LEARNING-INDUCED CHANGES IN MUSCARINIC RECEPTOR BINDING DENSITY AS A FUNCTION OF COGNITIVE STRATEGY

AN ABSTRACT SUBMITTED ON THE THIRD DAY OF APRIL 2013
TO THE NEUROSCIENCE PROGRAM IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE SCHOOL OF SCIENCE AND ENGINEERING OF TULANE UNIVERSITY FOR THE DEGREE OF
MASTER OF SCIENCE
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Abstract

Evidence from previous studies on the multiple memory systems model suggests that specific brain regions cooperate and compete to mediate the navigational strategies used to locate a goal in a spatial environment. Specifically, the cholinergic system within these discrete brain regions plays a key role in balancing this mediation such that acetylcholine release, genomic changes, and receptor regulation at cholinergic synapses are altered following learning and subsequent memory consolidation. Based on previous findings, we proposed to test learning-induced changes in muscarinic receptor binding expression in adult male rats following training on a water maze task guided either by a cue proximal to the escape platform (stimulus-response strategy), by cues surrounding the maze (place strategy) or by alternating between the two strategies (strategy-switching). The primary findings of the current study demonstrate that adult male rats that navigated to an escape platform guided by cues surrounding a water maze (place-trained) learned the task at a significantly slower rate than males that were guided by a cue proximal to the platform (stimulus-response-trained) or males that were required to switch strategies on alternating days. Additionally, males that were required to switch strategies over alternating days expressed higher ratios of muscarinic binding in the hippocampus relative to the striatum compared to place-trained rats, stimulus-response-trained rats, and swim-only controls. These results indicate that the use of a place learning strategy slows acquisition of a water maze task while the requirement to switch strategies as the demands of the task change over days engages the cholinergic system in the hippocampus most heavily. Taken together, the results from the current study further confirm the

involvement of cholinergic function in regulating the balance between multiple memory systems.

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Introduction

Brain Systems Implicated in Multiple Memory Systems

Specific brain regions in both humans and rodents process qualitatively different types of information when navigating toward a goal in a three-dimensional environment (McDonald and White, 1994; Packard, 1999; Packard and McGaugh, 1996). The learning and memory processes that guide the goal-directed behaviors are categorized into distinct types of learning strategies (Packard 2009). A place learning strategy relies upon the relationships between extra maze cues and a goal and is contingent upon optimal functioning of the hippocampus (O'Keefe and Nadel, 1978; Packard, 1999; Packard and McGaugh, 1996). Execution of a place learning strategy is impaired following either permanent lesions of the hippocampus, temporary inactivation of sodium channels in the hippocampus, or lesions of the afferent inputs to the hippocampus that project from the basal forebrain (Carli et al., 1997; Davoodi et al., 2009; Kaut and Bunsey, 2001; Koboyashi et al., 2000; McDonald and White, 1994). Alternatively, the dorsolateral striatum mediates execution of both response and stimulus-response navigational strategies, which depend on either specific body turns toward a goal or upon a discrete cue proximal to a goal that signals its location, respectively (Packard 1999; Packard and McGaugh, 1996). Accordingly, damage to the striatum results in impaired performance on response or stimulus-response learning tasks (Compton, 2004; Packard and McGaugh, 1992).

The results of a double dissociation experiment employing a place version and a stimulus-response version of a two-platform water maze task indicated that the hippocampus and striatum mediate different types of learning and memory in an

independent fashion (Packard and McGaugh, 1992). In their experiment, the two submerged platforms were affixed to rubber balls with different visual patterns. However, only one of the two platforms could be mounted for escape. On the spatial or place version, the correct escape platform was located in the same quadrant for each trial and the visual pattern varied across trials. On the cued or stimulus-response version, the correct escape platform had the same visual pattern on each trial and varied in spatial location across trials. Lesions to the fimbria-fornix, which provides input to the hippocampus, resulted in poorer performance on the place version but not on the stimulus-response version, while animals with lesions to the striatum performed poorer on the stimulus-response version but not the place version (Packard and McGaugh, 1992). Interestingly, on learning tasks that can be solved by relying on either brain structure, inactivation of the striatum leads to execution of a place strategy rather than a response strategy on a dual solution task in which rats could use either a place or response strategy (Packard and McGaugh, 1996).

In addition to the independent nature of the two brain structures, there also is evidence that the hippocampus-based and striatum-based memory systems can interact in either a cooperative or competitive fashion to coordinate learning. For instance, early in training, adult male rats tend to adopt a place learning strategy but defer to a striatum-dependent learning strategy as training progresses (Packard, 1999; Packard and McGaugh, 1996), which indicates cooperation between memory systems. Alternatively, dysregulation of hippocampus function results in enhanced performance on a striatum-dependent task (Chang and Gold, 2003; Compton, 2004; Lee et al., 2008; Schroeder et al., 2002), which indicates competition between the two memory systems. Furthermore,

competition between the hippocampus and dorsolateral striatum appears to be bi-directional, such that excitotoxic lesions to the striatum result in an enhancement in performance on a hippocampus-dependent task (Compton, 2004; Lee et al., 2008).

Interestingly, although the amygdala serves as the primary brain structure for emotional information processing (LeDoux, 1993; Makarchuk et al., 1981; Paz and Pare, 2013), there is evidence that the amygdala works in cooperation with the striatum and in competition with the hippocampus to modulate navigational strategies (Wingard and Packard, 2008). Post-training injections of an anxiogenic drug into the basolateral amygdala impaired place learning on the hippocampus-based water version of the T-maze task, in which the escape platform was always in the same location, and enhanced response learning on the striatum-dependent version, in which a specific body turn was necessary to reach the escape platform (Wingard and Packard, 2008). Furthermore, administration of bupivacaine, which inactivates cellular activity, into the basolateral amygdala blocked both the enhancement of response learning and impairment of place learning, which suggests that a functional basolateral amygdala is essential for the effects of anxiogenics in modulating hippocampus-dependent and striatum-dependent learning (Packard and Gabriele, 2009). Given that the hippocampus can interfere with striatum-dependent learning, the enhancing modulatory effect of the amygdala on striatum-dependent learning may be mediated by an impairment in the functioning of the hippocampus, which results in a redirection of control over learning by the striatum-based memory system (Wingard and Packard, 2008).

In addition to the execution of either hippocampus-based or striatum-based navigational strategies, rats exhibit an ability to switch between strategies as the demands

of the task change (Havekes et al., 2006; McAlanon and Brown, 2003; Ragozzino et al., 2003; Ragozzino et al., 2008). Behavioral flexibility reflects the ability to efficiently switch from executing one learning strategy to another and is governed by sub-regions of the prefrontal cortex as well as the dorsomedial striatum (McAlanon and Brown, 2003; Ragozzino et al., 2003; Ragozzino et al., 2008). The two types of behavioral flexibility are referred to as reversals and switches (Young and Shapiro, 2010). Strategy reversal refers to changes within a strategy or cognitive set while a strategy switch refers to a change across two different strategies or cognitive sets. For example, during a place reversal, the spatial contingency changes as the goal is repositioned to a different location within the maze. On a T-maze task, a rat is first trained with the goal located in the east quadrant and then the rat is required to inhibit execution of this goal location and switch to the a goal location in the west quadrant. An example of a response reversal requires a rat to initially make a body turn to the right to reach the goal followed by a contingency change in which the rat is required to turn left to find the goal. Inactivation of either the rat prelimbic area or the orbitofrontal cortex result in an increase in perseverative errors, such that rats are unable to inhibit the previously acquired learning strategy when confronted by reversals (McAlonan and Brown, 2003; Ragozzino et al., 1999). Learning impairments also were observed on a strategy switching task that required rats to switch between odor discrimination and place discrimination to locate a food reward, such that inhibition of the previously relevant strategy was impaired following inactivation of the prelimbic-infralimbic sub-region (Ragozzino et al., 2003).

During execution of a task that requires behavioral flexibility, the prefrontal regions modulate performance during the initial reversal phase as the demands of the task

change via inhibition of the previously relevant strategy. Both the orbitofrontal cortex and prelimbic area send afferent projections to the dorsomedial striatum. However, inactivation of the dorsomedial striatum using a sodium channel blocker did not impair the ability of rodents to inhibit the use of the previously learned place strategy shortly after place learning contingencies changed (Ragozzino and Choi, 2004). Nevertheless, following the initial reversal phase, the number of regressive errors increased, as the reliable execution of the new place learning strategy was impaired, which indicates that the dorsomedial striatum is involved in execution of the new strategy (Ragozzino and Choi, 2004). A response reversal is defined by reversing the body turn to the goal such that a right turn is no longer applicable and rats must learn to turn left to reach the goal. Similar to a place reversal, transient lesions to the dorsomedial striatum impair a response reversal such that regressive errors are increased (Ragozzino et al., 2002). Taken together, the hippocampus, dorsolateral and dorsomedial striatum, as well as sub-regions of the prefrontal cortex and amygdala have been implicated in regulating the balance between multiple memory systems.

