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HIGHER-ORDER HABIT STRENGTH AND SPATIAL LEARNING

By

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Abstract

Higher-Order Habit Strength and Spatial Learning

Paige Michener

This study examined the amount of acquisition trials necessary for higher-order habit formation to occur in the Morris water maze. After 8 days of acquisition, learning of the original platform location was predicted to be hippocampal-based, while after 16 days, learning was predicted to be a striatal-based higher-order habit. 16 male Long-Evans hooded rats were used. Rats were trained to find a platform in the water maze for either 8 or 16 days. Rats then experienced a 12-trial retraining session to a new platform location, followed by a probe test with quadrant preference measured. Both groups learned the platform locations at an equal rate. During the probe test, both groups showed a preference for the new location, indicating that learning of the original platform location may not have become habitual. Future research needs to extend the amount of acquisition training or determine a different paradigm for testing higher-order habit formation.

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Chapter One:

Introduction

The striatum is an important brain structure involved in forming new habits and choosing actions to take in response to stimuli. The striatum plays a large role in forming associations between stimuli and choosing a response in reaction to these associations. Over time, these associations and their subsequent responses become habitual. These associations and responses are important for determining the processes that allow habits to be formed, specifically higher-order habits. This review will discuss a commonly used method for testing spatial learning (Morris water maze) and then the process of how associations are strengthened in the brain. The importance and role of the striatum will be discussed, as well as the role of the hippocampus in spatial learning. Relevant literature examining the hippocampus and the striatum will then be described, concluding with an overview of the findings and a description of the present study including methods, results, and discussion.

One of the most commonly used methods of testing habit formation and spatial learning is the Morris water maze task, originally described by Morris (1981). In the Morris water maze task, rats are placed in a round white pool containing a platform in one of the four quadrants that is submerged under the water, which is made opaque. Rats are placed into the pool at one of four start points, with the start point typically changing for each acquisition trial. Training occurs for a set number of days, and finally, the test trial, or probe test, is performed. During the probe test, the rat is allowed to swim freely around the maze without the platform present. The time spent in each quadrant would be measured to see where the rats spend most of their time searching for the platform. This

paradigm has been used in multiple studies involving the water maze, with some slight variations in methodology being utilized in some studies. However, this is the task used for most of the following studies described, unless otherwise specified.

When learning the water maze, a rat experiences multiple training trials. This repeated exposure will cause the connection between the spatial environment of the water maze and the platform location to strengthen over time. The strengthening of associations in the brain through repeated exposure is known as long-term potentiation, which is defined as "a stable and enduring increase in the effectiveness of synapses following repeated strong stimulation" (Watson & Breedlove, 2012, p. 376). In other words, repeated exposure to a stimulus will increase the synaptic connections in the brain that are associated with that stimulus, and this will increase the power and strength of the learning. As new associations are learned, and new connections are formed in the striatum, these connections are strengthened. These connections then become stronger with more exposure, and as a result, habits are formed. This process allows for greater consolidation of the learning, which increases over time as exposure increases.

Striatum and Hippocampus

The dorsal striatum has two main functions: cognitive control and habit formation (Devan, Hong, & McDonald, 2011). Cognitive control refers to a stimulus-stimulus relationship, which forms a response. It refers to the specific responses that are performed when necessary, and eventually become automatic, habitual responses (Devan et al., 2011). An example of this would be for a rat to be exposed to two different stimuli and learn to approach one of these stimuli upon presentation.

Habit formation refers to the formation of stimulus-response associations as a form of reinforcement. The strength of the stimulus-response association is very important in terms of habit formation (Devan et al., 2011). The more often the stimulus-response relationship is presented and the greater the strength of the association, the stronger the habit formation will be. Spatial cues in the environment, such as posters on the walls or the experimenter standing in the corner of the room, are often used to form the stimulus-response relationship. These cues are especially important for learning this relationship when the cues have high visibility, for example, in a well-illuminated room (Restle, 1957). Egocentric orientation can also be an important contributor to spatial navigation. Egocentric cues involve using internal vestibular cues to provide input to the striatum and can be used in habit formation (Devan et al., 2011). An example of using an egocentric cue would be knowing that a left turn is required after swimming in the water maze for approximately 5 s from a given start point. This would rely on internal processes to learn the spatial environment instead of relying on the spatial cues in the environment. The dorso-lateral striatum (DLS) is an important structure for using these egocentric cues, and it has been shown that lesions to the DLS in rats disrupt the ability to use these cues (Rice, Wallace, & Hamilton, 2015). Spatial and egocentric cues seem to work together because learning the environment is dependent on many different cues across all modalities, as described in the multiple cue theory (see Restle, 1957, for a review).

The striatum appears to be involved in a number of tasks involving spatial learning and responses. It has been found that rats with lesions to the DLS experienced difficulty with discrimination tasks that required making voluntary movements when

presented with specific cues (Devan et al., 2011). For example, the rats would need to approach a specific object when the object was presented. This finding provides evidence that the striatum is important for cognitive control and producing necessary responses. Even rats without lesions to the DLS require large amounts of training on such tasks for a behavior to be described as habitual (Devan et al., 2011). This shows that habit formation is a process that requires a large amount of training before a response becomes automatic. It has also been found that rats with damage to the DLS were impaired at operant tasks with interval schedules of reinforcement. These tasks are thought to become habitual after prolonged training, and the impairment was specific to the late stages of training when the response would typically appear to have become habitual (Devan et al., 2011). This shows that the striatum, specifically the DLS, is important for habit formation. However, the type of task used was a response-outcome task, rather than a stimulus-response task, because no cue was presented that indicated to the rat that it needed to be performing a specific behavior. In contrast, the water maze most likely requires subjects to use allocentric, or spatial cues to find the platform, due to different start points requiring different turning responses to directly find the platform. Allocentric cues are spatial cues that are used in the environment to find the location of the platform (Devan, Goad, & Petri, 1996; Devan et al., 2011). An example of an allocentric cue would be to use a poster or a shelf on the wall in the room to know where the platform is located, or to use the experimenter as a cue that the platform is directly in front of the experimenter, or that the platform is always to the left of the experimenter.

Multiple studies (see Devan et al., 2011) have been conducted that have examined the role of the striatum in the learning of spatial information, as well as the effects of

various methods that interrupt the functioning of the striatum. Because the hippocampus and the striatum appear to work together to learn a spatial environment, the role of the hippocampus in the learning of spatial environments has also been examined. The hippocampus is believed to create new connections in the spatial environment between the cues presented and the location of objects in the environment (Eichenbaum, Stewart, & Morris, 1990; Morris, Garrud, Rawlins, & O'Keefe, 1982), and as a result, many studies have examined how manipulating the hippocampus can affect learning a spatial environment. For example, Broadbent, Squire, and Clark (2006) found that when the hippocampus was temporarily inactivated, it inhibited learning of the spatial environment of the water maze. Compton (2004) also found that lesions to the hippocampus had an effect on rats learning a location in the Greek cross version of the Morris water maze when this learning was dependent on the processing of multiple stimulus cues associated with that location. In addition, lesions of the dorsal striatum impaired the rats' ability to perform a correct response in the maze.

McDonald et al. (2005) examined whether N-methyl-d-aspartate (NMDA)-mediated LTP is necessary for the consolidation of information rather than the initial learning of the information, which was studied using a competitive place task with rats in a water maze. This task was first used by Morris and Doyle (1985), who trained rats to find a platform in the Morris water maze for 30 trials spread out over a number of days. The rats were then split into three groups and either given no further training, a mass training session of eight retraining trials in one day to find a new platform location, or one training trial per day for eight days to find the new platform location. The rats were then given four probe tests, with the first occurring 2 hr after the last retraining trial and

the rest occurring multiple days after this retraining. Morris and Doyle found that the group that received mass training of the new location showed a preference for that location on the first probe test, but showed a preference for the old location on the next three probe tests. The group that received retraining trials that were spread out across eight days showed a preference for the new location on every probe test. Morris and Doyle suggest that the rats that received the mass training session in one day and were then given a probe test on that same day were holding the new information about the environment and the platform location in their spatial working memory. As this information was never reinforced again after these mass training trials, the information about the new platform location did not become a part of the spatial representation of the environment for the rats. For the group with retraining spread out across eight days, the spatial representation of the environment was altered to replace the old platform location with the new platform location.

