ASSESSMENT OF SPATIAL RESPONSE INITIATION IN THE MORRIS WATER MAZE

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Abstract

Two facets of Obsessive Compulsive Disorder are obsessions and compulsions. Compulsions are strong, maladaptive habit behaviors exhibited to reduce obsessions, or unwanted thoughts. Compulsions in OCD, in part, are a product of dysfunctional communication between the striatum/habit memory system and higher areas of cortex which creates strong habit responses that are unable to be inhibited. Successful behavioral rodent models for these strong compulsive habits have been created through overtraining and repeated trials. The present study intended to create a behavioral paradigm of compulsive checking behavior in rodents through rats receiving four, eight, and sixteen days of training on a platform location in the Morris water maze. Eight and sixteen days of training were intended to produce overtraining and spatial response initiation. Results are discussed in terms of the animals’ self-initiated spatial response towards a previously learned location after being placed in a novel location.
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Introduction

The classic place learning task in a water maze developed by Morris (1981) involves animals navigating by swimming to a hidden platform submerged in a pool of water. Designed to eliminate any proximal or local cues, this task tests the animals’ ability to utilize distal cues in the environment for spatial localization. Morris found that rats could locate the platform by learning these distal cues, proving support for O’Keefe and Nadel’s (1978) theory of cognitive mapping. This theory states that the hippocampus organizes spatial and allocentric cues to form a “cognitive map” of a given environment. Many other studies have supported this theory due to induced hippocampal damage causing a deficit in learning place tasks in comparison to control animals (Morris, Garrud, Rawlins, O’Keefe, 1982; Morris, Schenk, Tweedie, Jarrard, 1990; Sutherland & Rudy, 1988; Sutherland, Whishaw & Kolb 1983). However, when hippocampal damaged rats were tested in a cued variation (visible platform), they were not impaired. This provides evidence for the existence of a second memory system at work in spatial navigation, believed to be independent of the hippocampus and rooted in habit (Mishkin & Petri 1984; Petri & Mishkin 1994). Habits are mostly considered to be adaptive in function by providing means for quick responses without using a considerable amount of cognitive resources, however, habits can also be maladaptive as seen in extensive research conducted in relation to Obsessive-Compulsive Disorder (OCD) (Graybiel, 2008).

This paper analyzes past and current literature on evidence for multiple memory systems and explains two common memory systems, specifically focusing on anatomy and physiology of the striatum. The present studies focus on creating a behavioral model
of checking behavior in rats, one of the maladaptive habits present in OCD, using the Morris Water Maze. These experiments will help provide a protocol useful for studying the underlying neurobiology of OCD in future research.

**History of Multiple Memory Systems**

The famous case of retrograde amnesia in H. M. sparked a new field of psychological research. In 1953, H. M. underwent psychosurgery to eliminate debilitating seizures in which the medial temporal lobe was removed bilaterally, essentially removing the entire hippocampal region as well as the amygdala and entorhinal cortex (Scoville & Milner, 1957). Upon psychological examination, it was found H. M. could not recall information up to two years before the surgery or form any new memories post-surgery; however, memories from early in life were still intact. H. M.’s memory reflected partial retrograde amnesia and severe anterograde amnesia (Scoville & Milner, 1957). Subsequent testing revealed deficits in cognitive memory tasks such as digit span and stylus-maze, but adequate performance and learning on habit related tasks testing procedural memory including mirror drawing (Milner, 1970), rotary pursuit, and bimanual tracking (Corkin, 1968). H. M.’s performance on the habit tasks improved across trials; however, he had no cognitive recollection of the tasks when presented with them each day. This suggests evidence for two separate memory systems at work in the brain, one controlling cognition based memory and another controlling procedural learning.

Subsequent animal experiments have consistently replicated this type of amnesia and support the idea of multiple memory systems. Lesioning or removing the hippocampus in monkeys causes severe deficits in a delayed-nonmatching-to sample
task, in which the goal is to choose the novel object that does not match one previously learned for reinforcement. These lesions seemed to have no effect on visual discrimination habit tasks such as pattern discrimination (Malamut, Saunders, & Mishkin, 1984; Parkinson, Murray & Mishkin, 1968; Zola-Morgan & Squire, 1986). Supported by these findings, Mishkin, Malamut and Bachevalier (1984) proposed a dual neural model of memory stored within separate cognitive and habit systems.