The Cholinergic System

The neurotransmitter acetlycholine (ACh) is an important chemical messenger in cognitive processes as well as many life-sustaining processes. ACh is formed from the two precursors choline and acetyl coenzyme A, a reaction catalyzed by the enzyme choline acetyltransferase (ChAT) (Meyer and Quenzer, 2005). The enzyme ChAT is only found in cholinergic neurons and is, therefore, often used to identify neurons that synthesize and release ACh. Following release from the presynaptic cell, ACh is broken down by the enzyme acetylcholinesterase (AChE), producing choline and acetic acid.

Following breakdown, choline transporters, which are located on the presynaptic membrane, return choline to the cholinergic neuron for future ACh synthesis. ACh is released from all preganglionic neurons of the autonomic nervous system, as well as postganglionic neurons of the parasympathetic nervous system. Within the central nervous system, there are several clusters of cholinergic neurons in the basal forebrain, which serve as the primary afferent source of ACh for the cerebral cortex, hippocampus, as well as other limbic areas. Alternatively, the sole source of ACh in the striatum is from the cholinergic interneurons positioned diffusely within the striatum.

ACh binds to both nicotinic ionotropic receptors, as well as muscarinic metabotropic receptors (Meyer and Quenzer, 2005). Nicotinic receptors are located on skeletal muscle cells at neuromusculuar junctions, on autonomic postganglionic cells, and in certain brain areas, while muscarinic receptors are located on cardiac muscle cells, many smooth muscle cells, and are widely distributed in the brain. In the brain, nicotinic receptors mediate fast excitatory responses and enhance the release of various neurotransmitters from their nerve terminals via presynaptic or postsynaptic modulation. Muscarinic receptors are located in the prefrontal cortex, basal forebrain, hippocampus, basolateral amygdala, and striatum, and mediate slower responses that lead to non-genomic and genomic actions, which bring about long-term changes in neuronal activity. Importantly, there are five known subtypes of muscarinic receptors with different pharmacological characteristics and varying activation of second-messenger systems. Given the role of ACh in cognition, both nicotinic and muscarinic receptors in the aforementioned brain areas are important modulators of various types of learning and memory (Deiana et al., 2010). However, muscarinic receptors may be particularly

important due to their slower modulatory effects that contribute to long-lasting changes thought to be involved in learning and memory formation (Deiana et al., 2010).

The Role of the Central Cholinergic System in Multiple Memory Systems

The central cholinergic system regulates a variety of cognitive processes, which include the balance between place and response learning strategies, and performance on spatial tasks that rely upon learning the relationships between extra-maze cues for optimal performance (O'Keefe and Nadel, 1978). When measured by *in vivo* microdialysis, ACh levels in the hippocampus were significantly increased from baseline during training on a single-solution place-learning task, which depends on the hippocampus (Pych et al., 2005). Interestingly, training on a single-solution response task, which depends on the striatum, caused a significant increase in the amount of ACh released in both the hippocampus and dorsolateral striatum (Pych et al., 2005). Given these results, the cholinergic system of the hippocampus appears to be engaged regardless of the cognitive demand imposed by the task.

Further investigations into the role of ACh release in the hippocampus on response learning tasks compared rats trained on the response version of the T-maze task under either cue-rich or cue-poor conditions. Although ACh release in the hippocampus was comparable under both cue conditions, release declined during training on the cue-poor condition (Pych et al., 2005). With regard to ACh release, these results suggest that the hippocampus relinquishes control when spatial processing is not conducive to solving the task. Further support for this hypothesis emerges from findings in which ACh release within the hippocampus was negatively correlated with performance on an amygdala-based task, such that an increase in ACh release in the hippocampus was correlated with a

greater impairment on the amygdala-based task (McIntyre et al., 2002). Conversely, ACh release within the amygdala was positively correlated with performance on a hippocampus-dependent task, such that an increase in amygdalar ACh output was matched with better performance on the hippocampus-based task (Ragozzino et al., 1996). Therefore, these results suggest that while the amygdala cooperates with hippocampus to enhance performance on hippocampus-based tasks, the hippocampus competes with amygdala processing and disrupts performance on amygdala-based tasks. With regard to behavioral flexibility, ACh release increased in the dorsomedial striatum but not the dorsolateral striatum during reversal learning, as rats began to adapt to a new learning contingency, which is consistent with the hypothesis that the dorsomedial striatum plays a role in the execution of a newly acquired learning strategy (Ragozzino and Choi, 2004; Ragozzino et al., 2008). These results taken together indicate the involvement of ACh release in coordinating multiple memory systems on navigational tasks.

In addition to ACh release, muscarinic receptors, which bind ACh, also play an important role in modulating the learning and memory processes that depend upon the hippocampus and striatum. Systemic administration of the muscarinic antagonist scopolamine impaired performance on both a response version and a spatial version of the spontaneous alternation task (McNaughton and Feldon, 1980). When administered directly into the hippocampus or striatum, scopolamine impaired use of a place strategy or a response strategy, respectively (Carli et al., 1997; Diaz del Guante et al., 1991). Additionally, antagonism of muscarinic receptors within the dorsomedial striatum with infusions of scopolamine impaired the ability of rats to effectively switch to executing a

new response learning strategy after previously learning a stimulus-response strategy (Ragozzino et al., 2002).

Although activation of muscarinic receptors has been implicated in a variety of cognitive processes, the expression of the receptor as a function of learning a particular task has received considerably less attention. Rats trained on a hippocampus-dependent spatial learning task exhibited a greater density of muscarinic receptors in the hippocampus than untrained control rats (Van der Zee et al., 1995). Consistent with previous reports examining ACh release (McIntyre et al., 2002; Pych et al., 2005; Ragozzino and Choi, 2004), muscarinic receptor binding was indicative of learning strategy preference in rats (Grissom et al., 2012). Specifically, rats that adopted a place learning strategy on a dual-solution learning task exhibited a greater ratio of muscarinic receptor binding in the hippocampus relative to the striatum than rats that adopted a stimulus-response strategy (Grissom et al., 2012). In addition, unpublished data from our laboratory further confirmed learning-induced changes in muscarinic receptor density. The memory for the context associated with a footshock assessed on an inhibitory avoidance task, was associated with greater muscarinic receptor binding in area CA1 of the dorsal hippocampus compared to rats that were never shocked or rats that were initially shocked but not reintroduced to the context of the footshock. Similarly, following training on a conditioned immobility task in which rats learned the context of a shock stressor, staining for muscarinic receptor immunoreactivity in the amygdala revealed a positive correlation with memory for the stressor (Van der Zee et al., 1997). Specifically, longer freezing bouts correlated with a higher expression of muscarinic receptors in the central nucleus of the amygdala.

Given that ACh release and binding of muscarinic receptors impacts performance on navigational tasks with distinctly different cognitive demands, the purpose of the present study was to examine the density of muscarinic receptor binding in the hippocampus, dorsolateral striatum, and dorsomedial striatum following training on either a place learning task or a stimulus-response learning task, or on a task that required rats to switch between executing place and stimulus-response learning strategies.

Specific Hypotheses

Based on previous findings, we proposed to test the following hypotheses in the current study:

H1: Adult male rats will learn to navigate to an escape platform at equivalent rates when learning is guided by a cue proximal to the platform (stimulus-response strategy) or by cues surrounding the maze (place strategy).

H2: Performance will be impaired when adult male rats must alternate between navigation to an escape platform guided by a cue proximal to the platform (stimulus-response strategy) and by cues surrounding the maze (place strategy).

H3: Adult male rats that are trained on a hidden platform version of the water maze task (place-trained) will express higher ratios of muscarinic binding in the hippocampus relative to the striatum compared to swim-only rats, stimulus-response-trained rats, and strategy-switching rats.

H4: Adult male rats that are trained on a cued platform version of the water maze task (stimulus-response-trained) and rats that must alternate between strategies (strategy-switching) will express similar ratios of muscarinic binding in the hippocampus relative to the striatum.

H5: Adult male rats that are trained on a cued platform version of the water maze task (stimulus-response-trained) or rats that must alternate between strategies (strategy-switching) will express higher ratios of muscarinic binding in the hippocampus relative to the striatum compared to swim-only rats, but less than rats trained on a hidden platform version (place-trained).

