose of this experiment was to address an outstanding issue in a recent report by Clark et al. (2007) examining the role of the hippocampus in retrograde memory in the water maze. Clark and coworkers developed a novel water maze task that required spatial memory, but did not require spatial navigation for escape. This task called for both the use of distal spatial cues and of proximal, ambiguous beacons in order to locate a hidden platform. The idea was that the rats would initially use distal spatial cues to locate the correct quadrant, and would then navigate to the platform using the one of four identical beacons that hung over the platform. Thus, rats could use distal cues to identify which beacon indicated the location of the platform, and then use that beacon to guide navigation to the hidden platform. They found rats with hippocampal damage could not use spatial cues to identify the correct quadrant, and further, did not adopt a search strategy involving the beacons to locate the vicinity of the platform. There are two possible reasons that the hippocampal rats did not use the beacons: 1) the lesioned rats had not been able to associate the beacon with the platform, indicating they had possibly forgotten there was a platform to find, or 2) the lesioned rats were unable to update their position in the water maze, reflecting a navigational performance deficit. Our findings suggest that rats with hippocampal system damage can in fact learn to associate beacons with the hidden platform. However, one difference between Clark et al. and our study was that Clark et al.'s four beacons were not distinguishable except by distal spatial cues, thus the lesioned rats could have retained the association of the beacons with the platform, but might not have used the beacons to guide navigation because they could not discriminate between them. Therefore, it seems likely that the "retrograde memory" deficit observed in the Clark et al. study may be due to a navigational performance deficit as the authors conclude, rather than a deficit in retrograde memory**.** Clark et al. (2007) therefore supports the idea that spatial memories, like other memories, are subject to consolidation.

Teixeira et al. (2006) further support the idea that spatial memories formed in the water maze can be consolidated from the hippocampus to neocortex. In this study, rats were trained and tested on three tasks in the water maze that involved both cued and spatial navigation. The immediate early gene *c-fos* was used to visualize activity levels in the hippocampus and neocortex with either a 1-day or a 1-month testing-retrieval interval. They found increased cortical activity at the 1-month interval as compared to the 1-day interval. Furthermore, remote spatial memory was disrupted when they administered reversible lesions to the cortex at 1-month, and recent memory was spared when the cortex was reversibly lesioned at the 1-day interval. These findings support Clark et al.'s (2007) explanation that the retrograde deficit in the water maze is due to an inability to express a consolidated memory. Together, these studies are consistent with the view that spatial memory consolidation in the water maze is no different from other nonspatial forms of memory, and that hippocampal-dependent memories are gradually reorganized and consolidated in the neocortex. While these memories can be represented independently of the hippocampus, the hippocampus may play an ongoing role in an animal's ability to update its position (or navigate) in the water maze.

Pedagogical Issues

This project was conducted by 12 students as part of the Laboratory in Behavioral Neuroscience: Learning and Memory course at Bowdoin College, a small, residential, liberal arts college. SR and NC have now taught this course 8 times, and a description of the course including a sample syllabus is described in Yates et al. (2006). Despite the logistical challenges and outside-of-class time requirement for conducting a real research project in a laboratory classroom setting, there are important advantages for both the students and faculty.

The students enrolled in this laboratory course are sophomores, juniors, and seniors. Prior to taking the laboratory, students are required to have taken introductory Psychology, an introductory Neuroscience course or Physiological Psychology, and a course in statistical analysis (co-requisite). For some students, this course is their first and only exposure to a real research endeavor. Inevitably, students are inspired to follow up on class projects as Independent Studies, or Honors Thesis Research. In either case, a relatively large number of students are exposed to the processes and techniques of real experimental research in behavioral neuroscience within the relative safety of the classroom environment. Students are more engaged and

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invested in the course when the experimental outcome is unknown. While doing a novel study, the students take ownership of the experiment and express a preference to this style of learning over the "canned" laboratories where the outcome of the experiment is known. Juniors and seniors have enough background information to flourish in the novel experimentation arena. Students come away with a fundamental understanding of how research is done, and a toolkit that includes behavioral testing, data analysis, basic surgical and histology techniques, scientific reading and writing skills, and not the least importantly, an understanding of how challenging and exciting research can be. Additionally, many students that complete this course are strong candidates for laboratory technician positions after graduation, the surgical and histological techniques they learn are marketable skills.

This model of laboratory class is also a unique opportunity for the faculty to conduct research central to their own research program. And we would like to emphasize that this kind of course works best when it can be linked to a broader research program. While it is not frequent to get publishable results from the students' first foray into research, it is possible. Further, the class often generates pilot data that can later be pursued by independent research students. As importantly, these research students have already been trained and are experienced in the basic techniques necessary to conduct independent research, thus reducing the amount of outside of the classroom training of research students (Hauptman & Curtis, 2009).

The pedagogical goals (Wiertelak, 2003) and how the course addresses them are summarized in Table 1. Briefly, since the goal of the course is to introduce students to the scientific endeavor from conception to publication, the emphasis is placed on scientific process and the content is focused on the background and interpretation of the project. For example, although the instructors design the experiment before the course starts (in large part so that animal use protocols can be approved and apparatus built prior to the start of the course) the students spend time discussing, and some cases, adjusting the experimental design. Importantly, students discuss what considerations were taken into account by the design, the appropriate controls, and then generate counterbalanced testing schedules. For the final project, students are required to produce a paper as if for publication. Therefore, we explicitly discuss how to write the different parts of the paper (e.g., Abstract, Introduction, Methods, appropriate citation, etc.) and the class as a whole discusses content of the different sections. For example, we ask students as a group to brainstorm a list elements to be included in the Methods, and then ask the students to discuss which items (and at what level of detail) need to be included. Likewise, the class works together during class time to conduct data analysis and interpretation of their results.