**Declarative Cognitive Memory System**

A classic experiment conducted by O’Keefe and Dostrovsky (1971) recorded neural firings in the rat hippocampus, specifically the CA1 sub-region. They found that these “place cells” fired when the animal was oriented a particular way on a platform in response to certain environmental stimuli. This experiment led to the development of the theory of the hippocampus as the site of cognitive maps by O’Keefe and Nadel (1978), who proposed the idea that the hippocampus is responsible for organizing these spatial stimuli and creating a mental map used to guide spatial navigation. Sutherland and Rudy (1989) support this idea by acknowledging the complex configural associations that take place in the hippocampus by combining different stimuli. This is the well-known idea of the hippocampus as a memory system being S-S (stimulus-stimulus) based. Various environmental stimuli become associated with each other to form lasting cognitions and memories. This memory system is responsible for the acquisition and storage of these associations and functions on a cortico-limbic neural pathway (Mishkin et al., 1984). Many studies have shown the importance of these S-S configurations in spatial navigation tasks. Morris et al. (1982) found that rats with hippocampal lesions showed significant impairment in a traditional place learning task in the water maze, compared to
rats with superficial cortical lesions and control sham lesioned rats. Although these rats did eventually improve in performance, they were still significantly impaired in comparison to the other groups.

**Procedural Habit Memory System**

The second memory system, centered around the striatum, is considered to be a procedural memory system based in habits. This is the memory system hypothesized to be responsible for the learning evident in cases of amnesia discussed above. Based on the S-R (stimulus-response) relationship, the cortico-striatal network develops non-cognitive associations based on repeated trials that strengthen this relationship (Mishkin & Petri, 1984). Cued variants of the water maze, in which rats swim to a visible platform, are used to test the S-R habit associations made by the striatum. Seeing the platform prompts the behavioral response of swimming towards it as a means of escape. Whishaw and Kolb (1984) conducted a study with both place and cued learning tasks in the water maze. In parallel with previous research, rats with lesioned hippocampi were unable to learn the traditional place task, but were able to swim to a cued (visible) platform. When a second group of rats received lesions to the basal ganglia, the collection of nuclei which includes the striatum, cue learning was eliminated. Many other studies have replicated these findings showing the involvement of the striatum in discriminate and cued learning tasks (Devan, Goad, & Petri, 1996; McDonald & White, 1994).

**Neuroanatomy of the Striatum**

The basal ganglia is a collection of subcortical nuclei which includes the caudate nucleus, putamen, globus pallidus and the substantia nigra (Devan, Hong, & McDonald, 2011; Wise, 1991). The caudate nucleus and the putamen are jointly referred to as the
striatum. In rats there is no clear separation of these two structures as in humans, so the terms caudate-putamen (CPu) complex or dorsal striatum (DS) are used interchangeably. The striatum is also broken down into dorsolateral (DLS), dorsomedial (DMS), and ventral striatal (VS) areas (Devan et al., 2011). Somatosensory and motor information from the parietal lobe project into the DLS, while auditory and visual information from the temporal and occipital lobe, respectively, project into the DMS. Also, areas of archaecortex including the entorhinal, hippocampus and amygdala send information to the VS (McGeorge & Faull, 1989).