H6: Adult male rats that are trained on a cued platform version of the water maze task (stimulus-response-trained) will express lower ratios of muscarinic binding in the dorsomedial striatum relative to the dorsolateral striatum compared to place-trained rats and strategy-switching rats.

Methods

Animals

A total of 32 male Long-Evans rats, 55 days of age upon arrival, were purchased from Harlan, Inc. (Indianapolis, IN, USA). Rats were housed individually, provided free access to food and water, and maintained under a standard 12 h:12 h light-dark cycle (lights on at 07:00 h). All procedures were approved by the Tulane University Institutional Animal Care and Use Committee in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (1996). Rats were acclimated to the housing conditions for approximately 1 week after arrival. Following the acclimation period, rats were handled for 1 min/day on four consecutive days in order to habituate to the experimenters.

Apparatus and General Behavioral Testing

Following handling, 8 rats were randomly assigned to each of the following testing conditions: swim-only control, place learning, stimulus-response learning, and

strategy switching. All water maze tasks were conducted in a white circular pool, which measured 180 cm in diameter and 60 cm in height, filled with water to a depth of 32 cm. Water was made opaque by the addition of non-toxic white paint and maintained at a temperature of approximately 25° C. Rats were subjected to 6 consecutive days of training. Eight trials per day were performed during which rats were placed into the pool twice from each of the 4 cardinal points in a pseudo-randomized order. If rats failed to locate the platform, trials were terminated after 60 s, at which point rats were guided to the platform where they remained for an additional 15 s. A 5-15 min inter-trial interval was maintained between all trials. Learning was indicated by increasingly shorter escape latencies as training progressed.

Stimulus-Response Learning Strategy: Cued Platform Water Maze Task (CPWM)

The 8 rats assigned to this condition were trained on a Cued Platform Water Maze Task (CPWM). An escape platform (15 cm length in length x 15 cm in width x 30 cm in height) was submerged 2 cm below the surface of the water and marked by a salient visual cue. The cue consisted of a black plastic ball (3 cm in diameter), affixed to a piece of threaded rod constructed of galvanized steel (1 cm in diameter x 11 cm in height), which was bolted to the center of the platform and covered horizontally with pieces of black and pink electrical tape (~ 2 cm each stripe). During training, the platform was located in the NW, NE, SW, or SE quadrants and relocated to a new quadrant in a pseudo-randomized order on each trial.

Place Learning Strategy: Hidden Platform Water Maze Task (HPWM)

The 8 rats in this condition were trained on a procedure that was performed identical to that described above for the CPWM task with two exceptions. The platform

for the HPWM task was replaced with an identical platform without the visual cue attached, and the platform remained in the SW quadrant of the pool across all trials.

Switching between Stimulus-Response and Place Learning Strategies: CPWM and HPWM Tasks

Eight rats were trained on alternating strategies, such that half of the rats were trained to execute a place strategy on the HPWM task described above on days 1, 3, 5 and a stimulus-response strategy on the CPWM on days 2, 4, 6 (*Place Odd/Stimulus-Response Even*). The other half of the rats were trained on the CPWM on days 1, 3, 5 and trained on the HPWM on days 2, 4, 6 (*Stimulus-Response Odd/Place Even*). Alternating the demands of the task between days required rats to successfully switch between stimulus-response and place strategies over the 8 trials.

Swim-Only Controls

Eight rats were placed in the water maze without an escape platform present for the same amount of time as a randomly selected experimental rat from one of the three training conditions. Specifically, swim-only control rats were yoked to 3 rats trained on the CPWM task, 3 rats trained on the HPWM task, and 2 rats trained on the task that required rats to switch between learning strategies.

Autoradiography

Rats were decapitated two hours following training. *In vitro* autoradiography for muscarinic receptor binding, using the muscarinic receptor antagonist [^{3}H] quinuclidinyl benzilate (QNB), was conducted according to procedures used in our laboratory previously (Dohanich et al., 1985; Grissom et al., 2012; Wolff et al., 2008). Brains were removed, frozen with powdered dry ice, and stored at -70°C. Frozen coronal sections (50

μm) were cut using a microtome cryostat, thaw-mounted on positively-charged microscope slides, and stored at -70°C. Sections were collected through the striatum (corresponding to bregma 1.00 mm through bregma 0.20 mm) to include the dorsolateral and dorsomedial areas, and the dorsal hippocampus (corresponding to bregma -3.00 mm through bregma -4.16 mm) and ventral hippocampus (corresponding to bregma -4.80 mm through bregma -5.30 mm) (Paxinos and Watson, 1998).

Immediately prior to incubation, sections were allowed to thaw and dry at room temperature. Three matched sections from each area (dorsomedial and dorsolateral striatum, dorsal hippocampus and ventral hippocampus) for each subject were incubated in 10 mL of 50 mM sodium-potassium phosphate buffer (pH = 7.4) containing the muscarinic receptor antagonist [^{3}H] QNB (Perking Elmer, Boston, MA; 51.0 Ci/mmol) for 1 h. Incubation medium was then poured off, followed by a cold rinse in fresh buffer for 5 min. Nonspecific binding was determined in parallel incubations containing excess atropine sulfate (1 μM) a muscarinic antagonist. Dry sections were placed in contact with autoradiographic film (Amersham Hyperfilm MP, Buckinghamshire, UK) and exposed in X-ray cassettes for 16 days. Autoradiographic film was developed with Kodak D-19 Developer for 2 min, rinsed in distilled water for 30 s, and fixed in Kodak Rapid Fixer for 2 min.

Autoradiographs were analyzed by computer-assisted densitometry (MCID Imaging Software, 7.0). To control for differences in the size of the brain areas measured, the same 20 X 20 pixel area was selected for each brain section and the relative optical densities. Images from sections incubated with atropine to determine non-specific binding were undetectable, therefore, optical densities from sections without

atropine were analyzed and total binding was used for statistical analysis (Wolff et al., 2008). Measurements were normalized by determining the density of each film sheet outside the exposure area to obtain a background value.

Statistical Analyses

A repeated measures analysis of variance (ANOVA) with a within-subject effect of day (days 1-6) and between-subject effect of learning condition (Place, Stimulus-Response, and Switching) was conducted to assess differences in escape latency across all days of training. Follow-up one-way ANOVAs were conducted to examine significant main and interactive effects. When appropriate, least squared differences (LSD) post-hoc tests were conducted. For rats assigned to switching between strategies, subsequent ANOVAs were conducted to examine effect of switch order (*Place Odd/Stimulus-Response Even and Stimulus-Response Odd/Place Even*). A Greenhouse-Geisser correction was implemented in instances where sphericity was violated.

ANOVAs were conducted to examine the effect on muscarinic binding as a function of task demand for the following brain areas: dorsomedial striatum, dorsolateral striatum, total striatum, dorsal dentate gyrus, dorsal CA3, dorsal CA1, total dorsal hippocampus, ventral dentate gyrus, ventral CA3, ventral CA1, total ventral hippocampus, and total hippocampus. Total striatum consisted of the sum of the aforementioned sub-regions of the striatum. Total dorsal hippocampus consisted of the sum of the aforementioned sub-regions of the dorsal hippocampus. Total ventral hippocampus consisted of the sum of sub-regions of the ventral hippocampus and total hippocampus consisted of the sum of total dorsal and total ventral hippocampus values. Following analyses of individual brain areas, analyses of hippocampus relative to areas of

the striatum as well as dorsomedial striatum relative to dorsolateral striatum were conducted. Post-hoc LSD analyses were conducted when appropriate.

