SPATIAL STRATEGY SELECTION IN THE SUBMERGED T-MAZE

by

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ABSTRACT

The ability to navigate in one’s environment is critical to survival, be it in order to acquire positive reinforcers—food, water, mates, shelter, and sleep—or to escape negative consequences—predation and pain. Spatial navigation is commonly examined using the T-maze, in which experimenters are able to dissociate two distinct strategies that are exhibited by animals when acquiring the location of a goal. The behavioral history and details of spatial navigation are introduced in Chapter 1.

Chapter 2’s behavioral data reveal that the emergence and progression of these spatial strategies are different if the animal is in an appetitive versus an aversive environment. Motivated by food, rats initially use the relational information in environmental cues (a “place strategy”) before switching to an automatic, movement-based tactic (a “response strategy”). Motivated to escape a noxious stimulus, as in the submerged T-maze, rats perform the opposite pattern.

Bolstered by several investigations, Chapter 3 introduces the concept of multiple memory systems, which suggests that, in the dry T-maze and Morris water maze, a place or spatial strategy is subserved by the dorsal hippocampus (HPC), whereas a stimulus-response (S-R) strategy is subserved by the dorsolateral striatum (DLS). Critically, our observation of the immediate expression of a response strategy and the gradual switch to a place strategy in the submerged T-maze initiates questions regarding the underlying neural circuitry.

Chapter 4 suggests that the dorsal hippocampus is unnecessary for the expression of the late place strategy, despite the same pharmacological manipulation causing impairments in a separate task previously shown to require the region. These results are
elucidated by the closer investigation of immediate early gene (IEG) expression in dorsal hippocampal subregions, and further discussed in a unifying theory of hippocampal function in Chapter 5. On the other hand, Chapter 6 proposes a necessary role of the dorsolateral striatum (DLS) in the acquisition, but not the expression, of the early response strategy, whereas Chapter 7 deliberates the broader implication of the striatum and basal ganglia. The modulatory roles of motivation and stress are purported in Chapter 8, with an emphasis on regulation by amygdalar and prefrontal regions in Chapter 9.

In utilizing multiple approaches, this research represents an innovative and important step in the converging exploration of the motivational modulation of behavior, specifically that of spatial navigation. This investigation is critical for understanding the strategies undertaken by individuals in stressful circumstances, and may be most relevant for military training and the rescue of missing persons. Furthermore, the implicated neural systems are involved in a variety of diseases, including both Parkinson’s and Alzheimer’s. Using maze learning paradigms, we combine these essential issues with the well-established rodent model. Notably, a general discussion expands our knowledge regarding how motivation modulates multiple aspects of the production and maintenance of behavior.

Advisor: Peter Holland

Internal Committee: Susan Courtney, Michela Gallagher, Patricia Janak

External Committee: Jay Baraban, Jeremiah Cohen, James Knierim
I cannot celebrate this accomplishment without first crediting those that have contributed to it. It is an impossible task to identify and adequately thank all of the people that have helped me throughout the years. There have been countless teachers, professors, and administrators that took added steps to invest in my personal education. After all of their effort, it pains me that such a short section in this dissertation can never convey the love and appreciation I have for people such as these.

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CHAPTER 1: INTRODUCTION

A critical requirement for an animal’s survival is the ability to navigate and reason within its environment, so as to procure resources, such as food, water, and mates. Notably, the environment is constantly changing—with shifting stimuli, stressors, consequences, and contingencies—within which the animal must adaptively guide its behavior.

To survive in any dynamic setting, organisms must be capable of efficiently selecting the most appropriate action among many possible alternatives. The necessary cognitive flexibility involves competition between various neurocognitive networks that control behavior. Thus, animals have the opportunity to attend to and utilize information from multiple sources to select the optimal behavior.