McDonald et al. (2005) used a variation of this competitive place task to study the learning and consolidation of place learning in the water maze. In their study, rats were given four days of training, with eight trials per day, to find a platform located in a specific location in the water maze, then split into two groups and given either an injection of an NMDR-receptor blockade or a saline injection into the hippocampus, and then trained to find a new platform location (mass training phase). During this mass training phase, the rats were given 16 training trials in one day to learn the new location. After this, the rats were placed in the water maze with either the original platform present or not present, and the time spent in each quadrant of the maze was measured to see which platform the rats were searching for. The results found that the rats injected with

the NMDR-receptor blockade performed just as well as the rats injected with saline when learning the location of a new platform. However, saline-injected rats performed significantly slower when being retrained to find the original location, showing that the learning of the new location interfered with the old location. The NMDR-receptor blockade injected rats did not experience any deficit in relearning the original location. This shows that acquiring and storing short-term spatial memory is not dependent on a NMDR form of memory and most likely occurs in the hippocampus. This study will be used to guide the present study in order to see how long training in the water maze needs to occur until it is no longer considered hippocampal based and it moves on to becoming a higher-order habit.

Because the hippocampus appears to create a spatial representation of the environment and the striatum controls the behaviors in the environment, Miyoshi et al. (2012) were interested in comparing the roles of both the hippocampus and the DLS in learning the Morris water maze and a cued water maze task. They conducted an experiment that examined whether or not lesions in both the hippocampus and the DLS would produce the same impairment as lesions in only one of the structures in rats when learning the two tasks. Four groups were used: a hippocampal lesion group, a DLS lesion group, a hippocampal and DLS lesion group, and a sham lesion group. Two weeks after receiving the lesions, the rats were trained on the Morris water maze and cued water maze tasks. The cued water maze procedure was the same as the Morris water maze procedure except that a small white ball sat on top of the platform as the cue. The results showed that the rats with hippocampal lesions were impaired on the Morris water maze task compared to the striatal lesions and the sham lesions. However, rats with striatal

lesions were not impaired on either version of the task. Rats with both hippocampal and striatal lesions were impaired on both versions of the task, showing that both the hippocampus and the DLS work together to learn both spatial and cued tasks. This is consistent with the theory that the hippocampus creates a contextual map of the spatial area being learned, while the DLS makes decisions about which actions to take in that environment. The hippocampus and the striatum also appear to work together to learn the spatial environment and respond appropriately to the environment.

Using a different task, Pistell et al. (2009) examined whether striatal lesions would inhibit performance in the 14-unit T-maze. In addition, they looked at the different effects of DLS versus dorso-medial striatal (DMS) lesions on acquiring and performing the task. Rats were given lesions in the DMS, DLS, or sham lesions. After receiving the lesions, the rats were pretrained to run along a straight runway from one box to another to avoid a foot shock. Following the pretraining, the rats were run through the 14-unit T-maze, which consisted of five main sections separated by guillotine doors. The rats needed to move into the next section after a certain amount of time to prevent being shocked. The results showed that both the DMS and DLS lesion groups committed more errors and did not improve during acquisition compared to the control group. In addition, both lesion groups took longer to run through the maze than the control group. These findings indicate that both the DMS and DLS are important for learning a spatial task, such as the 14-unit T-maze.

Using yet another maze task, Moussa, Poucet, Amalric, and Sargolini (2011) examined the roles of both the DMS and DLS on a T-maze task. Rats were given lesions in either the DMS, the DLS, or sham lesions and then trained and tested on a continuous

spatial alternation task. The rats were required to run in the T-maze in a specific sequence, with errors being counted. The results showed that DMS lesions impaired performance on acquiring the spatial alternation task. Rats with DMS lesions also committed significantly more errors than the DLS lesion and sham lesion rats. In contrast, the rats with DLS lesions showed a faster learning rate as well as a more rapid extinction rate compared to the other two groups. This demonstrates that the DMS is important for the acquisition of the spatial information. The DLS, which is involved in habit formation and the development of motor sequences, actually increased the learning and extinction rate when inhibited. Moussa et al. suggest that the disruption of habit formation facilitated goal-directed strategies instead, which minimized the effect of external stimuli on the response, instead increasing action-reward responses. In other words, the rats were acting only in response to the rewards received rather than the spatial environment itself.

Looking at just one area of the striatum, Braun and Hauber (2011) described how the DMS plays a role in stimulus-guided action selection, evaluation of actions, and value-based decision-making. They examined the effects of lesions in the DMS on an effort-based decision-making task in rats using a T-maze task. In one arm of the T-maze, the rats could receive four pellets (high reward) for climbing over a barrier, compared to two pellets in the other arm of the maze with no barrier. After extensive training on the task to ensure that the rats were showing preference for the high-reward arm, the rats received either DMS lesions or sham lesions. The rats' choice behavior was then tested on multiple tasks: four pellets with a barrier versus two pellets with no barrier (Block B), four pellets with a barrier versus two pellets with a barrier (Block C), and two pellets

with a barrier versus four pellets with no barrier (Block D). The results showed that the rats with DMS lesions exhibited equal performance to the sham-lesioned rats on both Block B and Block C trials. However, when the location of the high-reward arm was reversed in Block D to test for spatial flexibility of choice behavior, the rats with DMS lesions showed a much slower increase in choosing the high-reward arm. Although the rats with DMS lesions were still able to exhibit choice behavior, the choice behavior was less flexible after the reversal of the reward arms. Braun and Hauber explain that the DMS may not be as important for effort and reward-related decision-making, but rather the flexibility of spatially guided behavior.

These findings were also supported by Lee, André, and Pittenger (2014), who compared the effects of DMS lesions and sham lesions in learning a cued and spatial water maze task in male C57Bl/6 mice. After receiving either DMS lesions or sham lesions, the mice were trained to find a cued platform and were then trained on either the cued or the spatial (hidden platform) water maze task. It was found that although the DMS lesions did not impair the animals' ability to learn the new task, the DMS lesioned rats took significantly longer to learn the platform location for both tasks. In addition, the DMS lesions did not impair cued learning, but instead impaired their behavioral flexibility capacity to change their behavior after learning the original platform location. These findings again provide support for the important role of the DMS in the flexibility of spatial behavior.

Pooters, Gantois, Vermaercke, and D'Hooge (2016) examined the effects of DLS and DMS lesions in learning the Morris water maze using female C57Bl/6 mice. After receiving lesions in either the DLS, DMS, or sham lesions, the mice were trained to find

a hidden platform in the water maze for 15 days (four training trials per day). The mice were given a probe trial after every five days of training without the platform present. The results found that during training, the DMS lesioned mice took longer to learn the platform location, but eventually showed learning similar to the other two groups by the end of the 15 days. However, on the probe trials, the mice with DMS lesions did not show a preference for the quadrant that contained the platform, while the other two groups did show a preference. Pooters et al. explain that these findings indicate that the DMS may be important for the acquisition of spatial information, while the DLS may be more important for the later, more habitual stages of learning. This finding is again in line with previously mentioned research, showing the importance of the striatum in higher-order habit formation.

The hippocampus and the striatum both play important roles in learning a spatial environment. The hippocampus is important for acquiring and storing short-term spatial memory, as well as forming a spatial map of the environment being learned. The striatum appears to be the central structure that forms habits through stimulus-response associations. The DMS and DLS are important for learning a spatial task. In addition, the DMS is important for the acquisition of spatial information and the flexibility of spatially guided behavior, which is especially important when two different platform locations have been learned and the response behavior needs to be altered to find the correct platform. The striatum, specifically the DLS, is also important for making the associations that form higher-order habits. Higher-order habit formation can occur in the striatum after large amounts of training on a task (Devan et al., 2011). The exact amount

of time needed to form a higher-order habit in the Morris water maze will need to be determined, which will be addressed in the following studies.