Results

Learning as a Function of Task Demand

Results indicate that rats assigned to a learning condition learned the location of the escape platform as depicted by a significant decrease in escape latency over the course of training, represented in Figure 1, [$F(3.04, 63.74) = 26.26$, $p < 0.001$]. A main effect of learning condition also was observed, which indicated that rats exhibited different escape latencies depending on the learning condition to which they were assigned [$F(2, 21) = 22.96$, $p < 0.001$]. Specifically, rats that were assigned to stimulus-response training exhibited significantly shorter escape latencies than both place-trained rats and rats trained to switch between strategies. In addition, rats trained to switch between strategies exhibited significantly shorter latencies than place-trained rats. An interactive effect of day and learning condition indicated that rats showed a significant decrease in escape latency, which varied across the 6 days of training as a function of learning condition [$F(6.07, 63.74) = 2.26$, $p < 0.05$]. Subsequently, ANOVAs were conducted to identify main effects of task demand within each day. LSD post-hoc analyses revealed significant differences among learning conditions on each training day. Specifically, on Day 1 [$F(2,21) = 4.93$, $p < 0.05$], rats trained on the place task exhibited significantly longer escape latencies than rats trained on the stimulus-response task ($p < 0.05$) as well as rats trained to switch between tasks ($p < 0.05$). On Day 2 [$F(2,21) = 10.20$, $p < 0.01$], stimulus-response-trained rats exhibited significantly shorter latencies than both place-trained rats ($p < 0.01$), as well as switch-trained rats ($p < 0.01$). On Day

3 [$F(2,21) = 6.00$, $p < 0.01$], place-trained rats had significantly longer latencies than stimulus-response-trained rats ($p < 0.01$) with a strong trend toward longer latencies than rats trained to switch between strategies ($p = 0.08$). On Day 4 of training [$F(2,21) = 5.27$, $p < 0.05$], stimulus-response-trained rats exhibited significantly shorter latencies as compared to rats trained on a place task ($p < 0.05$), as well as switch-trained rats ($p < 0.05$). Similarly, on Day 5 [$F(2,21) = 3.02$, $p = 0.07$], rats trained on a stimulus-response task exhibited significantly shorter latencies as compared to switch-trained rats ($p < 0.05$). On Day 6 of training [$F(2,21) = 6.18$, $p < 0.01$], stimulus-response-trained rats exhibited significantly shorter latencies to the escape platform as compared to place-trained rats ($p < 0.01$) and switch-trained rats ($p < 0.05$).

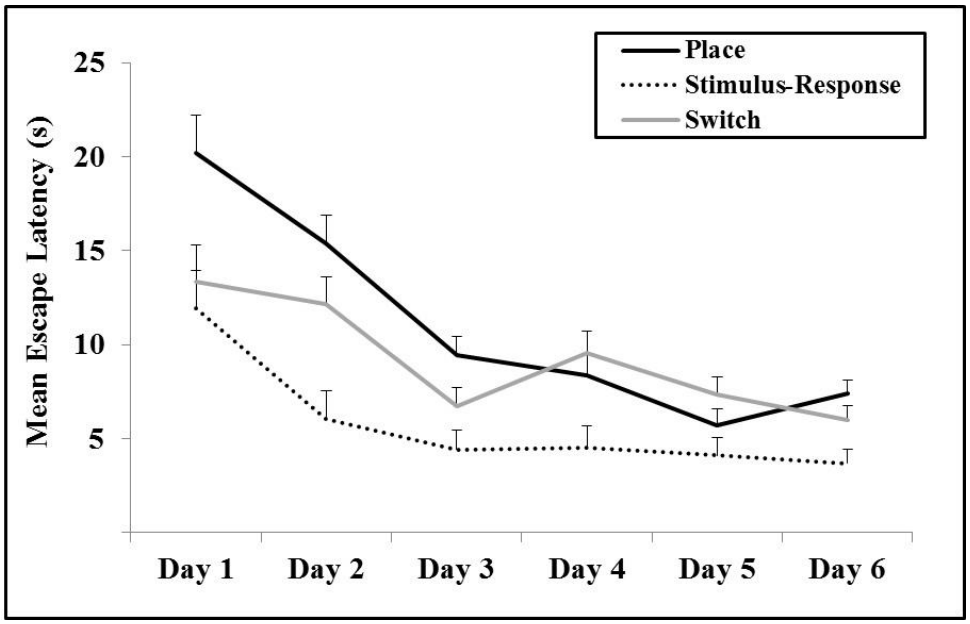

Figure 1. Mean escape latencies as a function of learning condition. Rats trained on a place task displayed significantly longer escape latencies as compared to rats trained on a stimulus-response task and rats trained to switch between tasks across all days of training ($p < 0.05$). Rats trained to switch between tasks displayed significantly longer escape latencies than rats trained on a stimulus-response task across all days of training ($p < 0.05$). See text for a description of group differences on each training day.

As represented in Figure 2, analyses for rats assigned to the switch-trained paradigm revealed a significant decrease in escape latency across days [F (2.08,12.46) = 8.31, $p < 0.01$]. Rats required to switch between place and stimulus-response tasks were divided into two groups such that half of switch-trained rats were trained on a place task on odd days and switched to a stimulus-response task on even days (*Place Odd/Stimulus-Response Even*). Conversely, the remaining rats in the switch group were trained on a stimulus-response task on odd days and switched to a place task on even days (*Stimulus-Response Odd/Place-Even*). ANOVA results indicated a significant interactive effect of task switching and switch order, such that rats assigned to the switch group had significantly different escape latencies across training depending on the task demand of the particular day [F(2.08,12.46) = 8.12, $p < 0.01$].

In order to discern differences between the switch conditions within each day of training, an independent samples *t*-test was conducted, and results indicated that when required to switch from a stimulus-response task to a place task, rats took longer to reach the escape platform. Specifically, on Day 1 [t(6) = 1.10, $p = 0.33$] and on Day 2 t(6) = 1.12, $p = 0.33$], rats in the switch-trained learning condition did not exhibit significant differences as a function of switch order. On Day 3, rats in the *Place Odd/Stimulus-Response Even* condition took significantly longer to reach the escape platform as compared to rats in the *Stimulus-Response Odd/Place Even* condition [t(6)=24.36, $p < 0.01$]. Additionally, a strong trend on Day 4 revealed that rats in the *Stimulus-Response Odd/Place Even* group took longer to escape than those in the *Place Odd/Stimulus-Response Even* group [t(6) = 5.02, $p = 0.07$]. On Day 5, rats in the *Place Odd/Stimulus-Response Even* condition took significantly longer to reach the escape platform than their

switch-trained counterparts [$t(6) = 6.32$, $p = 0.05$]. On day 6, rats did not exhibit significant differences between switch order conditions [$t(6) = 2.83$, $p = 0.14$].

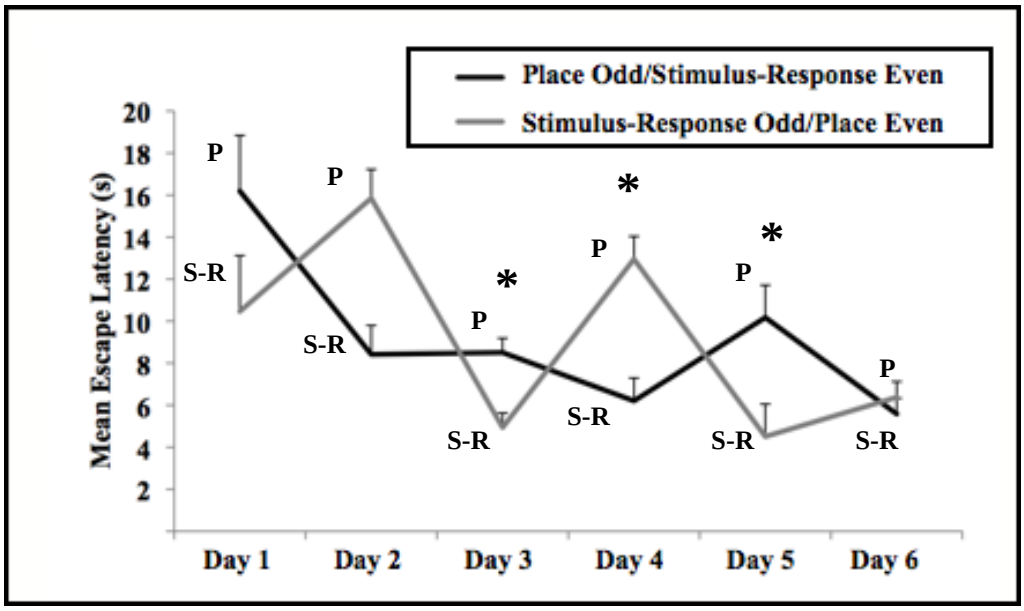

Figure 2. Mean escape latencies for rats trained to switch between a stimulus-response and a place task as a function of switch order. Rats assigned to the *Place Odd/Stimulus-Response Even* condition were trained on a place task on days 1, 3, and 5 and a stimulus-response task on days 2, 4, and 6. Rats assigned to the *Stimulus-Response Odd/Place Even* condition were trained on a stimulus-response task on days 1, 3, and 5 and a place task on days 2, 4, and 6. Overall, rats exhibited significantly longer latencies on days when required to switch from a stimulus-response to a place strategy ($p < 0.01$).