**Neurophysiology of the Striatum**

Traditional views of memory label the basal ganglia and striatum as mechanisms of habit memory. Assuming this is true, research on sub-regions of the DS has shown puzzling findings contradictory to this idea. Devan, McDonald, and White (1999) conducted an experiment to test the idea of heterogeneity of function within the DS itself. Three groups of rats were assigned to either lesions of the dorsolateral CPu, dorsomedial CPu or sham surgery. These rats were tested in the Morris water maze on traditional place learning, cued learning, and place retention tasks. They found that in the place learning (hidden platform) task, rats with dorsomedial CPu lesions took significantly longer to escape in initial training than the other two groups. After ten days of trials, these rats were not significantly different and had appeared to learn this task. However, in initial training of the cued task, the dorsomedial lesioned CPu rats were impaired. Following these trials, a competition test was conducted including both a hidden and visible platform in the pool. This showed that control rats escaped more frequently onto the visible platform, while rats with dorsolateral CPu lesions escaped more often on the
hidden platform, and dorsomedial CPu rats escaped significantly more often on the visible platform rather than hidden. Since the rats with dorsomedial CPu lesions showed a bias for the visible platform over the hidden, it was concluded that damage to the medial portion induced deficits in spatial navigation to a hidden platform. In contrast, dorsolateral CPu lesioned rats exhibited a stronger response to the hidden platform which suggests impairment in discriminatory learning in the visible platform task. This provides evidence that there are functional differences between the dorsomedial and dorsolateral CPu.

Devan and White (1999) replicated these findings and showed that even though rats with dorsolateral lesions were eventually able to learn the cued task, they showed significant behavioral bias for the hidden platform in a competition test; therefore, learning of the cued task was impaired. Rats with dorsomedial lesions showed a significant bias for the visible platform, which shows lesioning this area significantly impairs learning of a place task. To further expand upon these findings, a second experiment was conducted using unilateral and asymmetric lesions. They lesioned the medial CPu unilaterally in one group and the hippocampus unilaterally in a second group. A third group received asymmetric lesions to both the medial CPu and hippocampus. They found that in a competition test, both unilaterally lesioned groups did not show a clear bias towards hidden or visible platforms. However, the asymmetrical group with both lesions showed a significant bias for the cued, visible task. This suggests that the dorsomedial CPu and hippocampus act cooperatively to produce spatial navigation. Rats with unilateral lesions were able to retain and show a bias for the hidden platform due to the other structure being intact, meaning that rats with medial lesions utilized the
hippocampus to learn the spatial task and vice versa. The asymmetrical lesions show that damaging both structures essentially eliminates the communication between the two, resulting in an impairment in spatial navigation. However, this leaves the dorsolateral CPu intact and functional to mediate learning of the cued task. These findings suggest cooperation between the dorsomedial CPu and hippocampus to accomplish spatial learning and the seemingly competitive interaction of the dorsomedial CPu with the lateral region which mediates habit memory. Also in this experiment, the lateral CPu lesions caused the rats to respond out of habit to where they had previously learned the hidden location before escaping onto the visible platform in the competition test (Devan & White, 1999). These findings show evidence for heterogeneity of function between striatal sub-regions and their complex roles in mediating goal-directed behavior in the water maze.

**Striatal Role in Obsessive-Compulsive Disorder**

Obsessive-Compulsive Disorder (OCD) is a neuropsychiatric disorder characterized by two hallmark symptoms: obsessions and compulsions. Obsessions are recurring unwanted thoughts, while compulsions are defined as repetitive and unwanted behaviors exhibited in efforts to reduce obsessions (APA, 2013). Compulsions can include repetitive behaviors centered around organizing, cleaning, and checking things such as that doors are locked. These behaviors are also related to high levels of anxiety (Burguière, Monterio, Mallet, Feng, & Graybiel, 2015). OCD is largely believed to be related to dysfunction in neural substrates, specifically along cortico-basal ganglia-thalamo-cortical loops (Szechtman et al., 2014; Wise & Rapoport, 1989). This feedback loop initiates through input of sensory and motor information from areas of cortex to the
basal ganglia and thalamus, which in turn relay information back to the cortex to plan and produce movement (Parent & Hazrati, 1995).