MEMORY IN MAZES: A SPATIAL FRAMEWORK

The domination of the study of learning and memory in experimental psychology has seen a variety of models. In nonhuman animals, one common approach uses maze learning, in which animals are trained to remember the location of one or more items in mazes of varying sizes, shapes, and complexity. Historically, a maze setting consists of environmental “extra-maze” cues planted around the apparatus, either in the room or on a surrounding curtain, and may also contain “intra-maze” landmarks within the apparatus itself. A plethora of literature shows that animals use external, visual cues and internal, movement cues to guide learning and performance.

In a typical radial arm maze, animals are trained to approach one or more goal locations, and start the maze from the same or different entry points. When tested for
memory of these goal locations, the extra-maze cues may be manipulated in a variety of ways. For example, these cues might be removed, replaced, or rotated, such that the relationship amongst them is maintained, as well as transposed, such that there is a new configuration of the cues. Researchers use manipulations such as these to assess questions about what animals learn in environments with cues that are proximal and/or distal to themselves.

The idea that animals learn a “place,” a particular location containing items in a spatial relationship, can be traced back several decades. Early evidence includes the observation that animals expect certain consequences in specific locations (Blodgett & McCutchan, 1944; Tolman et al., 1946), suggesting that, not only are animals able to recognize different places, they are also able to bind information to those sites.

The need for environmental, or extra-maze, cues and, in particular, their spatial arrangement, has been observed many times over. Maze performance is disrupted by the transposition of stimuli, or the scrambling of their configuration, but is relatively unaffected by their rotation (Suzuki et al., 1980) or selective removal, which maintains their original formation (Knierim & Hamilton, 2011). These results suggest that the relationship amongst the items is critical, and not any one individual cue itself.

Although animals may be biased to the use of relational information, they can also be guided by single, salient intra-maze cues, or “landmarks,” that are predictive of a particular location (e.g., McDonald & White, 1994). These landmarks may form independent representations, driving behavior singularly. One experiment capitalized on this characteristic, showing that predictive landmarks can block learning to new ones (Stahlman & Blaisdell, 2009). In this open-field foraging task, Phase 1 involved training
rats that Landmark A signaled the location of hidden food (A+ trials). In Phase 2, AX+ trials included a redundant spatial cue (Landmark X). Using a within-subjects overshadowing control (BY+ trials), later test trials showed that subjects took longer to find a goal location in the presence of the superfluous Landmark X than the control Landmark Y.

A unique version of the radial arm maze, in which extra-maze cues are visible from the maze arms, but not from the center of the maze, shows disruption of performance after rotation, suggesting that the local cues, near the center of the maze, were guiding behavior (Brown & Bing, 1997). These cues seem to be salient enough to warrant representations independent of relationship information, arguing against the observation that spatial relationships are critical (Suzuki et al., 1980). These discordant results were elucidated by data showing that the transposition of local cues resulted in greater disruption than either the rotation or removal of them (Vollmer-Conna & Lemon, 1998), similar to the aforementioned results involving extra-maze cues. These observations suggest that intra-maze cues are not only utilized, but configured into a spatial representation.

Although intra-maze control can come from visual stimuli, such as landmarks, it may also come from the animal itself, such as vestibular or kinesthetic information (Brown & Moore, 1997). When explicit intra-maze visual cues were moved from trained locations to novel ones, disrupted maze performance suggests that extrinsic cues (visual stimuli) and intrinsic cues (kinesthetic, vestibular information) may compete with one another for expression (Brown & Moore, 1997; Knierim & Hamilton, 2011; Deshmukh & Knierim, 2013).
Although animals may utilize either intrinsic or extrinsic information, experimental subjects seem biased toward environmental spatial cues. In the dual-solution T-maze, acquisition of the task could mean learning to approach the “place” of the goal, according to the environmental cues, or learning to make the necessary motor movement (i.e., turning right or left). In such a task, in which rats can perform accurately by using either a place (spatial) or response (action) strategy, rats are biased to produce a place strategy (Brown & Humphrey, 1955; Chang & Gold, 2004; Schwabe et al., 2008). Critically, this spatial choice is more accurately purported to be a bias and not an unchanging preference: animals are capable of using other strategies to solve the task. If these spatial stimuli are unavailable, performance is initially disrupted, but animals eventually rely on response chaining to solve the task (Suzuki et al., 1980; Chang & Gold, 2004).