Muscarinic Receptor Density in the Hippocampus and Striatum as a Function of Task Demand

The results depicted in Figure 3 indicate a significantly higher ratio of muscarinic receptor binding in the dorsal hippocampus relative the striatum for rats trained in the task-switching paradigm [$F(3,25) = 2.98$, $p = 0.05$]. Specifically, rats trained on the task-switching paradigm showed a significantly higher ratio of binding in the dorsal hippocampus relative to total striatum as compared to swim-only control ($p < 0.05$),

place-trained rats ($p < 0.05$), and stimulus-response-trained rats ($p < 0.05$). Detailed analyses of muscarinic receptor binding in the dorsal hippocampus and corresponding sub-regions independent of muscarinic receptor binding in the striatum are presented in Table 1a. Detailed analyses of muscarinic receptor binding in the dorsal hippocampus and corresponding sub-regions relative to muscarinic receptor binding in the striatum are presented in Table 2a.

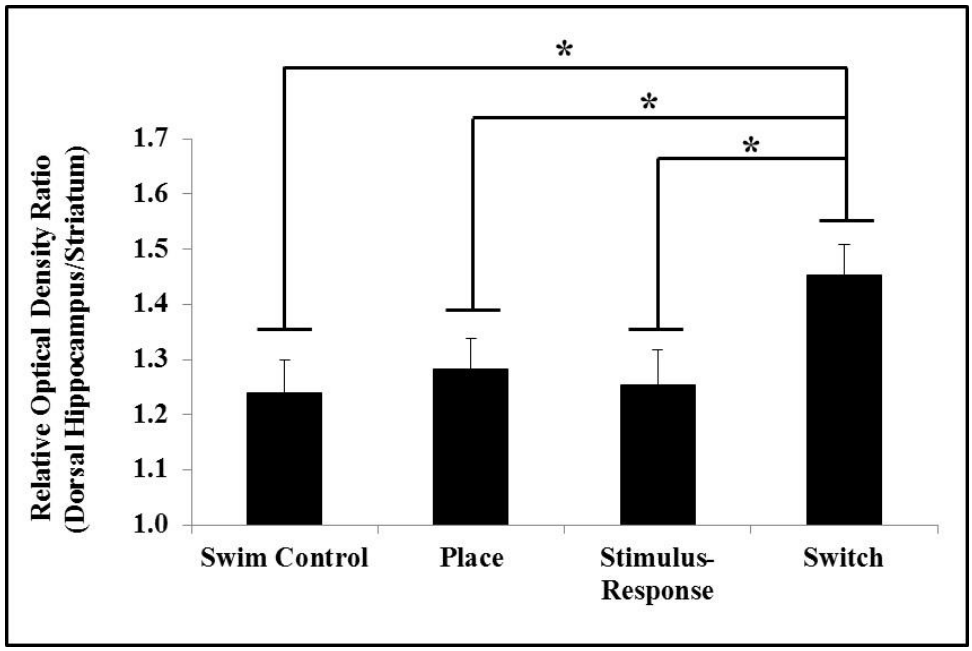

Figure 3. Ratios of muscarinic receptor binding densities in the dorsal hippocampus relative to the total striatum as a function of learning condition. Rats trained to switch between tasks exhibited significantly higher ratios as compared to all groups ($p < 0.05$).

In Figure 4, analyses of muscarinic receptor binding in the ventral hippocampus relative to the striatum revealed significantly higher ratios for rats trained on the task-switching paradigm as compared to place-trained rats ($p < 0.05$), as well as rats trained on a stimulus-response task ($p< 0.01$). Detailed analyses of muscarinic receptor binding in the ventral hippocampus and corresponding sub-regions independent of muscarinic

receptor receptor binding in the striatum are presented in Table 1b. Detailed analyses of muscarinic receptor receptor binding in the ventral hippocampus and corresponding sub-regions relative to the muscarinic receptor binding in the striatum are presented in Table 2b.

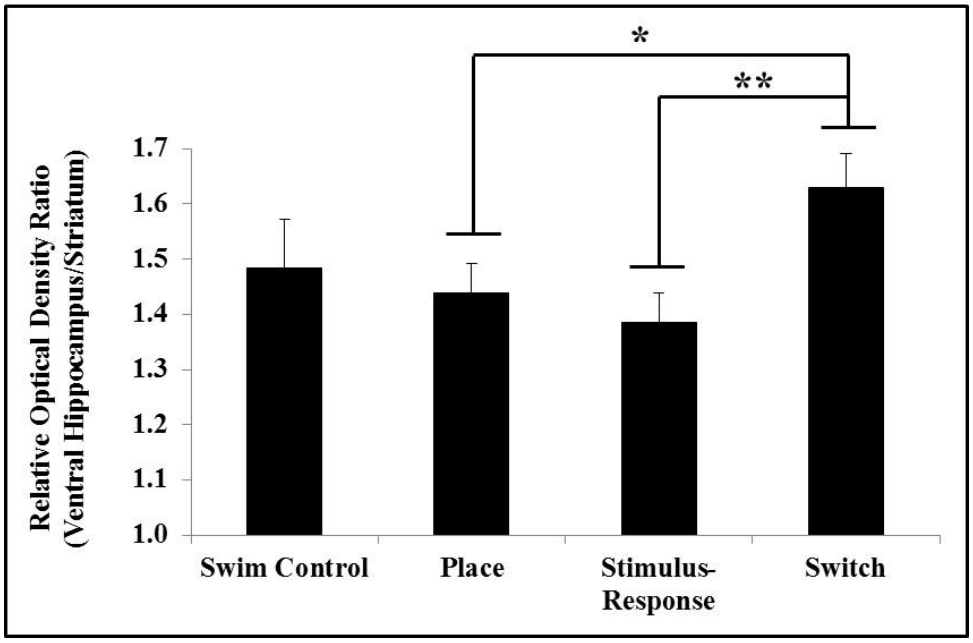

Figure 4. Ratios of muscarinic receptor binding densities in the ventral hippocampus relative to the total striatum as a function of learning condition. Rats trained to switch between tasks exhibited significantly greater binding ratios compared to rats trained on a stimulus-response task ($p < 0.01$), as well as rats trained on a place task ($p < 0.05$).

As represented in Figure 5, analyses of binding ratios in the total hippocampus relative to the total striatum indicate a trend towards significant differences between groups [$F(3,20) = 4.92$, $p = 0.09$]. Post-hoc analyses, revealed that rats trained to switch tasks showed a significantly greater ratio of muscarinic receptor binding in the total hippocampus relative to total striatum, when compared to place-trained rats ($p < 0.05$) and stimulus-response-trained rats ($p < 0.05$). Further, binding in the total hippocampus revealed significant differences between rats trained to switch strategies as compared to

all groups as represented in Table 1b [F(3,20) = 4.92, $p < 0.05$]. Detailed analyses of the muscarinic receptor binding in the total hippocampus independent of the muscarinic receptor binding in the striatum are presented in Table 1b. Detailed analyses of the muscarinic receptor binding in the total hippocampus relative to muscarinic receptor binding in the total striatum as well as the sub-regions of the striatum are presented in Table 2c.

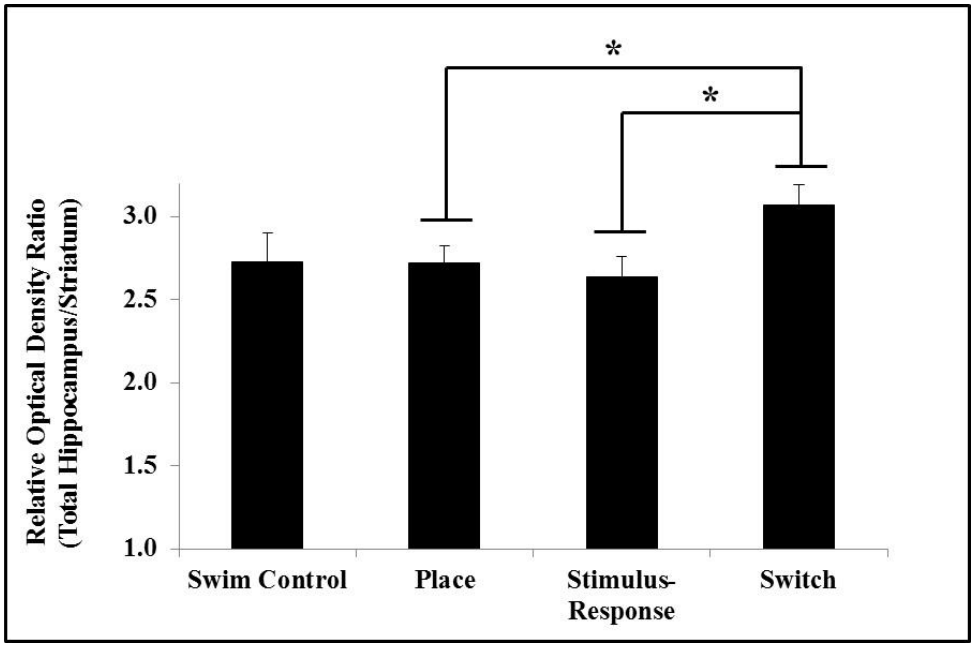

Figure 5. Ratios of muscarinic receptor binding densities in the total hippocampus relative to the total striatum as a function of learning condition. Rats trained to switch between strategies exhibited significantly higher binding ratios as compared to rats trained on a stimulus-response task, as well as rats trained on a place task. ($p < 0.05$).