Evidence supporting this dysfunction has been found through functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) techniques which provide information regarding network connectivity in the brain. Specifically, functional connections between the caudate nucleus, orbitofrontal and anterior cingulate cortices were stronger in OCD patients than in controls, while connections of the caudate nucleus and temporal cortex were significantly weaker (Harrison, et al., 2013). Zhong et al. (2014) used DTI to compare functionality of white matter tracts in unmedicated OCD patients to controls. OCD patients had significantly lower efficiency in connections between the striatum, frontal lobe, and parietal lobe; specifically, connections to the precentral gyrus, the primary motor cortex, were weaker. These findings show physiological differences in the striatum of OCD patients, which supports the idea that dysfunctional communication between the striatum and areas of cortex could contribute to OCD symptoms.

The “habit hypothesis” of OCD acknowledges the role of the striatum in producing ritualized, repetitive checking habits through dysfunction of its neural circuitry. Graybiel (2008) defines habits as a learned ritual that occurs repeatedly over time in response to specific environmental stimuli. In OCD, compulsions become habits due to their repetitive nature which strengthens the stimulus-response relationship over time. Similar to all other habit formation, these responses eventually become automatic and motoric in nature. In the case of OCD however, these habits are considered maladaptive (Graybiel, 2008) due to clinical patients’ inability to control their checking
behavior. Checking behavior notably is centered around repeating a behavior in efforts to eliminate possible harms (Rachman, 2002) and reduce uncertainty in the environment (Burguière, et al., 2015).

This ritualistic behavior has also been attributed to lack of behavioral flexibility in OCD patients, as they are largely unable to change their behavior upon being presented with new stimuli. This has been tested in experimental reversal designs involving goal-directed behavior to avoid an aversive stimulus. Gillan, et al. (2014) examined this phenomenon through a reversal design using a shock paradigm where clinical OCD patients and control individuals were trained to depress pedals with either their left or right foot to avoid shocks to their hand of the same side. Being presented with a blue stimulus was indicative of a shock to the right hand which could be avoided by depressing the right pedal, while a red stimulus indicated a shock to the left hand and could be avoided with a pedal on the left side. After brief training of 3 trials per stimulus, both groups successfully learned the correct responses and the stimulus was devalued by disconnecting the electrodes to one hand. A test of devaluation revealed significantly more responding to the active stimulus than the devalued one in both groups, showing the OCD patients were able to learn the devalued stimulus meant being safe from shock.

Following devaluation, a habit test was conducted after both groups received overtraining for 30 trials per stimulus. In a final devaluation phase, the electrodes were again detached on one hand in plain view of the participants. The results of the habit test showed OCD patients responded significantly more frequently to the devalued stimulus after being overtrained than control participants. Even though they could learn devaluation initially, their reflexive habit responses were strengthened extensively.
through overtraining resulting in failure to inhibit their response to the devalued stimulus. This study shows support for behavioral inflexibility in OCD as well as the failure to control strong habits in relation to goal directed behavior through avoidance of shock.

**Rodent Models of Obsessive-Compulsive Disorder**

Compulsive checking behavior has also been shown in rodent models, which is characterized by fixation with one particular place in an environment (Dvorkin et al., 2010). Multiple studies have examined this behavior by inducing OCD symptomology pharmacologically with the drug quinpirole in an open field test paradigm, showing a more extensive distance traveled by treated rats and compulsive spatial responses to fixed objects within the environment (Dvorkin, Perreault, & Szechtman, 2006; Dvorkin et al., 2010). Tucci et al. (2014) conducted an experiment using the open field paradigm in conjunction with a surgical and pharmacological manipulation to produce rodent checking behavior. They found that rats which received a bilateral lesion to the nucleus accumbens core (NaC), a part of the basal ganglia that makes up the ventral striatum, engaged in checking behavior patterns similar to rats that were given quinpirole (Dvorkin et al., 2006; Dvorkin et al., 2010) and checked significantly more than control sham lesioned rats (Tucci et al., 2014).