THE T-MAZE

As previously described, in a spatial navigation framework, animals can attend to either external or internal sources of information—for example, environmental cues or self-based movement—and early psychological studies debated the relative usage of each in guiding behavior. In particular, psychologists have long questioned the extent to which animals use cues from either external location or internal movement to guide their behavior in maze learning tasks (Tolman, 1948; Tolman & Gleitman, 1949). Put another way, does the animal learn to approach the location of reward or to make an appropriate series of turns?
For decades, it has been widely accepted that animals initially learn about and utilize relational information in the configuration of environmental cues (a “place strategy”) and subsequently switch to the more automatic and less cognitively effortful “response strategy” after repeated exposure (Restle, 1957; Restle, 1962).

For example, in a common dual-solution T-maze, which can be solved by either “place” or “response” strategies (Tolman et al., 1946; Tolman & Gleitman, 1949), rats are trained to find a food reward in one arm (e.g., West) of a 4-arm maze. Rats are started in the South arm and access to the North arm is blocked.

Rats readily solve this task, exhibiting a decline in the latency to reach the goal location as well as in the number of errors. To assess the solution strategy utilized, probe tests, in which the South arm is blocked and the rats are started from the North arm, are interspersed throughout training. If a rat enters the previously trained arm, the use of place information is inferred, but if it enters the previously untrained arm, a response strategy is inferred.

We see similar strategies in humans, using, admittedly, much more complex maze scenarios; some participants are biased toward a map-like representation (place learning), whereas others are predisposed to an action sequence (response learning). Critically, participants fall along a continuum, forming a relatively normal distribution of the frequency of strategy choice (Marchette et al., 2011; Furman et al., 2014). Very few people are pure place or response learners: participants seem to employ both strategies, but with a bias toward one versus the other. Furthermore, this preference may be manipulated by the introduction of factors, including stress (Schwabe et al., 2007; Schwabe & Wolf, 2009).
Collectively, these observations suggest that animals may use one of many strategies to solve a task. In cases in which performance indicates a particular strategy, it is possible that the animal had access to information necessary for the other, with some form of internal or external factor biasing the production of the observed behavior.

GENERAL EXPERIMENTAL METHODS

Young male Long-Evans rats were procured from Charles River Laboratories (Raleigh, NC) for all experiments. According to our submerged T-maze protocol (Figure 1), subjects are habituated to the apparatus: they are placed in the South arm (Start) and allowed to explore the maze, without the escape platform, for two trials of two minutes each. Access to the North arm is blocked during habituation and training trials.

During training sessions, the Goal Arm is baited with an escape platform, located just below the water’s surface. Each rat receives two training trials per day: they are placed in the Start arm and allowed to traverse the maze to locate the hidden platform. The experimenter records the latency to the platform, as well as the number of errors; for our purposes, entering the incorrect arm and/or re-entering the Start arm are construed as errors.

To assay the strategy utilized, single-trial probe tests are interspersed at regular intervals throughout training (Experiments 1, 2, 8), clustered “early,” “intermediate,” “late,” or “final” during training (Experiments 3, 6), or administered once “early” or “late” in training (Experiment 5). On probe tests, there is no platform available and access to the original Start arm (South) is blocked. The rat is placed in the opposite arm (North) and is allowed to choose between the two possible Goal Arms.
Each rat’s choice is determined by the arm initially entered. Rats approaching the reinforced extra-maze cues, and entering the previously trained arm, are designated as “place learners,” whereas rats producing the reinforced response, and entering the previously untrained arm, are designated as “response learners.” Using this information, it is possible to determine the probability of observing the number of rats expressing each strategy based on the binomial distribution. We can then compare the observed proportions under different conditions by using a Chi-squared statistical test for between-subject comparisons and a sign test for within-subject comparisons.