As represented by Figure 6, analyses of the muscarinic receptor binding ratios in the dorsomedial striatum relative to the dorsolateral striatum did not reveal significant differences as a function of learning condition [$F(3,27) = 1.34$, $p = 0.28$]. Analyses of the muscarinic receptor binding in the total striatum independent of either sub-region of

the striatum did not reveal significant differences between groups as a function of learning condition [$F(3,27) = 1.89$, $p = 0.16$]. Analyses of the muscarinic receptor binding in the dorsomedial striatum independent of the dorsolateral striatum revealed a trend between the rats trained on a stimulus-response task and rats trained to switch between tasks [$F(3,27) = 2.52$, $p = 0.08$]. Specifically, rats trained on stimulus-response task showed significantly more binding in the dorsomedial striatum as compared to rats trained to switch between tasks ($p < 0.05$]. Analyses of the dorsolateral striatum independent of other areas of the striatum did not reveal significant differences in muscarinic receptor binding as a function of learning task [$F(3,28) = 1.21$, $p = 0.32$]. Analyses of muscarinic receptor binding in the striatum and corresponding sub-regions are presented in Table 1c.

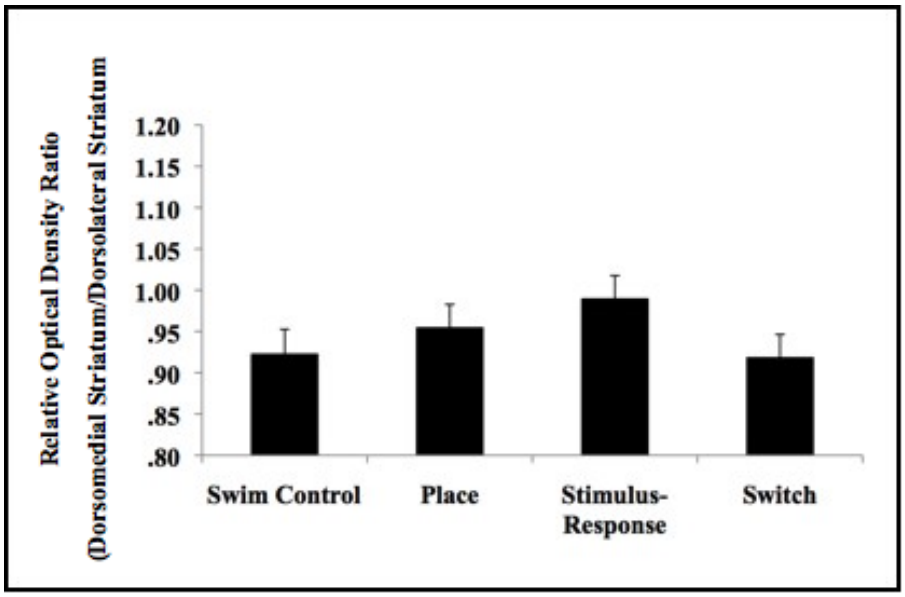

Figure 6. Ratios of muscarinic receptor density in the dorsomedial striatum relative to the dorsolateral striatum as a function of learning strategy. Analyses did not reveal any significant differences among groups ($p = 0.29$).

Table 1. Muscarinic Binding in (a) Dorsal Hippocampus, (b) Ventral Hippocampus and Total Hippocampus, and (c) Striatum. (Values represent relative optical density means ± SE. Significant ANOVA values in bold, notable trends (≤ 0.10) in italics; significant post-hoc differences between groups indicated by matching superscript letters in bold, trends in italics).

Area of Dorsal Hippocampus	**Swim Control**	**Place**	**Stimulus-Response**	**Switch**	**ANOVA**
Dorsal Dentate Gyrus	0.24±0.01	0.24±0.01	0.24±0.01	0.26±0.01	F (3,26)=1.01, p = 0.41
Dorsal CA3	0.14±0.01	0.14±0.01	0.16±0.01	0.15±0.01	F (3,26)=1.89, p = 0.16
Dorsal CA1	0.25±0.01	0.25±0.01	0.25±0.01	0.27±0.01	F (3,26)=1.64, p = 0.21
Total Dorsal Hippocampus	0.62±0.02	0.63±0.02	0.65±0.02	0.68±0.02	F (3,26)=1.99, p = 0.14

Table 1a

Area of Ventral Hippocampus	**Swim Control**	**Place**	**Stimulus-Response**	**Switch**	**ANOVA**
Ventral Dentate Gyrus	0.27±0.01	0.25±0.01^{a}	0.26±0.01^{b}	0.28±0.01^{a,b}	F(3,22)=2.34, p = *0.10*
Ventral CA3	0.18±0.01	0.17±0.00^{a}	0.17±0.00^{b}	0.19±0.01^{a,b}	F(3,22)=3.59, ***p* = 0.03**
Ventral CA1	0.30±0.01	0.29±0.01	0.29±0.01	0.31±0.01	F(3,22)=1.97, p = 0.15
Total Ventral Hippocampus	0.75±0.02	0.71±0.01^{a}	0.72±0.01^{b}	0.78±0.02^{a,b}	F(3,22)=3.56, ***p* = 0.03**
Total Hippocampus	1.35±0.03^{a}	1.34±0.02^{b}	1.37±0.02^{c}	1.46±0.02^{a,b,c}	F(3,20)=4.92, ***p* = 0.01**

Table 1b

Area of Striatum	**Swim Control**	**Place**	**Stimulus-Response**	**Switch**	**ANOVA**
Dorsomedial Striatum	0.24±0.01	0.24±0.01	0.26±0.01^{a}	0.22±0.01^{a}	F(3,27)=2.52, p = *0.08*
Dorsolateral Striatum	0.27±0.01	0.25±0.01	0.26±0.01	0.25±0.01	F(3,28)=1.21, p = 0.32
Total Striatum	0.51±0.02	0.50±0.02	0.53±0.02	0.47±0.02	F(3,27)=1.89, p = 0.16

Table 1c

Table 2. Ratios of Muscarinic Receptor Binding (a) Dorsal Hippocampus including Dorsal Dentate Gyrus (dDG), Dorsal CA3 (dCA3), Dorsal CA1 (dCA1), and Total Dorsal Hippocampus (dHPC) (b) Ventral Hippocampus including Ventral Dentate Gyrus (vDG), Ventral CA3 (vCA3), Ventral CA1 and Total Ventral Hippocampus (vHPC), as well as Total Hippocampus (HPC). Areas of the Hippocampus are analyzed relative to areas of the Striatum including Dorsomedial Striatum (DM), Dorsolateral Striatum (DL), and Total Striatum (STR) (Values represent relative optical density means ± SE. Significant ANOVA in bold, notable trends ≤ 0.10 in italics; significant post-hoc differences between groups indicated by matching superscript letters).