Behavioral manipulations have also successfully produced compulsive responding by using reversal designs, in which rats are presented with a novel stimulus and the previously learned stimulus no longer provides reinforcement. These designs have been used in conjunction with the signal attenuation model which states that these compulsive behaviors are due to procedural deficits in completing goal-directed responses (Fineberg, Chamberlain, Hollander, Boulougouris, Robbins, 2011). Joel and Avisar
(2001) used these models in a study where rats were trained to press a lever to receive a food reward. The presentation of the food was also paired with a light and sound compound stimulus. During signal attenuation, upon pressing the lever rats were only presented with the compound stimulus and no food reward, devaluing the stimuli. The results showed rats excessively pressing the lever, even after they had learned there was no reward of food available. This behavior can be attributed to the lack of successful feedback connections between the basal ganglia and cortex (Szechtman et al., 2014), and it also reflects upon the lack of behavioral flexibility seen in the human OCD population when responding to new stimuli (Gillan, et al., 2014). They are unable to inhibit the habit that became strengthened through repeated trials, especially since this paradigm includes the goal-directed behavior of receiving food (Fineberg, et al., 2011).

**Experiment 1**

Behavioral paradigms have been successfully utilized to produce compulsive checking behavior in rats involving both goal-directed behavior and responses towards specific locations in an environment (Fineberg, et al. 2011; Tucci, et al. 2014.) However, most of the literature has examined these phenomena individually. Testing compulsive spatial responding in a particular environment has largely been completed using an open field test, while testing malfunctions in goal-directed behavior includes using food rewards in typical S-R paradigms such as bar pressing. The present study was designed to combine both features of OCD by testing them in the Morris water maze using a variation of the competitive place task designed by McDonald, Hong, Craig, Holahan, Louis, and Muller in 2005. Inherently, the water maze motivates goal-directed behavior in rats by using water as an aversive stimulus. Rats swim through the water with the goal
of finding means for escape onto the hidden platform. Using the variation of the competitive place task provides a way to test checking behavior between two locations. Rats were trained or overtrained on one initial place in the maze, and were then be placed onto a platform in a new location for a long duration and left to respond freely with their spatial response initiation being measured. After being placed on the platform, rats should begin to experience anxiety upon not being removed from the platform after a long delay. This anxiety is theorized to be representative of the anxiety present in OCD (Burguière, et al., 2015), since the rats are safe on a platform but will become anxious and want to check for another means of escape. They then should go back to the old location to check for the original platform. A strong spatial response initiation would be defined as the rats leaving the new platform to swim to the old location in search of escape, and spending a significant amount of time in the old quadrant relative to the new quadrant. A strong spatial response initiation would be evident when rats pass through the original platform location a greater number of times. I hypothesized the rats that were overtrained would show a stronger compulsive spatial response initiation towards the old location in both time spent in the original quadrant and amount of times passing through the original platform location. This would be the result of the overtrained group building a stronger and possibly compulsive habit response towards the old location, reflecting compulsive checking behaviors in OCD (Graybiel, 2008).

Method

Subjects

Sixteen Long-Evans hooded rats (Harlan Sprague Dawley), approximately three to four months of age, were single housed in a colony room that was maintained on a
12:12 hour light-dark cycle with a temperature of 21 ± 1° C. All animals had access to food and water *ad libitum*. Prior to water maze training, animals were experimentally handled for three days to acclimate them to the procedure. Training was conducted at approximately the same time each day during the light phase.

**Apparatus**

The Morris water maze used in this experiment was a white, circular pool with a diameter of 172 cm and a height of 63 cm, elevated 20 cm from the floor. The water maze is centered in the testing room which contains several environmental cues such as a door, ladder, computer desk, sink, and overhead lights. Devan, Tobin, Dunn, and Magalis (2016) provide an in-depth description of the testing room for further reference. The pool water was made opaque with white non-toxic paint (Crayola) and measured approximately 38 cm in height. The water temperature was maintained at 23 ± 2° C. The white platform, a circle 10 cm in diameter, was submerged 1-2 cm below water level.

A ceiling mounted video camera accompanied by a VHS recorder was used to record experimental trials. HVS Image Video Tracking software will record various performance measures such as escape latency, swim speed, and time spent in designated quadrants.