*Figure 1.* Submerged T-maze used in all experiments. Here, the West (left) Goal Arm is baited. This variable is counterbalanced in all experiments.
CHAPTER 2: MOTIVATION MODULATES SPATIAL STRATEGY SELECTION IN “DRY” VS. “WET” T-MAZE

Demonstrations of the place-to-response strategy shift are dominated by appetitively motivated tasks, within which the animal learns to approach food reinforcement. Importantly, it has yet to be explicated how these same strategies are acquired, expressed, and maintained in an aversively motivated task.

Although there are established protocols in the dry T-maze, the submerged T-maze is not widely used. Thus, the subsequent experiments benefit from earlier pilot experiments (not reported here) that sought to provide such a protocol by determining the appropriate amount of training and frequency of interspersed probe tests.

In Experiment 1, we examined performance in an aversively motivated task: rats escaped a submerged T-maze by swimming to a hidden platform. Remarkably, we observed a response strategy early in training followed by a switch to a place strategy, which persisted over extended training. This observation is opposite of the pattern of data described in the appetitive T-maze (Packard & McGaugh, 1996). In Experiment 2, we used the same protocol and apparatus, but with food reward rather than water escape, and replicated Packard and McGaugh’s (1996) observation that normal rats initially displayed a place strategy and later adopted a response strategy.

Experiments 1 and 2 Methods

Subjects

Male Long-Evans rats (275-325g; Charles River Laboratories; Raleigh, NC) were housed individually in a temperature-controlled room, with lights on 7 A.M. to 7 P.M.
Rats in Experiment 1 (n = 34) had free access to food (Teklad Chow 2018, Harlan Laboratories; Madison, WI) and water. Weights of subjects prior to Experiment 1 were 350-415g. Rats in Experiment 2 (n = 16) were maintained at 85% of their *ad libitum* weights by limiting access to food. Weights of subjects prior to Experiment 2 were 350-415g.

The 10 rats in Group Single in Experiment 1 previously received pairings of an 80-db white noise with 45-mg sucrose pellets (Formula 5TUT, Test Diets; Richmond, IN) and extinction of this association while under food restriction; they were given two weeks to recover. All other rats were experimentally naïve.

*Apparatus*

A white, circular, galvanized steel water tank (h = 57cm, d = 173cm) was surrounded by a circular black curtain (h = 180cm) with attached white shape cues. A 4-arm Plexiglas plus maze (arms: l = 72.5cm, w = 20cm) was placed inside the tank. Removable Plexiglas inserts were used to block access to the individual arms when necessary.

*Submerged T-Maze.* For Experiment 1, the tank was filled with water (depth = 52.5cm, 27 ± 1°C) and non-toxic, white Premium Tempura paint (Blick Art Materials; Galesburg, IL) to obscure the escape platform’s (h = 50cm, d = 12cm) location, positioned 5cm from the side of the tank and 1.5cm below the water’s surface.

*Dry T-Maze.* For Experiment 2, the water was removed from the tank, and a Plexiglas floor was added to the maze, giving it walls (h = 12cm). An aluminum reward cup (h = 2cm, d = 6cm) was secured at the ends of both the East and West arms.
Experiment 1 Procedures

Habituation. Rats were placed in the South (Start) arm for two 2-min habituation trials. Access to the North arm was blocked during habituation and training trials.

Training. Subjects received two training trials each day, on which they were placed in the South arm, facing the wall, and trained to locate the hidden platform in either the East or West arm (counterbalanced). A correction procedure was applied, such that rats making an error were allowed to subsequently locate the platform. Errors included entries into the incorrect, untrained arm and re-entries into the Start arm.

Rats were required to remain on the escape platform for 10s before being removed, towel-dried, and placed in a holding cage. Rats were run in squads of four subjects, such that each rat received its first trial before the second trial was administered (McDonald & White, 1994). The two remaining rats in Group Single were run in an additional squad. Thus, the intertrial interval (ITI) depended on the performance of all rats in a squad (range = 2-5min).