Dorsal Hippocampus Ratio	**Swim Control**	**Place**	**Stimulus-Response**	**Switch**	**ANOVA**
dDG/DM	0.99±0.06^{b}	1.00±0.06^{c}	0.93±0.07^{a}	1.16±0.06^{a,b,c}	$F(3,25)=2.64$, *p=0.07*
dDG/DL	0.90±0.05	0.95±0.05	0.93±0.05	1.06±0.05	$F(3,26)=1.92$, $p=0.15$
dDG/STR	0.47±0.03	0.49±0.03	0.47±0.03	0.55±0.03	$F(3,25)=2.25$, $p=0.11$
dCA3/DM	0.59±0.03	0.60±0.03	0.60±0.03	0.69±0.03	$F(3,25)=2.27$, $p=0.11$
dCA3/DL	0.53±0.03^{a}	0.57±0.03	0.60±0.03	0.63±0.03^{a}	$F(3,26)=2.68$, *p=0.07*
dCA3/STR	0.28±0.01	0.29±0.01	0.30±0.01	0.33±0.01	$F(3,25)=2.24$, $p=0.11$
dCA1/DM	1.01±0.06^{a}	1.04±0.05^{b}	0.98±0.06^{c}	1.20±0.05^{a,b,c}	$F(3,25)=3.50$, **$p<0.05$**
dCA1/DL	0.93±0.05^{a}	0.99±0.05^{b}	0.97±0.06^{c}	1.10±0.05^{a,b,c}	$F(3,26)=2.32$, *p=0.10*
dCA1/STR	0.48±0.03^{a}	0.50±0.02^{b}	0.49±0.03^{c}	0.57±0.02^{a,b,c}	$F(3,25)=3.05$, **$p<0.05$**
dHPC/DM	2.58±0.14^{a}	2.64±0.13^{b}	2.51±0.15^{c}	3.05±0.13^{a,b,c}	$F(3,25)=3.27$, **$p<0.05$**
dHPC/DL	2.36±0.11^{a}	2.50±0.11^{b}	2.50±0.13	2.79±0.11^{a,b}	$F(3,26)=2.60$, *p=0.07*
dHPC/STR	1.24±0.06^{a}	1.28±0.06^{b}	1.25±0.06^{c}	1.45±0.06^{a,b,c}	$F(3,25)=2.98$, **$p=0.05$**

Table 2a

Ventral Hippocampus Ratio	**Swim Control**	**Place**	**Stimulus-Response**	**Switch**	**ANOVA**
vDG/DM	1.11±0.08	1.06±0.05^{a}	1.02±0.05^{b}	1.43±0.06^{a,b}	$F(3,21)=3.37$, **$p<0.05$**
vDG/DL	1.02±0.05	1.01±0.04	1.00±0.04	1.10±0.04	$F(3,22)=1.27$, $p=0.31$
vDG/STR	0.53±0.03	0.52±0.02^{a}	0.50±0.02^{b}	0.59±0.02^{a,b}	$F(3,21)=2.50$, *p=0.09*
vCA3/DM	0.76±0.05	0.71±0.03^{a}	0.67±0.03^{b}	0.84±0.04^{a,b}	$F(3,21)=4.52$, **$p<0.05$**
vCA3/DL	0.69±0.04	0.67±0.03	0.66±0.03	0.75±0.03	$F(3,22)=1.48$, $p=0.25$
vCA3/STR	0.36±0.02	0.34±0.02^{a}	0.33±0.02^{b}	0.40±0.02^{a,b}	$F(3,21)=3.01$, **$p=0.05$**
vCA1/DM	1.24±0.09	1.20±0.05^{a}	1.11±0.05^{b}	1.29±0.06^{a,b}	$F(3,21)=3.82$, **$p<0.05$**
vCA1/DL	1.15±0.06	1.13±0.04	1.09±0.04	1.22±0.05	$F(3,22)=1.47$, $p=0.25$
vCA1/STR	0.59±0.04	0.58±0.02	0.55±0.02^{a}	0.65±0.03^{a}	$F(3,21)=2.88$, *p=0.06*
vHPC/DM	3.11±0.21	2.96±0.13^{a}	2.80±0.13^{b}	3.48±0.15^{a,b}	$F(3,21)=4.19$, **$p<0.05$**
vHPC/DL	2.86±0.14	2.81±0.10	2.75±0.10	3.07±0.12	$F(3,22)=1.57$, $p=0.23$
vHPC/STR	1.48±0.09	1.44±0.05^{a}	1.39±0.05^{b}	1.63±0.06^{a,b}	$F(3,21)=3.12$, **$p<0.05$**

Table 2b

Total Hippocampus Ratio	**Swim Control**	**Place**	**Stimulus-Response**	**Switch**	**ANOVA**
HPC/DM	5.71±0.41	5.06±0.25^{a}	5.29±0.29^{b}	6.55±0.29^{a,b}	$F(3,19)=3.43$, **$p<0.05$**
HPC/DL	5.20±0.27	5.31±0.19	5.27±0.22	5.79±0.22	$F(3,20)=1.39$, $p=0.28$
HPC/STR	2.73±0.17	2.72±0.10^{a}	2.64±0.12^{b}	3.07±0.12^{a,b}	$F(3,19)=2.53$, *p=0.09*

Table 2c

Discussion

The primary findings of the current study demonstrate that adult male rats that navigated to an escape platform guided by cues surrounding a water maze (place-trained) learned the task at a significantly slower rate than males that were guided by a cue proximal to the platform (stimulus-response-trained) or males that were required to switch strategies on alternating days. Further, males trained to switch strategies throughout training learned the task at a significantly slower rate than males assigned to the stimulus-response task learning condition. Additionally, males that were required to switch strategies over alternating days expressed higher ratios of muscarinic binding in the hippocampus relative to the striatum compared to place-trained rats, stimulus-response-trained rats, and swim-only controls. These results indicate that the use of a place learning strategy slows acquisition of a water maze task while the requirement to switch strategies as the demands of the task change over days engages the cholinergic system in the hippocampus most heavily.

Analyses of muscarinic receptor binding ratios of the dorsal hippocampus relative to the striatum revealed significantly greater ratios in rats trained to switch between tasks compared to the other rats. Specifically, rats trained to alternate between a place task and stimulus-response task expressed more binding in the dorsal hippocampus relative to the total striatum compared to swim-only controls, place-trained rats, and stimulus-response-trained rats. Similarly, rats trained to switch between tasks expressed more binding in the ventral hippocampus, as well as total hippocampus, relative to total striatum compared to both place-trained and stimulus-response-trained rats. However, differences in the binding ratios of the ventral hippocampus relative to the total striatum and the total

hippocampus relative to the total striatum, between the rats trained to switch between tasks and swim controls, did not quite reach significance. Further, analyses of muscarinic receptor binding in the dorsomedial striatum relative to the dorsolateral striatum did not differ as a function of learning condition.

Learning as a Function of Task Demand

Results indicate that rats assigned to the place task learning condition had significantly longer escape latencies across all days of training than rats trained on the stimulus-response task on the task switching paradigm. In agreement with the differences observed between the place-trained group and the stimulus-response-trained group, there also was a difference in the latency to reach the platform within the group of rats that were trained to switch between place and stimulus-response learning tasks on alternating days. Specifically, results indicated that rats took longer to locate the escape platform on days when they were required to execute a place task. These results are consistent with the hypothesis that executing a place task requires more cognitive effort as compared to executing a stimulus-response task, which is more habitual in nature while executing a place task requires development of a cognitive map (O'Keefe and Nadel, 1978; Pych et al., 2005). Development of a cognitive map involves more complex processing such that multiple brain areas must work together to make a singular representation of an animal's environment (Bancquet et al., 2005). This spatial representation depends on developing relationships between the place and various cues within the environment. Specifically, a cognitive map appears to include possible changes in contingencies in order for the animal to successfully adapt to these changes in the environment (O'Keefe and Nadel, 1978). For example, in the current study, when a

rat was placed in the maze from an alternate cardinal point, it was required to adapt to its new position in relation to the hidden platform and the extra-maze cues in order to successfully escape from the water. Taken together, previous studies and the current results suggest that a place task takes more cognitive effort requiring more complex processing compared to the procedural learning that is associated with a stimulus-response task (Pych et al., 2005).

Learning-Induced Changes in Muscarinic Receptor Density in the Hippocampus

Analyses of muscarinic receptor binding in the dorsal hippocampus relative to total striatum revealed significant differences between switch-trained rats as compared to swim-only controls, stimulus-response-trained rats, and place-trained rats. Specifically, switch-trained rats expressed higher binding ratios in total dorsal hippocampus relative to total striatum as compared to all other groups of rats. Hasselmo and McGaughy (2004) suggested that there is an increase in ACh release during encoding and a decrease during consolidation. Specifically, ACh efflux was associated with acquisition of a task and this release was positively correlated with spatial memory performance (Fadda et al., 2000). Further, previous research has shown that an increase in available ACh, following administration of cholinesterase inhibitors, was associated with muscarinic receptor down-regulation (Lerer et al., 1984). Taken together, the model proposed that an increase in ACh is associated with muscarinic receptor down-regulation and a decrease of ACh is associated with consolidation. Therefore, in rats required to switch between tasks a decrease in ACh release during consolidation of the memory for both types of learning tasks may induce an up-regulation of muscarinic receptor binding in the dorsal hippocampus relative to the striatum in the present study.