Study 1

Study 1 examined higher-order habit strength in relation to spatial working memory in rats. Previous studies that have looked at spatial working memory in the Morris water maze paradigm used a competitive place task in the water maze (Morris & Doyle, 1985), which has been used to assess NMDA receptor blockades within the hippocampus after extensive place training, which occurred in the first (acquisition) phase (McDonald et al., 2005). During this phase, rats learned the location of a platform in the water maze after four days of acquisition with eight training trials per day. This was followed by a mass training session (second phase) within a restricted amount of time (30 min). The rats learned a different platform location, which conflicted with the previous knowledge about the first platform. Finally, the third (place competition test) phase consisted of a probe test with no goal platform present in the water maze. This tested which area of the maze the rats were more likely to spend time in and which platform location they were searching for.

Study 1 utilized more extensive training in the acquisition phase (Phase 1). Training was conducted for both four and eight days, with eight trials per day, and the relative preference for place locations in the rats was assessed. The end goal was to determine how long the rats would need to be trained on acquisition of the platform location in order for this learning to become striatal based and therefore a higher-order habit. The rats with four days of training were expected to show greater difficulty learning the new platform location because learning of both the old and new locations

would have been hippocampal based. Therefore, the new platform location would have been competing with the old location, making it more difficult to learn the new location. In contrast, the rats with eight days of training were expected to show no difficulty learning the new platform location because this hippocampal-based learning would not be interfering with the old location because learning of the old location would have become striatal based. During the probe test, the rats with four days of training were expected to show a preference for the new platform location because the learning of this new location should have superseded the learning of the old location. This would occur because the learning of both locations was hippocampal based. However, the rats with eight days of acquisition training should prefer the old location because at this point, the learning should have become striatal based and finding the original platform should have become a higher-order habit. Therefore the learning of the old location would have superseded the learning of the new location for this group.

Chapter 2:

Method

Subjects

Sixteen male Long-Evans hooded rats were used in Study 1. The rats were about 75 days old at the start of the acquisition trials. Once the rats arrived in the lab, they were placed into quarantine for one week. After this quarantine, the rats were acclimated to the facilities and the housing room and were then handled daily by the experimenters for another week. The rats were acclimated to the experimental procedure for three days prior to beginning the acquisition trials. During this acclimation period, the rats were placed into holding cages and carried around an experimental room (which was different from the room where the experiment actually took place) four times per day to acclimate them to being carried and handled during the actual experimental trials.

Materials

All training and testing occurred in a Morris water maze which was 6 ft in diameter. There was a platform in the maze that was submerged approximately 1-2 cm below the surface of the water. The water was maintained at 25±2 °C. Nontoxic tempera white paint was added to the water so that it became opaque and hid the platform. A video camera, VHS recorder and HVS tracking system were used to obtain several performance measures including distance and time in predefined pool areas, escape latency, and quadrant preference.

Procedure

The rats were randomly assigned to one of two experimental groups. One group received acquisition training for four days and the other group for eight days. The rats

received eight training trials per day, in two blocks of four trials each. All behavioral training and testing was conducted at approximately the same time each day during the light phase of the 12:12 hr light/dark cycle. The animals had ad lib access to food (Purina Lab Chow) and water throughout the course of the experiment. On each training day, the experimenter brought the rats from the housing room into the experimental room. The rats were each placed into a holding cage, and were then placed individually into the pool, facing the wall of the pool. Once released, the rats were allowed to swim in the pool and search for the platform (located in Quadrant 1) for a maximum of 60 s per trial. If the rat did not find the platform within 60 s, the experimenter gently guided it there by hand. The animals were allowed to remain on the platform for 5 s before being returned to the holding cage until the next trial. Each trial began at one of four different start points in the pool, with start points occurring in a different order each day. Each rat experienced one trial before the first rat experienced the next trial, allowing for each rat to have a small break between trials. Once all trials had been completed each day, the rats were placed back into their home cages and returned to the housing room.

After the training trials had been completed, the animals experienced a retraining session, which lasted for one day. During this session, each rat experienced eight trials (two blocks of four trials each) of learning a new platform location in the pool (in Quadrant 3). This retraining followed the same protocol as the acquisition training. The day after this retraining session, the animals experienced a probe test. The rats were placed in at a start point that was different from any of the previous start points so that this did not factor into their platform preference. During the probe test, the animals were allowed to swim freely around the pool with no platform present for 60 s, with time spent

in each quadrant of the pool (preference score) measured to see where the animals were spending the most time (around the new or old platform location).

Chapter 3:

Results

Mauchly's tests of sphericity and Levene's tests of homogeneity of variance were not violated for any of the following analyses, *p*s > .05, so the following results were reported with sphericity assumed.

Acquisition

A 2 x 4 (Group x Day) Factorial ANOVA Mixed Design was run on the escape latency for the acquisition trials. The ANOVA revealed a significant main effect of Day, $F(3, 42) = 63.49$, $p < .001$, $\eta^2_p = .82$ [90% CI: .71, 86]. Bonferroni post hoc comparisons showed that escape latency decreased significantly across the acquisition days (see Figure 1). Escape latency on Day 1 ($M = 33.38$, $SD = 9.24$) was significantly higher than on Day 2 ($M = 17.72$, $SD = 7.08$), Day 3 ($M = 12.59$, $SD = 5.91$), and Day 4 ($M = 9.04$, $SD = 3.44$). In addition, escape latency was significantly lower on Days 3 and 4 compared to Day 2. The ANOVA revealed no significant main effect of Group, $F(1, 14) = 0.76$, $p = .399$, $\eta^2_p = .05$ [90% CI: .00, .16]. In addition, the ANOVA revealed no significant Day X Group interaction effect, $F(3, 42) = 1.19$, $p = .327$, $\eta^2_p = .08$ [90% CI: .00, .18].

Retraining

A 2 x 2 (Group x Trial Block) Factorial ANOVA Mixed Design was run on the escape latency for the retraining trial. As shown in Figure 2, the ANOVA revealed a significant main effect of Trial Block, $F(1, 14) = 59.11$, $p < .001$, $\eta^2_p = .81$ [90% CI: .58, .87], with escape latency being significantly lower in the second Trial Block ($M = 10.01$, $SD = 6.75$) than in the first Trial Block ($M = 26.91$, $SD = 10.77$). However, the ANOVA revealed no significant effect of Group, $F(1, 14) = 0.06$, $p = .805$, $\eta^2_p = .01$ [90% CI: .00,

.14]. In addition, the ANOVA revealed no significant Group X Trial Block interaction effect, $F(1, 14) = 0.50$, $p = .493$, $\eta^2_p = .03$ [90% CI: .00, .24].

Probe Test

A 2 x 2 (Group x Quadrant) Factorial ANOVA Mixed Design was run on the quadrant time (preference score) during the probe test. The ANOVA revealed no significant main effect of Group, $F(1, 14) = 0.86$, $p = .369$, $\eta^2_p = .06$ [90% CI: .00, .28]. In addition, the ANOVA revealed no significant main effect of Quadrant, $F(1, 14) = 3.13$, $p = .099$, $\eta^2_p = .18$ [90% CI: .00, .43]. As shown in Figure 3, the ANOVA also revealed no significant Group X Quadrant interaction effect, $F(1, 14) = 0.04$, $p = .850$, $\eta^2_p = .00$ [90% CI: .00, .10].

Chapter 4:

Discussion

During acquisition trials, the groups showed no difference in escape latency across the first four days of acquisition. However, escape latency significantly decreased across the four days. During retraining, escape latency significantly decreased from the first trial block to the second trial block, but the groups showed no difference in performance. During the probe test, there was no difference in preference score between the groups for either of the quadrants.