**Procedure**

**Phase 1: Acquisition Training.** Animals were individually placed into the water maze facing the pool wall, ensuring that the head did not become submerged. Upon release, they were allotted a maximum time of 60 seconds per trial to escape onto the hidden platform. If they did not escape within 60 seconds, the experimenter gently guided them to the platform by hand. After escaping, the animals were required to
remain on the platform for five seconds before being removed from the pool and placed in a holding cage until the next trial. The 16 rats were randomly assigned to one of two groups, receiving four trials per day for either 4 (Group 1) or 8 (Group 2) consecutive days. Four start points in the maze were counterbalanced each day, while the position of the hidden platform remained fixed in one particular quadrant.

**Phase 2: Spatial Response Initiation Test.** Following acquisition, both groups received a single test trial involving placement on a partially submerged platform in the quadrant diagonally opposite to the original platform location. After placement, the trial ran for four minutes, including the time taken to initiate swimming. During the trial, rats have been observed to initiate a spatial response towards the original platform.

**Results**

**Phase 1: Acquisition Training**

A 2 (Group: 1 vs. 2) X 2 (Trial Block: Block 4 vs. Block 4) between-subjects analysis of variance was conducted on escape latency to ensure equal level of learning in both groups after 4 initial days of acquisition. The analysis revealed no significant difference in escape latency, $F(1,14) = 0.86, p = .368, \eta^2_p = .06$ [90% CI: .00, .28], such that Group 1 ($M = 16.97, SD = 9.24$) and Group 2 ($M = 13.43, SE = 5.55$) had achieved similar levels of learning after 4 days of training. An independent $t$-test was also conducted on escape latency to compare the last trial block of Group 1 (Block 4) and Group 2 (Block 8) to determine if overtraining in Group 2 provided further learning. The analysis showed a significant difference in escape latency between the groups, $t(14) = 3.25, p = .006, d = 1.64$ [95% CI: -1.87, 5.15], such that Group 2 ($M = 5.98, SD = 2.52$) showed further learning and improved performance after four extra days of training in
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comparison to Group 1 ($M = 16.97, SD = 9.24$), as demonstrated by a lower escape latency (see Figure 1).

![Figure 1. Means for Final Block of Acquisition Training per Group](image)

**Phase 2: Spatial Response Initiation Test**

An independent $t$-test performed on the mean time spent in the original quadrant revealed no significant differences between the groups, $t(14) = 0.36, p = .726, d = 0.19$ [95% CI: -2.08, 2.46], meaning that Group 1 ($M = 7.89, SD = 4.01$) and Group 2 ($M = 8.78, SD = 5.76$) spent equal time in the original quadrant. A second independent $t$-test was conducted to compare mean number of times each group passed through the original platform location within the original quadrant and showed no significant difference, $t(14) = 1.48, p = .160, d = 0.75$ [95% CI: -0.48, 1.99]. Group 2 ($M = 4.88, SD = 3.40$) did, in fact, pass through the original location more than Group 1 ($M = 2.88, SD = 1.73$), but not significantly more.

**Discussion**

These data show that eight days of acquisition training was unsuccessful in producing overtraining effects and compulsive checking behavior in rodents, since rats with four and eight days of acquisition training did not significantly differ on any of the recorded measures. The hypothesis of equal learning levels between groups after four initial days of training was supported, due to the groups intentionally being
experimentally identical. However, no further hypotheses were supported as the group with extra training did not exhibit greater spatial response initiation toward the old platform location.

**Experiment 2**

The procedure in Experiment 1 was unsuccessful in creating spatial response initiation due to a lack of extensive training; therefore, a second experiment was conducted with the goal of producing the desired behavior. A within-subjects design was used approximately four weeks following Experiment 1 by administering an additional 16 days of acquisition training to both Group 1 and Group 2. It is expected that both groups will show an increase in spatial response initiation behavior, with Group 2 showing a higher increase due to total extra four days of training over Group 1.

**Method**

**Subjects**

All sixteen Long-Evans hooded rats used in Experiment 1 were used in Experiment 2 in their respective pre-assigned groups. The apparatus and materials used in Experiment 2 were also identical to those used in Experiment 1.