A similar relationship was found in the subfields of the dorsal hippocampus, such that rats trained on the strategy-switch paradigm showed significantly higher ratios of muscarinic receptor binding in the dorsal CA1 relative to the total striatum as well as dorsal CA1 relative to the dorsomedial striatum, as compared to swim-only controls, place-trained rats, and the stimulus-response-trained rats. Area CA1 has not only been implicated in long-term memory storage via long-term potentiation, but also has been associated with behavioral flexibility such that protein kinase A in area CA1 was found to be increased following acquisition on a version of the Y-maze in which task demands required switching between learning strategies (Havekes et al., 2007; Xu et al., 1998). Consistent with the results from the current study, and the idea that the dorsal hippocampus plays a role in behavioral flexibility, the regulation of calcineurin, a calcium-dependent phosphatase previously associated with learning and memory, was enhanced in the dorsal hippocampus during reversal training on the Y-maze task (Havekes, et al., 2006). The current study is the first to show changes in muscarinic receptor binding in the hippocampus following training on the behavioral flexibility task, which required rats to switch between two navigational strategies. Taken together, results indicate that the expression of various proteins in area CA1 of the hippocampus is responsive to training on a task that requires behavioral flexibility.

The current study revealed no significant differences in binding ratios when comparing place-trained rats to swim-only controls, which may be due to the binding of ACh to specific muscarinic receptor subtypes. Previous studies have shown varying effects of ACh such that its modulation of synaptic transmission depends on the site of release, receptor subtypes, and the target neuronal population. For example, presynaptic

muscarinic receptors (M2, M4), are often inhibitory and act as inhibitory autoreceptors on cholinergic terminals (Picciotto et al., 2011). Alternatively, post-synaptic muscarinic receptors can either be excitatory (M1, M3, M5) or inhibitory (M2, M4). Given that tritiated QNB, the radioactive ligand used in the current study, is not specific to any one muscarinic receptor subtype, the observed up-regulation within the dorsal hippocampus relative to the striatum could be due to the predominance of any one of the five muscarinic receptor subtypes expressed in the hippocampus. Moreover, because the M1 subtype is thought to play an important role in spatial learning and memory (Hagan et al., 1987; Herrera-Morales, 2007), it is conceivable that an up-regulation of M1 receptors following training on the place task was not detected by QNB.

Rats trained to switch between learning strategies also exhibited significantly higher binding ratios in the ventral hippocampus relative to total striatum, as well as a trend in the total hippocampus relative to the striatum, compared to both stimulus-response-trained rats and place-trained rats, but not swim-only controls. Importantly, muscarinic receptor binding ratios in the ventral hippocampus relative to the striatum were not significantly different when comparing rats trained to switch between strategies to swim controls. Conceivably, this effect may be attributed to the stress following placement into the water maze with no means of escape and the greater involvement of the ventral hippocampus in modulating stress responses (Degroot and Treit, 2002). Interestingly, differences in muscarinic receptor binding between the rats trained to switch between strategies relative to place-trained rats, stimulus-response-trained rats, and swim-only controls were observed only in the ratios that compared the dorsal hippocampus to the striatum, and not the ventral hippocampus (or total hippocampus) to

the striatum. Consistent with previous studies, the dorsal hippocampus appears to play a greater role in spatial learning and memory (Moser, et al., 1995) and behavioral flexibility (Satvat 2011), whereas cholinergic function in particular within the ventral portion of the hippocampus has been implicated more strongly in emotionality (Degroot and Treit, 2002).

Analysis of the total hippocampus relative to the total striatum revealed that rats trained to switch strategies expressed higher ratios of muscarinic receptor binding. Specifically, there was a trend towards significance in the total hippocampus relative to total striatum such that rats trained to switch between strategies expressed higher binding ratios compared to stimulus-response-trained rats and place-trained rats. Analysis of total hippocampus relative to total striatum showed a trend when comparing rats trained to switch between strategies to swim-only controls such that switch-trained rats expressed higher binding ratios. Independent of muscarinic binding in the striatum, it is worth noting that the analysis of the total hippocampus alone revealed significantly more binding for rats trained in the switch group as compared to all other groups. The key role of the hippocampus in behavioral flexibility observed in the current study is consistent with previous results analyzing the roles of immediate early genes (IEG), such as Arc and zif268, in the hippocampus. Arc is an IEG that is thought to directly modulate specific cellular functions, which in turn affect downstream genes to facilitate modulation of synapses (Guzowski et al., 2001), while zif268 has more broad influences depending on which downstream gene is regulated (Satvat et al., 2011). The IEG zif268 was found to increase in the dorsal dentate gyrus following a switch between place and response versions of the plus maze task (Satvat et al., 2011). Further, Arc RNA expression within

the hippocampus was found to increase following training on a spatial version of the water maze as well as following training on a navigational task that required behavioral flexibility, which adds further support to the hypotheses that the hippocampus is not only involved in spatial learning, but also behavioral flexibility (Guzowski et al., 2001).

Learning-Induced Changes in Muscarinic Receptor Density in the Striatum

Following training on a strategy-switching paradigm, we expected to observe a significant change in muscarinic receptor binding expression within the dorsomedial striatum relative to swim controls, which was based on findings suggesting that training on a task that requires behavioral flexibility is associated with an increase in ACh release in the dorsomedial striatum (Brown et al., 2010; Ragozzino and Choi, 2004; Ragozzino, 2007). Ratio analyses of the dorsomedial relative to the dorsolateral striatum did not uncover any significant differences in muscarinic receptor binding among groups. However, analyses of muscarinic binding in individual areas of the striatum indicated differences among groups. Rats trained to switch between strategies did not express a difference in muscarinic receptor binding in the dorsomedial striatum when compared to swim controls. However, rats trained on a stimulus-response task showed significantly higher muscarinic receptor binding in the dorsomedial striatum as compared to rats trained on the strategy-switching condition, but not to swim-only controls. Although the results of the current study should be interpreted cautiously, that the stimulus-response-trained rats exhibited higher binding in the dorsomedial striatum is consistent with results following training on a response version of the T-maze that showed training is associated with increased expression of the immediate early gene (IEG) *c*-Fos in both sub-regions of the dorsal striatum, rather than solely the lateral region (Gill et al., 2006). Therefore,

training on a stimulus-response task can induce an up-regulation of IEGs as well as muscarinic receptor binding in the dorsomedial striatum.

The contribution of the striatal sub-regions in procedural learning and memory is complex and depends on multiple factors pertaining to the specific demands of the learning task. Anatomical connectivity shows that somatosensory and motor information from the neocortex is relayed to the dorsolateral striatum, while visual and auditory information is relayed to the dorsomedial striatum (McGeorge and Faull, 1989). In a previous study, in which rats were trained on a conditioned avoidance response task while neural activity in the dorsal striatum was recorded, specific populations of cells responded to the auditory stimulus (stimulus-related cells), to the lever-release response (response-related), or to both (stimulus/response-related) (White and Rebec, 1993). Specifically, the response-related cells were located in the dorsolateral striatum while the stimulus/response-related cells were located in the dorsomedial striatum (White and Rebec, 1993), which indicated an association between the anatomical and functional connectivity of these sub-regions. Additional findings suggest that processing of specific combinations of movement, odor, and varying levels of reward combinations occurred in both sub-regions of the striatum (Stalnaker et al., 2007). Specifically, measurement of neural activity within the dorsolateral and dorsomedial striatum following training on different stimulus-response associations with varying levels of food reward indicated that the dorsolateral striatum mediates simple stimulus-response associations while the dorsomedial is more likely involved in goal-directed-response associations. Notably, the task demands in the cued version of the water maze task in the present study required both goal-directed-responses as well as stimulus-response associations, such that locating

and swimming to the cued-platform necessitated the development of a stimulus-response-reward association. Further, additional studies have examined differences in the anterior versus posterior dorsomedial striatum and indicated that goal-directed behavior is impaired following lesions to the posterior dorsomedial striatum (Yin et al., 2005). Therefore, it is important to take in to account both medial/lateral and anterior/posterior sub-regions of the striatum as well as the specific modalities of task demands when interpreting neurochemical changes within the striatum following training on a stimulus-response task that requires both stimulus-response and goal-directed-response associations.

Conclusions

Results from the current study implicate changes in muscarinic receptor binding in the hippocampus relative to the striatum in the use of learning specific navigational strategies. Specifically, muscarinic receptors in the dorsal hippocampus are important for switching between a stimulus-response task and place task. Additionally, the role of muscarinic receptors in the striatum in stimulus-response learning and switching between strategies is more complex than originally thought, such that the medial and lateral subdivisions may overlap in their contributions. Taken together, the results from the current study provide further support for the involvement of cholinergic function in regulating the balance between multiple memory systems (Gold, 2004).

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