For the first four days of acquisition, the groups showed equal rates of learning, which was expected. Escape latency decreased across the first four days, demonstrating that the animals were learning the platform location and learning at the same rate across both groups. However, escape latency did not differ during the retraining trials as expected. The group with four days of training was predicted to show more difficulty learning the new platform location, and therefore show a greater escape latency. In contrast, the group with eight days of training was predicted to have an easier time learning the new platform location, and therefore show a reduced escape latency. However, this is not what was found. Both groups learned the new platform location at an equal rate. This may have been because the learning of the old location had not yet become a higher-order habit for the group with eight days of training. The equal rates of learning suggest that the old platform location was still being processed by the hippocampus for both groups, and as a result, both groups seemed to experience difficulty with learning the new platform location. This was also demonstrated in the performance on the probe test between the two groups. Neither group showed a

preference for the old nor the new location, which suggested that the learning of these two locations had not undergone enough consolidation as a result of the stimulus-response relationship not being presented enough times. The group with eight days of training was predicted to show a preference for the old location because going to this location was thought to have become a higher-order habit. In contrast, the group with four days of training was predicted to show a preference for the new location because this learning was expected to have superseded the learning of the old location. However, only having eight retraining trials may not have been enough for the group with four days of training to have this learning override the learning of the old location. For the group with eight days of training, the old location may not have become a striatal-based habit yet, so the learning of the new location may have been interfering with the old location. Learning the two different platform locations allowed for potential competition between the hippocampal-based spatial working memory and the habit-based striatal learning, which may have resulted in the rats searching both locations for the platform. This may have caused the rats to swim between the two locations during the probe test, and as a result, they did not spend more time in one platform location over the other.

The findings of the present study differed from the results of previous research. McDonald et al. (2005) found that using the same acquisition procedure with eight training trials per day for four days, rats showed a preference for the new platform location when given the probe test. In the present study, no preference was shown for either the old or new platform location for the rats with four or eight days of training. However, McDonald et al. used a different procedure during the retraining phase of their experiment. The rats were given 16 training trials within a short amount of time, while

the present study used only eight retraining trials. This difference in methodologies may have caused the inconsistencies between the findings. In the present study, eight training trials may not have been sufficient for the rats to have learned the new platform location. The rats instead received the same number of trials that they had experienced each day during acquisition, and this may not have provided the necessary overtraining in order to ensure learning of the new platform location. With more retraining trials, the new location would have possibly been able to override the old location, causing the rats to show preference for the new location. However, it is important to note that behavior can be difficult to replicate for a number of reasons. In a study that examined mouse behavior on a number of tasks using different strains across three different laboratories, Crabbe, Wahlsten, and Dudek (1999) found that there were strain differences across different tasks, as well as differences between experimenters and differences in the laboratory environment. Differences between our laboratory and laboratories where previous research was conducted could have had an effect on the failure to replicate previous findings in studies that were similar to Study 1.

There were several limitations in Study 1. Due to time constraints that prevented the rats from receiving a longer amount of training, the rats were unable to demonstrate behavior similar to that of previous research. In addition, the sample size of 16 was small but also consistent with other animal research. However, this small sample size did not provide adequate power, and perhaps with a larger amount of rats in each group, an effect may have been seen. In addition, the rats were only 75 days old at the start of training and may not have been old enough to be able to fully learn the task due to the hippocampus being underdeveloped (Martin & Berthoz, 2002). Previous research (Devan et al, 1996;

Rice et al., 2015) used rats that were at least 90 days old before training began, and this may have allowed for better learning of the task.

A follow-up pilot study was run to address these issues. This study utilized acquisition trials that spanned a greater number of days but decreased the number of trials per day. Two groups were run for either eight or 16 days of acquisition trials, and four trials were run each day for both groups, which could potentially allow for greater consolidation to occur over the extended number of days. Sixteen days of acquisition trials was predicted to be enough trials for higher-order habit formation to occur because of this opportunity for greater consolidation. During retraining, the rats instead received 12 trials of learning the new platform location, which could allow for increased learning of this location. With this amount of retraining, the rats with eight days of acquisition trials were predicted to show a preference for the new location when given the probe test, while the rats with 16 days of acquisition trials were predicted to show a preference for the old location because this location should have become a higher-order habit.

Chapter 5:

Study 2

Study 2 again examined higher-order habit strength in relation to spatial working memory in rats. The current study was a pilot study that utilized more extensive training in the acquisition phase. Training was conducted for both eight and 16 days, with four trials per day, and the relative preference for place locations in the rats was assessed. The end goal was to create a version of the competitive place task in the water maze that would assess striatal-based higher-order habit formation in relation to hippocampal-based spatial working memory for future studies, using temporary and permanent lesions.

Method

Subjects

Sixteen male Long-Evans hooded rats were used in the study. Subjects were randomly assigned to one of two experimental groups. The rats were about 5 months old at the start of the acquisition trials. The rats were acclimated to the experimental procedure for three days prior to beginning the acquisition trials. During this acclimation period, the rats were placed into holding cages and carried around the room (which was different from the room where the experiment was actually held) four times per day to acclimate them to being carried and handled during the actual experimental trials.

Materials

All materials were identical to the materials used in Study 1.

Procedure

The procedure was identical to the procedure used in Study 1. All acquisition, retraining, and probe test procedures were the same, with the exception of the groups receiving either eight or 16 days of acquisition training and 12 retraining trials.

Chapter 6:

Results

Acquisition

A 2 x 8 (Group x Day) Factorial ANOVA Mixed Design was run on the escape latency for the acquisition trials. Mauchly's test of sphericity was significant, $p < .001$, so a Greenhouse-Geisser correction was used. The ANOVA revealed a significant main effect of Day, $F(2.84, 39.80) = 12.86$, $p < .001$, $\eta^2_p = .48$ [90% CI: .25, .59]. Bonferroni post hoc comparisons revealed that escape latency decreased significantly across the acquisition days (see Figure 4). The escape latency on Day 1 ($M = 11.28$, $SD = 5.43$) was significantly higher than the escape latency on Day 2 ($M = 6.54$, $SD = 2.59$), Day 4 ($M = 5.80$, $SD = 1.73$), Day 5 ($M = 5.35$, $SD = 1.83$), Day 6 ($M = 4.66$, $SD = 1.07$), Day 7 ($M = 4.33$, $SD = 0.75$), and Day 8 ($M = 5.44$, $SD = 2.49$). In addition, the escape latency on Day 3 ($M = 7.39$, $SD = 3.33$) was significantly higher than on Day 7. The escape latency on Day 4 was significantly higher than on Days 6 and 7. The ANOVA revealed no significant main effect of Group, $F(1, 14) = 4.46$, $p = .053$, $\eta^2_p = .24$ [90% CI: .00, .48]. In addition, the ANOVA revealed no significant Day X Group interaction effect, $F(2.84, 39.80) = 0.57$, $p = .631$, $\eta^2_p = .04$ [90% CI: .00, .11].

Retraining

A 2 x 3 (Group x Trial Block) Factorial ANOVA Mixed Design was run on the escape latency for the retraining trial. Mauchly's test of sphericity was significant, $p < .001$, so a Greenhouse-Geisser correction was used. As shown in Figure 5, the ANOVA revealed a significant main effect of Trial Block, $F(1.13, 15.82) = 96.96$, $p < .001$, $\eta^2_p = .87$ [90% CI: .73, .91]. Bonferroni post hoc comparisons showed that escape latency was

significantly lower in Trial Block 2 (M = 7.12, SD = 2.45) than in Trial Block 1 (M = 26.48, SD = 9.25) and significantly lower in Trial Block 3 (M = 5.76, SD = 2.36) than in Trial Block 1. However, the ANOVA revealed no significant effect of Group, $F(1, 14) = 0.66$, $p = .432$, $\eta^2_p = .05$ [90% CI: .00, .26]. In addition, the ANOVA revealed no significant Group X Trial Block interaction effect, $F(1.13, 15.82) = 1.52$, $p = .240$, $\eta^2_p = .10$ [90% CI: .00, .33].

Probe Test

A 2 x 2 (Group x Quadrant) Factorial ANOVA Mixed Design was run on the quadrant time (preference score) during the probe test. The ANOVA revealed no significant main effect of Group, $F(1, 14) = 0.76$, $p = .787$, $\eta^2_p = .01$ [90% CI: .00, .27]. However, as shown in Figure 6, the ANOVA revealed a significant main effect of Quadrant, $F(1, 14) = 13.64$, $p = .002$, $\eta^2_p = .49$ [90% CI: .14, .67]. The ANOVA revealed no significant Group X Quadrant interaction effect, $F(1, 14) = 0.00$, $p = 1.00$, $\eta^2_p = .00$ [90% CI: .00, .00]. Figure 7 illustrates the individual preference scores for each rat in Quadrants 1 and 3 during the probe test. As seen in the figure, all but three rats spent more time searching in the new quadrant location during the probe test than in the old location.