**Procedure**

**Phase 1: Acquisition Training.** Daily procedures for Experiment 2 were identical to those used in Experiment 1. Subjects were individually placed in the pool, facing the pool wall, and were given a maximum time of 60 seconds to escape onto the hidden platform. All sixteen subjects completed four trials a day, starting from four different counterbalanced start points in the pool, and completed 16 days of acquisition training. Therefore Group 1, which initially completed four days of acquisition training
in Experiment 1, received an additional 16 days of training resulting in a total of 20 days of training on the hidden platform location. Group 2 received 8 days of initial training in Experiment 1, and also received an additional 16 days of training in Experiment 2 to make a total of 24 days spent learning the hidden platform location.

**Phase 2: Spatial Response Initiation Test.** On the 17th day of experimental testing following acquisition, all 16 subjects completed a single test trial in which they were placed on a second platform location. The procedure on this day was identical to the test day in Experiment 1.

**Results**

**Phase 1: Acquisition Training**

A mixed Repeated Measures ANOVA was conducted with the between-subjects factor of group (Group 1 vs. Group 2) and the within subjects factor 16 trial blocks in order to ensure an equal level of learning over time. This analysis revealed a significant within-subject effect of escape latency, $F(15,210) = 15.52, p < .001, \eta^2_p = .53 [90\% CI: .42, .56], \text{Power} = 1.00$, showing that escape latency significantly decrease from day 1 of training to day 16, regardless of group membership. Tests of between-subjects effects showed no difference between the groups in escape latency, showing that they achieved equal levels of learning as expected (see Figure 2).
Phase 2: Spatial Response Initiation Test

A 2 between (Group 1 vs. 2) and 2 within (Experiment 1 vs. Experiment 2) mixed repeated measures analysis of variance was conducted on the mean percentage of time spent by both groups in the original quadrant. The analysis showed no significant within subject effect of Experiment, $F(1,14) = 1.08, p = .317, \eta_p^2 = .07$ [90% CI: .00, .31], Power = .16, such that each group performed similarly across experiments. The analysis showed no significant between subject effect of Group, $F(1,14) = 0.83, p = .379, \eta_p^2 = .06$ [90% CI: .00, .16], Power = .135, and no significant interaction of Group X Experiment, $F(1,14) = 1.41, p = .254, \eta_p^2 = .07$ [90% CI: .00, .34], Power = .20.

A second 2 between (Group 1 vs. 2) and 2 within (Experiment 1 vs. Experiment 2) mixed repeated measures analysis of variance was conducted to analyze the number of times each group passed through the original quadrant platform location. The analysis showed no significant main effect of Group, $F(1,14) = 1.82, p = .199, \eta_p^2 = .12$ [90% CI: .00, .36], Power = .24; however, a significant main effect of Experiment was found, $F(1,14) = 5.02, p = .042, \eta_p^2 = .41$ [90% CI: .01, .50], Power = .55, which can be explained by an Experiment by Group interaction, $F(1,14) = 4.29, p = .05, \eta_p^2 = .24$ [90% CI: .00, .41].
CI: .00, .40] Power = .49 (see Figure 3). Simple effects dependent \( t \)-tests indicated that Group 1 passed through the original platform location significantly more in Experiment 2 than Experiment 1, \( t(7) = -3.90, p = .007, d = 1.34 \) [95% CI: 0.55, 2.13], but Group 2 passed through the location equally in both experiments, \( t(7) = .10, p = .921, d = 0.04 \) [95% CI: -0.75, 0.82]. An independent \( t \)-test was conducted on both group performance in Experiment 2 and showed that Group 1 \( (M = 9.25, SD = 3.92) \) passed through the original location more than Group 2 \( (M = 5.13, SD = 3.87) \), \( t(14) = 2.12, p = .05, d = 1.13 \) [95% CI: -0.65, 2.92].