Rats in all groups received two training sessions, followed by a probe test. The Single group (n = 10) received no further training or testing. The Extended group (n = 24) was divided into two subgroups, matched by performance over the first two training sessions, and received protracted training, as well as additional probe tests after every two (Frequent, n = 12) or four (Infrequent, n = 2) training sessions. We varied probe test frequency due to concerns for counterconditioning. Repeated exposure to the probe test contingencies, i.e., novel start location and removal of platform, might result in discrimination learning between training conditions and probe test conditions.
**Probe Tests.** On single-trial probe tests, there was no escape platform, and access to the South arm was blocked. Rats were placed in the North arm and allowed to choose between the previously trained or untrained arm. We reported the first arm entered by each rat. Rats approaching the reinforced extra-maze cues and entering the previously trained arm were designated as “place learners,” whereas rats producing the reinforced response and entering the previously untrained arm were designated as “response learners.” Upon reaching the end of the arm, rats were removed from the maze and towel-dried, beginning the next training session after approximately 30min.

**Experiment 2 Procedures**

In Experiment 2, we used the same apparatus and training procedures as in Experiment 1, but with food reward rather than water escape contingencies.

**Habituation.** Habituation was conducted as in Experiment 1, except that 45-mg sucrose pellets (Formula 5TU, Test Diets; Richmond, IN) were distributed throughout the South, East, and West arms, as well as in the secured aluminum reward cups.

**Training.** Training was conducted as in Experiment 1, except that the reward was four 45-mg sucrose pellets, placed in one of the aluminum cups. If a rat “timed out” by failing to complete the task in 5min, the subject was removed, without reward, and assigned a latency of 300s.

**Probe Tests.** Rats were probed either after every four (Infrequent, n = 8) or eight (Rare, n = 8) training sessions. We reduced the frequency of probe tests, relative to Experiment 1, because a preliminary experiment suggested slower learning in the food-rewarded maze and some evidence of counterconditioning with more frequent probe
tests. No sucrose reward was available on probe tests.

Data Analysis

An overhead camera and computer-assisted tracking system recorded each rat’s position in the maze. During training, an experimenter recorded each rat’s latency to reach the platform, as well as errors accrued. For the Single group in Experiment 1, measures were analyzed with Goal Arm (East, West) x Training Day mixed-design ANOVAs. Data from the Extended groups in Experiment 1 and Experiment 2 were subjected to Group (Frequent, Infrequent) x Goal Arm (East, West) x Training Day mixed-design ANOVAs. If all main effects and interactions involving the Goal Arm counterbalancing variable were nonsignificant, we omitted that variable in subsequent analyses. All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

For each probe test, we determined the probability of observing the recorded number of “response learners” in each group, based on the binomial distribution. We also analyzed the change in probe test performance over training using mixed-design ANOVAs across Test, as described above (except in Group Single, which was only tested once). Although ANOVA is often considered inappropriate for dichotomous data, D’Agostino (1971) found that, with adequate degrees of freedom, and when mean cell proportions range between .25 and .75 (as was the case here), the assumptions of ANOVA are not violated.
Experiments 1 and 2 Results

Experiment 1 (Submerged T-Maze)

Acquisition. In the Single group, we observed a marginally significant decrease in latency to reach the platform, from $29.94 \pm 5.14$ s [mean ± sem] on the first trial, to $15.86 \pm 3.00$ s on the second trial ($F(1,9) = 4.090, p = .074$), and a nonsignificant decrease in the number of errors, from $1.50 \pm 0.48$ errors to $0.75 \pm 0.24$ errors ($F(1,9) = 1.833, p = .209$). On the probe trial, 8 of 10 rats entered the previously untrained arm, suggesting the rapid acquisition of the escape-reinforced motor response ($p = .055$).

We also observed rapid acquisition during training of the two Extended subgroups, evidenced by asymptotic mean latency and number of errors to the platform after Day 3 (Figures 2A and 2B). Group x Goal Arm x Training Day ANOVAs over all training sessions showed a significant effect of Training Day for both latency ($F(19,380) = 20.125, p < .001$) and number of errors ($F(19,380) = 14.705, p < .001$).