Chapter 7:

Discussion

During acquisition trials, escape latency decreased across the first eight days for both groups. During the retraining phase, escape latency decreased from the first trial block to the second and third trial blocks. During the probe test, both groups showed a preference for the new platform location by spending more time in Quadrant 3, where the new platform was located.

For the first eight days of acquisition, the groups demonstrated equal performance, suggesting that both groups were learning the platform location at the same rate. At this point, both groups had received equal amounts of training and were expected to learn the platform location at the same rate. During the retraining phase, the groups showed no difference in escape latency, which decreased from Trial Block 1 to Trial Blocks 2 and 3. On the probe test, both groups showed a preference for the new platform location, which suggests that the learning of the original platform had not become a higher-order habit for the group with 16 days of acquisition training. Instead, the learning of the original platform location may have still been hippocampal based and was overridden by the learning of the new platform location, causing both groups to show a preference for the new platform location. It is possible that the learning of the original platform still had not become a higher-order habit, as habits take a very large amount of training and repeated exposure to form (Devan et al., 2011).

Another possible explanation for these findings was suggested by Devan et al. (1996) in response to their findings that rats with dorsal striatal lesions were impaired on acquisition of a platform location in the water maze, but rats with hippocampal lesions

were impaired on a probe task compared to controls and striatal-lesioned rats. These findings suggest that acquisition of a platform location in the water maze is more sensitive to habit learning, associated with the striatum, while the probe test is more sensitive to spatial performance, which is associated with the hippocampus. In this case, this paradigm in the water maze may not adequately measure higher-order habit formation in the water maze based on probe test behavior. If the probe test measures spatial performance associated with the hippocampus, then higher-order habits that may have been formed may not be expressed during the probe test.

One possible procedural change that could be made to this paradigm would be to place the two platforms in quadrants that are adjacent to each other, rather than quadrants that are diagonal. This could potentially remove the time that the rats spend in the irrelevant quadrants as they swim between the two locations searching for the platform. If the two quadrants where the platforms are located are only separated by the quadrant boundaries, this could give a much more sensitive measure of where the rats are focusing their search for the platform and potentially decrease time spent traveling between the relevant quadrants.

The findings of the present study were consistent with the findings of previous research to an extent. McDonald et al. (2005) found that after training rats to find a platform in the Morris water maze for four days with eight trials per day and then giving 16 retraining trials to a new platform location, the rats showed a preference for the new platform location during the probe test. The present study found that the new platform location was also preferred during the probe test, but fewer trials were run during the retraining phase in the present study. These findings also differ from preliminary findings

reported by Devan, Chaban, Piscopello, Deibel, and McDonald (2016). Using the same paradigm in the Morris water maze, they found that after 18 days of acquisition training and eight retraining trials, saline-injected rats made more passes through the old radial quadrant than the new radial quadrant on the probe test, showing more searching behavior in the old location rather than the new location. However, Morris and Doyle (1985) also found that rats preferred the new quadrant location on a probe test that occurred two hours after an eight-trial mass training session, which is similar to the findings of the present study.

The findings of the present study also differed from the findings of Study 1, in which the rats did not show a preference for either quadrant during the probe test. However, Study 1 only used eight trials during the retraining phase, which may not have allowed for adequate learning and overtraining of the new platform location. The difficulty in replicating behavioral findings due to environmental differences is also important here again (Crabbe et al, 1999). Although one experimenter ran all of the training and testing in Study 1, scheduling conflicts caused multiple experimenters to run the training and testing in the present study. While the same experimenter ran most of the trials, a different experimenter ran acquisition training on occasion. This inconsistency may have produced changes in the environment that affected the rats' behavior.

The present findings should also be interpreted with a word of caution for a number of other reasons. Due to limited resources, the same rats that were used in Study 1 were used again in the present study. Therefore, the rats in Study 2 were not naïve rats. They had already experienced this paradigm before and were able to pick up the procedure in the water maze very quickly. However, the rats were about 5 months old at

the time of Study 2. This is consistent with prior research that utilized rats that were at least 90 days old in maze procedures (Rice et al., 2015) or that were at least 5 months old (Compton, 2004), which is the same as the present study. The rats were now older and likely had fully developed hippocampi, which could have contributed to their better spatial learning of the new platform location and their preference for this location (Martin & Bertoz, 2002). However, this acquisition training in the present study was also building onto the training from Study 1, and this combined training could have contributed to the increased learning of the platform location. The sample size was also small but comparable to typical animal research.

The findings of Study 2 may have shed some light on the use of the competitive place task in examining higher-order habit formation in the Morris water maze. Although higher-order habits may not have been formed, if they were, their expression may have not been adequately measured by the probe test paradigm used. Future research that utilizes adjustments to the paradigm by changing the platform locations and the probe test process should be made in order to best examine higher-order habit formation in the Morris water maze.

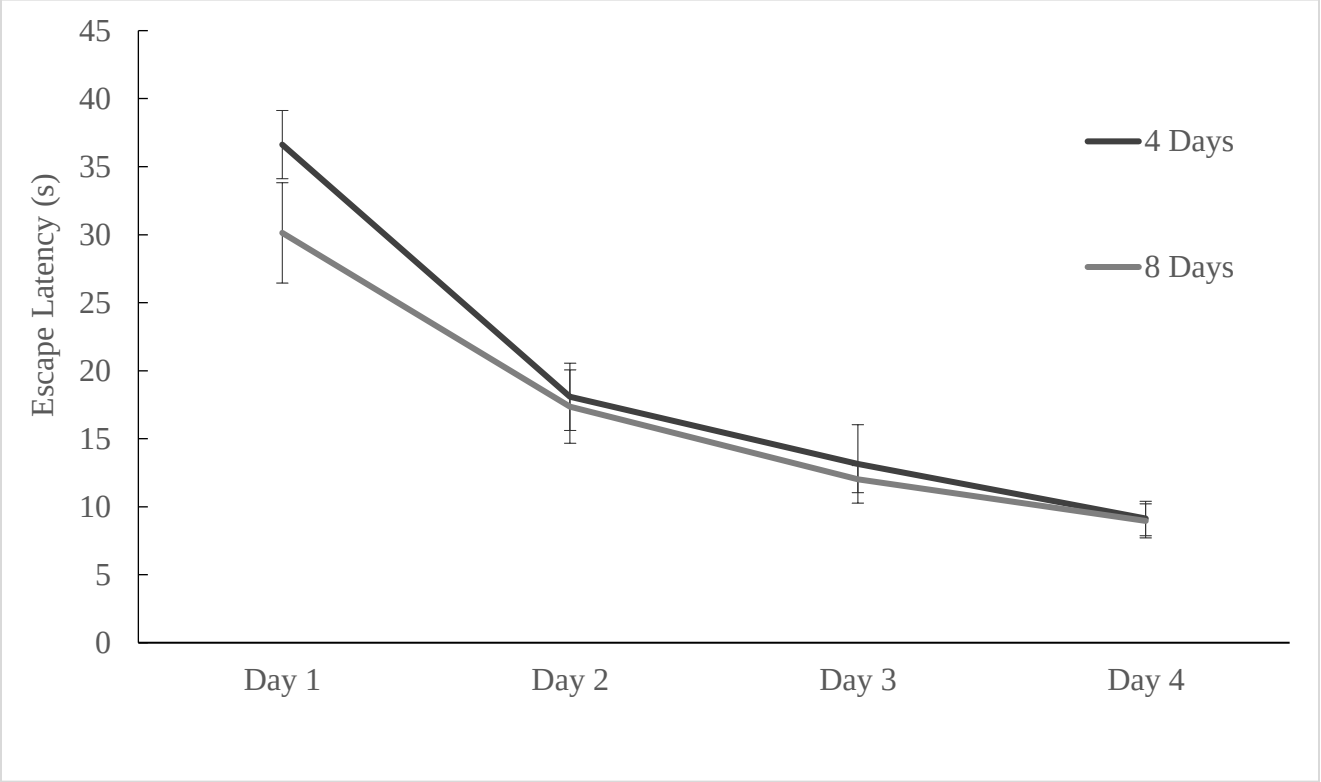

Figure 1. This figure illustrates the escape latency across the first four days of acquisition. Escape latency decreased over each day, with Days 2, 3, and 4 decreasing significantly from Day 1, and Days 3 and 4 decreasing significantly from Day 2. The groups did not differ on escape latency across the four days, showing equal amounts of learning. The standard error bars represent an estimate of the extent escape latency deviated from the average escape latency.