![Figure 3. Ordinal Group by Experiment Interaction in Group 1](image)

**Discussion**

Following null findings in Experiment 1, Experiment 2 was conducted to determine if a more extensive period of acquisition training would produce a stronger spatial response initiation towards the original platform location. Both experimental groups were given an additional 16 days of acquisition training on the original platform location, with the expectation of having both groups increase their spatial response initiation towards the old location after being placed on a new platform. It was also
expected that Group 2 would demonstrate greater spatial response initiation due to receiving more extensive acquisition training lasting a total of 24 days, versus the 20 days for Group 1.

In Experiment 2 both groups learned equally in acquisition, but did not perform equally on the spatial response initiation test. Analysis of the spatial response initiation measures showed that Group 1 and Group 2 spent an equal amount of time in the quadrant containing the original platform, but that Group 1 passed through the specific platform location a greater number of times than Group 2. Group 2 passed through the location more frequently and rapidly, since they did not spend a greater amount of time in the quadrant. This is indicative of checking behavior because the rats swam to the original location to check if the platform was there as a means of escape and continued to leave and return again, similar to checking bouts. They did not stay in the location for an extensive duration.

Although the interaction effect was $p = .05$ and not below that criteria, the graph and $t$-tests conducted show a significant difference in Group 1’s performance across the experiments. Even though Group 2 received more training overall and were expected to show a greater behavioral response, adding an extra 16 days to the eight days they had already experienced did not give them a greater response. This could be attributed to these rats having a longer exposure to acquisition in Experiment 1 and therefore, they would respond similarly after any number of additional days spent training. Group 1, rather, received a brief exposure to acquisition in Experiment 1 and showed a marked increase in their spatial response initiation in Experiment 2. These data are contradictory to what was expected and suggest that a protocol to produce this behavior could be
conducted in stages, and should not merely focus on adding a substantial number of days making the training more extensive.

One possible explanation for the varied behavioral responses across the experimental groups is the competitive dynamic between the dorsomedial and dorsolateral sections of the striatum. Past findings have shown the dorsomedial striatum to be involved with spatial learning, whereas the dorsolateral striatum contributes to habitual learning (Devan, McDonald, & White, 1999; Devan & White, 1999). In the present study, rats were expected to show a strong habit response towards the original platform location through a spatial response initiation; however, as shown by the results, not all rats exhibited this response. This is potentially due to the cognitive control of the dorsomedial striatum influencing rats to not initiate a response toward the old location since they were safe on the new platform. The rats that showed a stronger spatial response could have been displaying effects from the dorsolateral striatum, causing them to use their habit memory to check the old location. These individual differences in behavioral responses would be useful to examine in future research to differentiate the contribution of the different striatal regions at work in spatial response initiation.

One prevalent limitation in rodent research is a small sample size, and these experiments are no exception. Several of the analyses conducted resulted in $p$ values that were at the .05 level and not below which is the conventional standard for significance testing. With a larger sample size to accompany this protocol, that level may have been achieved. Also, a small sample size leaves room for individual differences among rodents which are difficult to account for and can often skew the data. This causes
greater variability within experimental groups and makes it more difficult to achieve the conventional levels of statistical significance.

Another limitation of the present study is that even though there was some success in creating spatial response initiation, a 20 or 24-day protocol is impractical to use as a model of this behavior simply in the interest of time. Future research should include focusing on developing a more efficient protocol that uses training stages, with a brief initial exposure to acquisition training followed by more training in stages of consecutive days. This would help to strengthen the habit responding to produce stronger checking behavior. A combination of behavioral and pharmacological interventions could also be used to produce this behavior in a more time efficient manner. Once a successful model has been produced, research efforts should focus on reducing the behavior with a possible pharmacological intervention.

Past research has shown successful behavioral manipulations for producing compulsive behavior in rats using locations in an environment as well as reversal designs (Fineberg et al., 2011), which are both aspects that can be included in the Morris water maze. The water maze provides the perfect venue to create a protocol for this behavior, but the specific methodology needs fine tuning. Improving upon this research by designing a methodology for OCD-like checking behavior in rodents could also give insight on how to stop these behaviors from occurring. Ultimately a pre-clinical rodent study such as this could help bring about new treatment implications for the OCD population.
References


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Curriculum Vita

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