Although we were concerned that counterconditioning or extinction from probe tests would affect subsequent training performance, there were no significant effects of Group on either latency ($F(1,20) = .267, p = .610$) or number of errors ($F(1,20) = .170, p = .684$). Additionally, no interactions were significant for latency ($ps > .344$) or number of errors ($ps > .741$), except a marginally significant Goal Arm x Training Day interaction for errors ($F(19,380) = 2.446, p = .088$).

Probe Tests. The Frequent group was first probed after two training sessions (Figure 2C). On this probe, 8 of 12 rats entered the previously untrained arm and were designated as response learners. Although this proportion of response learners did not differ significantly from chance ($p = .193$), combined with the 10 rats in Group Single,
which were also probed after two training sessions, 18 of 24 rats exhibited a response strategy ($p = .011$). By contrast, on the final probe test, only one rat in Group Frequent ($p = .003$) and two rats in Group Infrequent ($p = .019$) entered the previously untrained arm, indicating that, by the end of training, a significantly greater than chance number of rats were place learners (21 of 24, $p < .001$).

We then evaluated the change in solution strategy over the course of the experiment. A Group x Test ANOVA of responding on the probe tests common to both groups (even-numbered probe tests in Figure 2C) showed significant effects of Test ($F(4,88) = 4.29, p = .003$), but no effect of Group ($F(1,22) = 1.15, p = .294$). An analysis of the decreasing linear trend in using the response strategy over these probe tests was significant for the two groups combined ($F(1,22) = 15.45, p < .001$) and for Group Frequent alone ($F(1,22) = 13.73, p = .001$), as well as marginally significant for Group Infrequent alone ($F(1,22) = 3.43, p = .077$). An ANOVA of responding over all 10 probe tests of the Frequent group also showed significant effects of Test ($F(9,99) = 2.55, p = .011$) and decreasing linear trend ($F(1,11) = 10.98, p = .007$).

Despite this change in solution strategy, the rats showed no significant change in their latencies to reach the escape platform on probe trials over the course of testing (data not shown). Our ANOVA showed no significant main effects or interactions ($ps > .295$), and these latencies were comparable to those observed on contemporaneous training sessions (Figure 2A).

In conclusion, in the submerged T-maze, rats exhibited a response strategy at the beginning of training and switched to a place strategy by the end of training, a pattern opposite to that reported with food-rewarded procedures (Packard & McGaugh, 1996).
Experiment 2 (Dry T-Maze)

Acquisition. During training, we observed much more gradual acquisition of maze performance, with asymptotic mean latency to the reward cup and number of errors after Day 14 (Figures 3A and 3B). Although much slower than dry maze acquisition (Experiment 1), our results are similar to those observed in other dry T-maze experiments. ANOVAs showed significant effects of Training Day for both latency ($F(39,468) = 48.189, p < .001$) and number of errors ($F(39,468) = 12.245, p < .001$). However, as in Experiment 1, there were no significant effects of Group (probe test frequency) for either latency ($F(1,12) = .636, p = .441$) or number of errors ($F(1,12) = .156, p = .700$). The main effect of the Goal Arm counterbalancing variable was significant for number of errors ($F(1,12) = 5.969, p = .031$), but not latency ($F(1,12) = 3.186, p = .100$). Finally, the Goal Arm x Training Day interaction was significant for latency ($F(39,468) = 2.595, p = .038$), but no other interactions were significant for either latency ($ps > .435$) or number of errors ($ps > .160$).

Probe Tests. After four days of training, the Infrequent group received its first probe test (Figure 3C). On this first probe test, 6 of 8 rats entered the previously trained arm, suggesting that the majority of rats were place learners (but not significantly, $p = .109$). The rats in the Rare group exhibited a similar tendency on their first probe test, which occurred after eight training sessions: 6 of 8 rats entered the previously trained arm ($p = .109$). Thus, combined over the two groups, 12 of 16 rats were place learners on their initial probe trial ($p = .028$). By contrast, on the final probe test, 6 of 8 rats in the Infrequent group ($p = .109$) and 7 of 8 rats in the Rare group ($p = .035$) entered the
previously untrained arm. Thus, combined over the two groups, 13 of 16 rats were response learners on their final probe trial (p = .011).