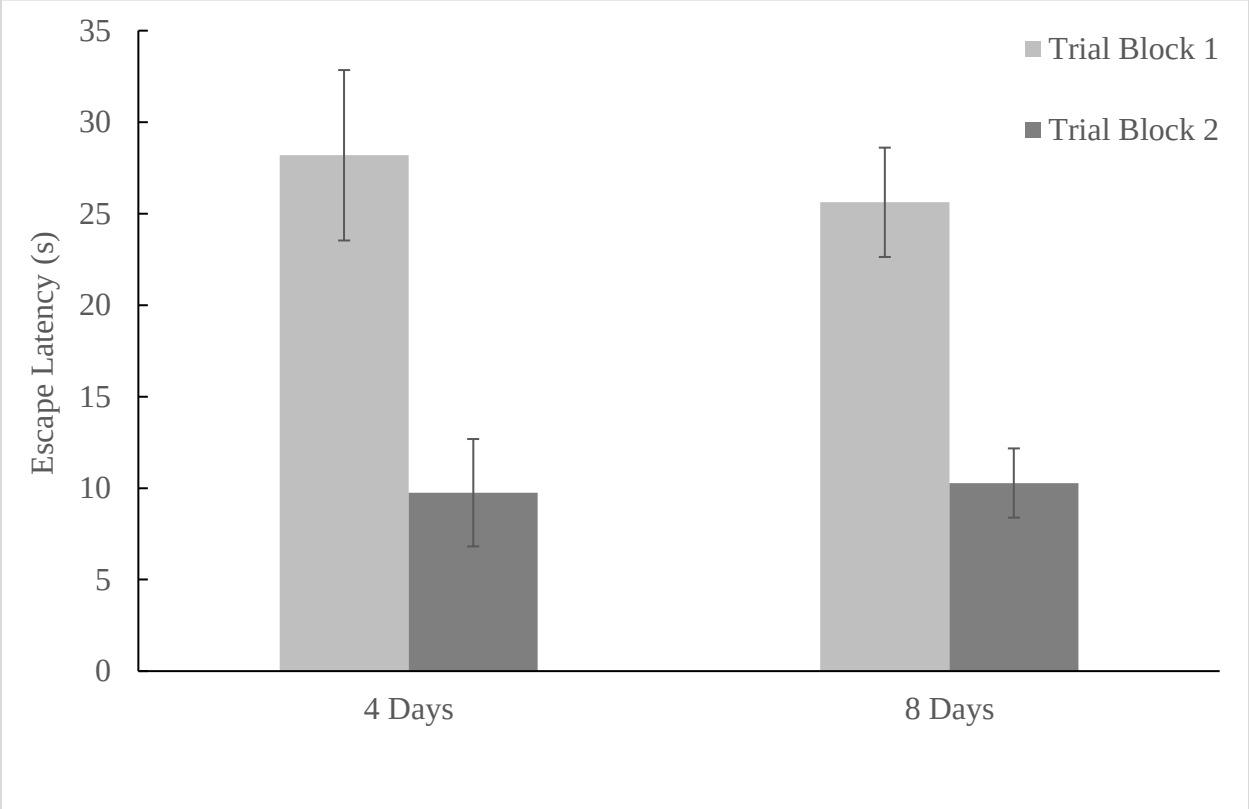

Figure 2. This figure illustrates the escape latencies for the two trial blocks during the retraining phase. Escape latency decreased significantly from Trial Block 1 to Trial Block 2. No differences were found between the two groups, showing that the group with four days of acquisition training and the group with eight days of acquisition training were learning the new platform location at an equal rate. The standard error bars represent an estimate of the extent escape latency deviated from the average escape latency.

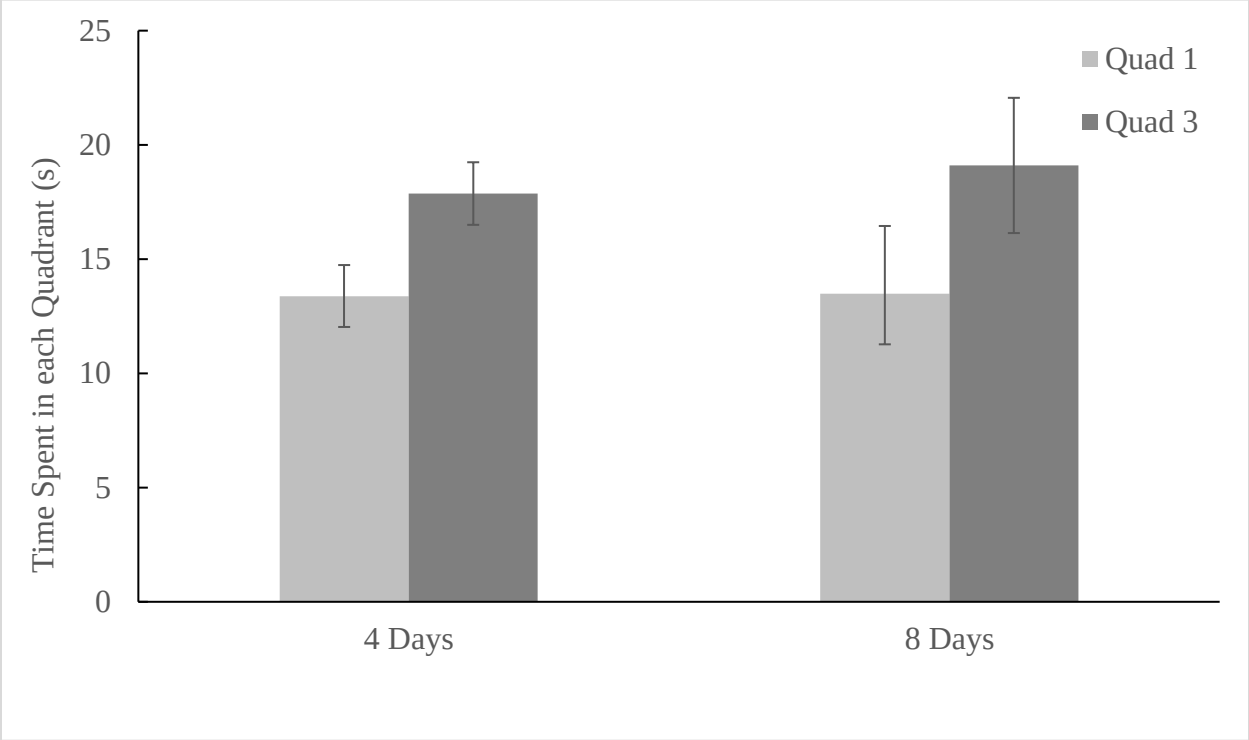

Figure 3. This figure illustrates the amount of time spent in Quadrants 1 and 3 during the probe test. The rats did not spend significantly more time in one quadrant over the other, and the groups (four versus eight days of acquisition training) did not differ on quadrant preference. The standard error bars represent an estimate of the extent quadrant preference deviated from the average quadrant preference.