Since the paper includes an Introduction and Discussion based on the literature, we also explicitly discuss how to read scientific papers, and throughout the course we hold journal clubs where students present and discuss journal articles relevant to the current project. To help the students write the paper, there is a midsemester, take-home exam where students are essentially asked to write an expanded introduction. For example, for the current study, students were asked 1) to describe their project, 2) describe the hippocampal memory system, and the kind of memory it subserves, and 3) the implications of temporally-graded retrograde memory for the ultimate storage site for memory. Students will ultimately need to pare their Introductions down to 500 words of the Journal of Neuroscience Brief Communication Format. At several points near the end of the semester, students participate in anonymous peer review of the different sections of their papers.

There are certain challenges associated with conducting research in the classroom. For example, on some years we have not had enough students enrolled in the course to be able to conduct a full project. One solution is to collaborate with courses taught at other institutions, or multiple sections at the same institution (Yates et al., 2006). This approach also helps to defray some of the financial burden associated with the course, especially those associated with getting n's large enough for statistical analysis. While we are fortunate enough to have the resources available for a video tracking system, one author has run water maze experiments with a kiddie pool and stopwatch. Faculty should also consider resources such as the Faculty for Undergraduate Neuroscience (FUN) Equipment Loan Program, http://www.funfaculty.org. This program allows members to borrow equipment for classroom and research use to gain the pilot data they would need for successful grant applications or to convince administrations of the usefulness of such expenditures.

Scheduling daily testing times (5 days/week at the same time each day) can also be a logistical challenge. But this challenge has been overcome for all of the students across the eight years that the course has been taught. In some cases, students have had to test animals early in the morning (at 6am) or in the evening (7pm) or have had to stagger the testing

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of their animals by an hour on different days. But our students have consistently expressed that the outside of class time demands are outweighed by the opportunity to participate in the project. However, Bowdoin is a residential college, and students at commuter institutions might find it especially difficult to schedule testing times in the early morning or late evening. To cope with the fact that behavioral testing happens throughout the day, 5 days/week, and the course meets only twice a week, students are required to post comments to an online discussion board after each testing session so that the faculty and other students can deal with problems as they arise (see Yates, et al., 2006 for a discussion of the online component of this course).

Another consideration is that because of time (and skill) constraints, we have chosen to use fornix lesions rather full hippocampal lesions. In fact, the lesions we use are small (two sites) and often result in incomplete transection of the fornix. However, these lesions have consistently produced sufficient deficits to get significant results in this laboratory course. Additionally, the duration of a single semester course often means that the project is necessarily modest. In the case of the current project, it would have been ideal to have a positive control to show that the lesions had an effect. In fact, we will be addressing these two issues in the upcoming semester, when the Laboratory in Behavioral Neuroscience: Learning and Memory will repeat this experiment using 4 lesion sites to more completely damage the fornix, and with only two weeks of discrimination training (rats reached asymptotic performance within 10 days of training). This would allow two weeks of training on the standard version of the water maze as a positive control. Thus, this course is serving as a pilot for the experiment to be run this year.

Finally, students could potentially have concerns about doing invasive work with live vertebrate animals. Although the Laboratory in Behavioral Neuroscience: Learning and Memory is a part of both the Psychology and Neuroscience curricula, this course is not required for graduation, and students may opt to take other laboratory courses that do not use rats. During the first week of the course, we also attempt to make sure that students completely understand what will be expected of them so that they can choose to drop the class early as early in the semester as possible. During the first class, we completely describe the proposed experiment and the procedures we will use in the laboratory. We also demonstrate a perfusion or a surgery at the end of the first day, so that students are immediately exposed to the most dramatic procedures. In our experience, students are less anxious about the procedures once they have observed them.

During the second and third classes, students complete a required training about the rules and regulations pertaining to animal care and use. We also hold discussions of the ethics, and importance, of humane animal use to the scientific process. While we have never had a student drop the course because of concerns about using animals, it is possible that students with these concerns do not register for this class.

Students are also informed at the outset of the course that their primary obligation in the class is to the animals they will be using. Any animal abuse, or unexcused failures to show up during agreed upon testing times, can result in a failing grade. Students take their obligations seriously. However, we have had several students who have had difficulty handling the rats or performing procedures. Our general rule is that to discharge their responsibility to the animals, students must be present for all testing and procedures. Students who are shy about handling rats are encouraged to choose lab partners who are more comfortable handling animals. In this way, they can be record-keepers while their partners do the actual testing.

Students who are uncomfortable with the prospect of performing procedures are likewise encouraged to find laboratory partners who are more excited about it. For surgeries and perfusions, students may elect not to perform the procedures themselves, but they must be present for the duration, even if it is to sit in the hallway outside of the lab. However, all students are strongly encouraged to try. In the 8 years we have taught the course, only two students have opted out of being in the room during the perfusions, and none have left the room for surgery. We should also note that on several occasions students have vomited or fainted while observing surgeries or perfusions. As noted above, to reduce the anxiety associated with these procedures, we demonstrate one during the first class. This has greatly reduced the number of adverse reactions, and in fact we have only had a single incident since moving the first demonstration to the first day of class. Nevertheless, we monitor students carefully during any procedure.

Together, we feel that this course serves as a model for exposing students to real research early in their careers as possible, and that the cost in time and resources is outweighed by the benefits to both students and faculty. While this approach is certainly challenging, it is also an ideal way to meet desired curriculum outcomes, including introducing students

to experimental methodology, design, and data analysis, advanced awareness of a particular field within neuroscience, critical and independent thought, effective communication skills, and ethics (Wiertelak, 2003).

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