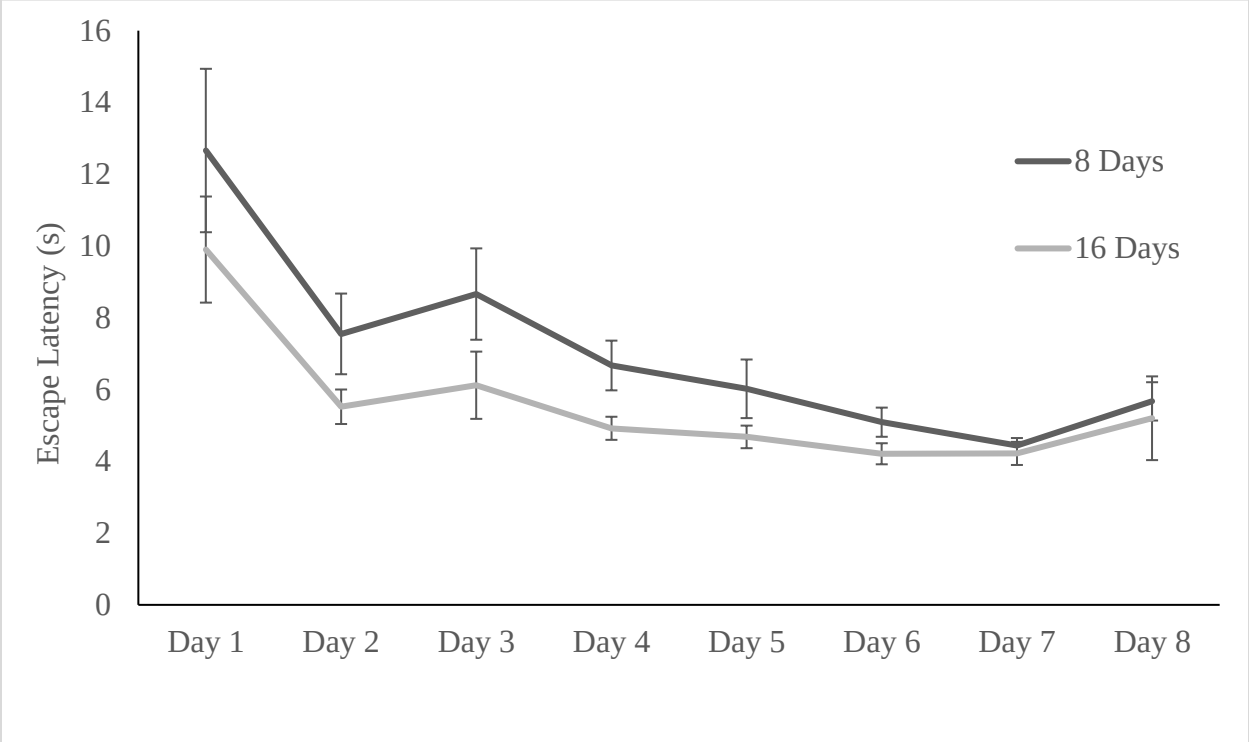

Figure 4. This figure illustrates the escape latency across the first eight days of acquisition. Escape latency decreased over each day, with Days 2, 4, 5, 6, 7, and 8 decreasing significantly from Day 1. Escape latency on Day 3 was significantly higher than on Day 7, and escape latency on Day 4 was significantly higher than on Day 6. The groups did not differ on escape latency across the eight days, showing equal rates of learning. The standard error bars represent an estimate of the extent escape latency deviated from the average escape latency.

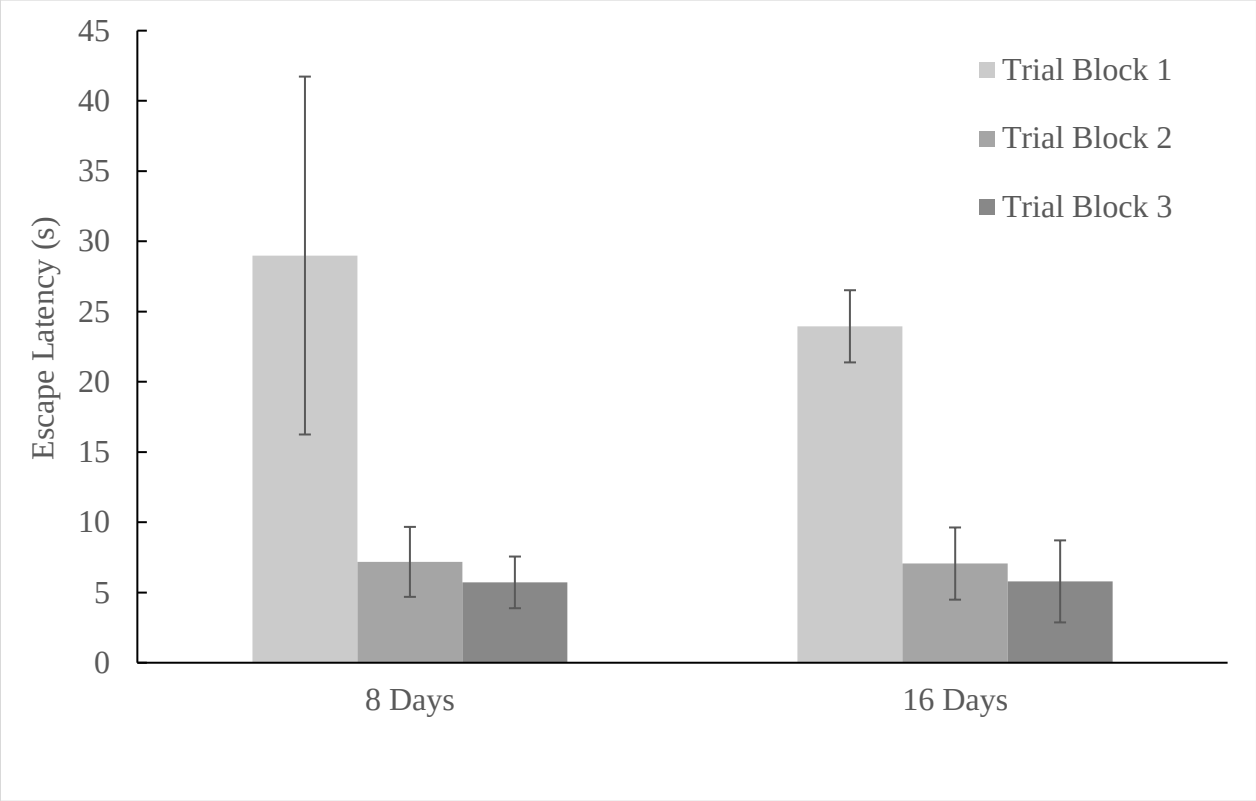

Figure 5. This figure illustrates the escape latencies for the three trial blocks during the retraining phase. Escape latency decreased significantly from Trial Block 1 to Trial Block 2 and from Trial Block 1 to Trial Block 3. Trial Block 2 and Trial Block 3 did not differ significantly on escape latency. No differences were found between the two groups, showing that the groups were learning the new platform location at an equal rate. The standard error bars represent an estimate of the extent escape latency deviated from the average escape latency.

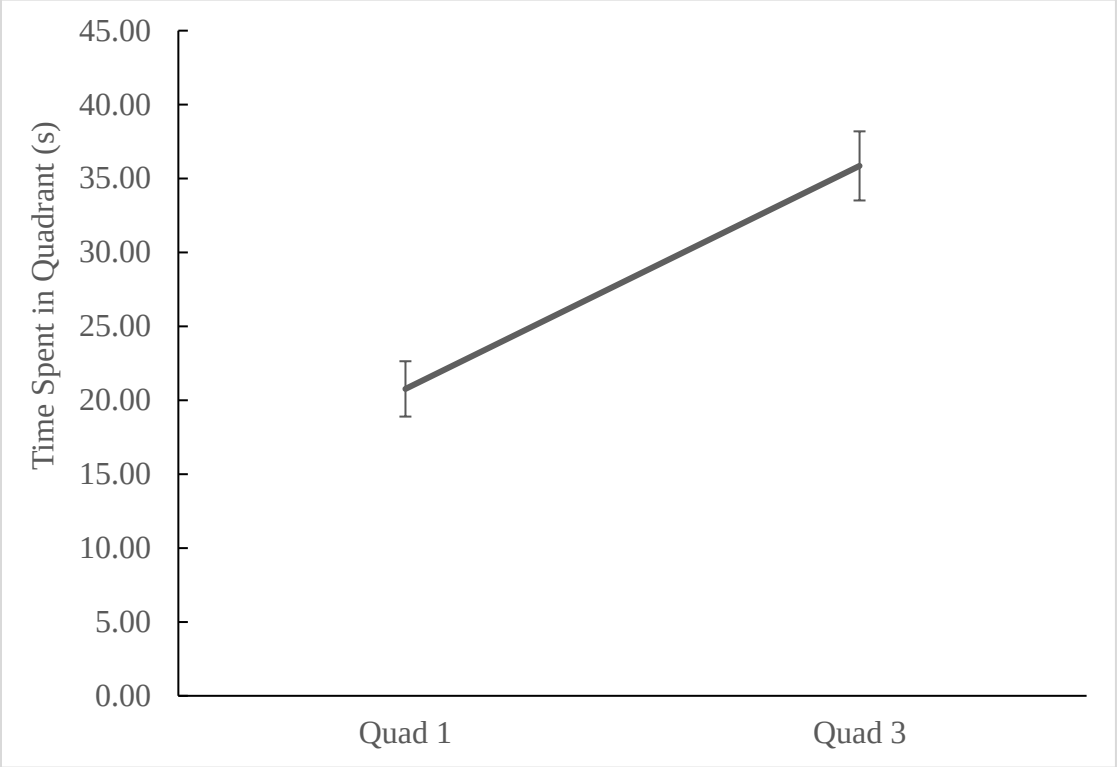

Figure 6. This figure illustrates the amount of time spent in Quadrants 1 and 3 during the probe test across both groups. The rats spent significantly more time in Quadrant 3 than in Quadrant 1. However, the groups did not differ on quadrant preference, with both groups showing a preference for Quadrant 3. The standard error bars represent an estimate of the extent quadrant preference deviated from the average quadrant preference.

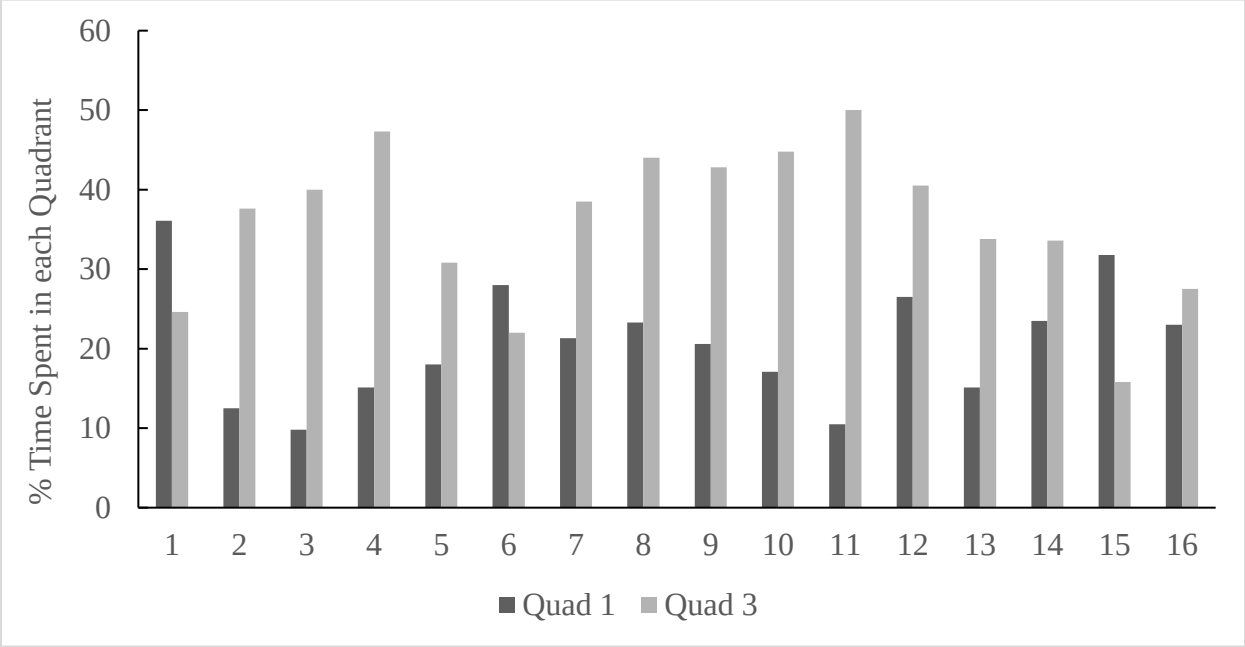

Figure 7. This figure illustrates the individual preference scores for each rat. Rats 1-8 received eight days of acquisition training, and Rats 9-16 received 16 days of acquisition training. As shown in the figure, three rats showed spent more time in the original quadrant location, while the rest of the rats spent more time in the new quadrant location.

Appendix

TOWSON UNIVERSITY

IACUC PROTOCOL: 07082014BD-01

To: Bryan Devan, PhD

From: Towson University Institutional Animal Care and Use Committee
Louis DeTolla, VMD, PhDl, DACLAM, IACUC Chairperson

Date: July 8th, 2014

RE: **IACUC PROTOCOL # 07082014BD-01**
Cognitive enhancement of learning and memory in rats

Office of Sponsored Programs
& Research
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www.towson.edu/ospr

This is to certify that the Institutional Animal Care and Use Committee has reviewed your protocol and granted FULL APPROVAL. The approval date for this protocol is July 8th, 2014.

Your protocol is approved for a period of 3 years; an annual report must be submitted to the IACUC six weeks before each anniversary of the protocol. Please note your protocol will expire July 7th, 2017. If you need to extend the protocol beyond this date, you must submit an Animal Care and Use form at least three months prior to the expiration.

If you have any questions, please do not hesitate to contact the IACUC Coordinator by email (ospr@towson.edu) or by phone (410.704.4488).

X Louis DeTolla

Louis J. DeTolla, VMD, PhD, DACLAM
Chairman, IACUC

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Paige N. Michener

[redacted]

Education

May 2017 (Expected)	**M.A., Experimental Psychology**, Towson University GPA: 3.89 Master's Thesis: *Higher-Order Habit Strength and Spatial Learning* Thesis Committee: Dr. Bryan Devan, Dr. Rick Parente, Dr. Mark Chachich
May 2015	**B.A., Psychology**, Bloomsburg University Overall GPA: 3.47 Undergraduate Thesis: *Evaluation of the Ability of Concord Grape Juice to Reverse Age-Related Latent Learning Impairment in Rats* Advisor: Dr. Eric Stouffer

Academic Honors

2014-15	Psi Chi, The International Honors Society in Psychology, Vice President
2013-15	Dean's List, Bloomsburg University

Research Interests

Learning and memory in rodent models; latent learning; dietary influences on learning and memory; age-related decline in learning and memory; higher-order habit formation of implicit memory

Research Experience

Current	Towson University Psychology Department, Lab of Comparative Neuropsychology, *Research Assistant*
2013-15	Bloomsburg University Psychology Department, Stouffer Lab, *Research Assistant*

Conferences

Stouffer, E. M., Warninger, E. E., & **Michener, P. N.** (2014). *A high-fat diet impairs hippocampal-dependent learning but spares hippocampal-independent learning.* Poster presented at the annual conference for the Society of Neuroscience (SfN), Washington, DC, November 15-19, 2014.

Research Software and Hardware

Experienced with SPSS statistical software, Microsoft Word, Excel, and PowerPoint. Experienced with using HVS Water Maze software. Familiar with the Biopac 150 and Biopac Acqknowledge analysis software used for measuring and analyzing electrodermal activity, respiration, electrocardiography, and impedance cardiography.

Manuscripts & Book Chapters

Schachtman, T. R., Richardson, R. A., & **Michener, P. N.** (in press). Stimulus-stimulus interactions and the habituation of neophobia. In S. Reilly (Ed.), *Food Neophobia*. Elsevier: Amsterdam.

Stouffer, E. M., Warninger, E. E., & **Michener, P. N.** (2015). A high-fat diet impairs learning that is dependent on the dorsal hippocampus but spares other forms of learning: High fat diet impairs hippocampal-dependent learning. *Hippocampus, 25*(12), 1-10.

Grants & Awards

2014	Bloomsburg University Travel Grant, Bloomsburg University ($350)
2014	Air Liquide America Foundation Scholarship ($1,000)
2014	Outstanding Poster Presentation in Social Sciences ($50)
2014	Undergraduate Research, Scholarship, and Creative Activities Award ($6,000)

Teaching Interests

Intro to Psychology; Experimental Psychology; Introductory Statistics; Learning and Memory; Research Methods

Teaching Experience

2017	Graduate Teaching Assistant, Physiological Psychology, Towson University (Psych 465)
2015	Teaching Assistant, General Psychology, Bloomsburg University (Psych 101)

Membership in Professional Organizations

2016-Present	Association for Psychological Science (APS)
2014-2015	Society for Neuroscience (SfN)