We then evaluated the change in solution strategy over the course of training. A Group x Test ANOVA of responding on the probe tests common to both groups (even-numbered probe tests in Figure 3C) showed no significant effects of Group (F(1,14) = 1.571, p = .231) or Test (F(4,56) = 2.00, p = .108), and no Group x Test interaction (F(4,56) = .71, p = .591). Nevertheless, an analysis of the increasing linear trend over these probe tests was significant for the two groups combined (F(1,14) = 7.06, p = .019) and for the Rare group alone (F(1,14) = 7.60, p = .015), but not for the Infrequent group alone (F(1,14) = 1.00, p = .333).

Thus, rats initially used a place learning strategy, but switched to a response strategy with further training, the same pattern observed in Packard and McGaugh (1996), but the opposite to that found in the submerged T-maze in Experiment 1. We directly compared the changes in solution strategies over the course of training in Experiments 1 and 2 by conducting an Experiment x Group x Test ANOVA on probe test performance (Figures 2C and 3C). This ANOVA showed a significant Experiment x Test interaction (F(4,144) = 5.76, p < .001), and a significant difference in the linear trends in the two experiments (F(1,36) = 20.67, p < .001). No other effect or interaction was significant.

Discussion

Rats rapidly learned to escape to a hidden platform in a submerged T-maze task. Probe tests indicated that the rats initially adopted a response strategy, but switched to a
place strategy as training continued. By contrast, with the same apparatus and procedures, rats rewarded by food began as place learners and switched to a response strategy, as described previously by Packard and McGaugh (1996) and others.

Previous investigations using the submerged dual-solution T-maze (Packard & Wingard, 2004; Elliott & Packard, 2008) focused on rats’ early use of a place strategy. In those experiments, the only probe test was administered after 12 training trials. The proportion of untreated rats that displayed a place strategy in that probe (~60%) was similar to that observed here after 12 training trials. By testing throughout training, the present study provides a more complete description of the course of strategy selection in the submerged dual-solution T-maze. Although it is possible that, after more training, rats in the submerged T-maze would revert to a response strategy, our Experiment 1 involved extensive post-asymptotic training without such a return. Thus, we conclude that the nature of reinforcement or motivation substantially affected spatial learning strategy selection and progression in this task.

Our results differ from those obtained in the submerged T-maze when rats are started from both North and South arms throughout training. Packard and Gabriele (2009) found that rats learn more rapidly in such “dual-start” tasks, when they are required to approach the same location, than when required to make the same response. Perhaps the changing stimulus situation in the dual-start task favors earlier selection of a place strategy.

Another variable that may have influenced our results is the intertrial interval (ITI). Packard and Goodman (2013) noted that short ITIs promote place learning and long ITIs promote response learning. Because, in our study, the ITIs were determined in
part by the rats’ performances, our rats received longer ITIs over the first few training sessions than over the later sessions. It might be argued that our observation in Experiment 1 of initial place learning followed later by response learning reflected this shift in ITIs.

However, this possibility seems unlikely for several reasons. First, our rats received only two trials during each training session, so variations in the within-session ITI were small compared to the 24-hr interval between each pair of trials. Experiments that have found these ITI effects used multiple trials in each session, and so within-session ITIs constituted a greater proportion of the experienced intervals. Second, although the ITIs in Experiment 2 were consistently short, the rats were heavily biased to use a response strategy. Critically, using identical training procedures in the dry T-maze, rats used a place strategy early in training, when the ITIs were longer, and switched to a response strategy later in training, when the ITIs were shorter.

The shift from a place to a response strategy in the food-rewarded T-maze has been described as a shift from an initial tendency to use relatively complex allocentric information to locate food resources, which are naturally distributed widely in space, to a cognitively more economical, and perhaps automatic, strategy of using egocentric response information to obtain reward efficiently.

By contrast, the escape-motivated submerged T-maze may immediately provoke a fight-or-flight response, including automatic motor movements, anxiety, and the release of stress hormones. These movements may be reinforced by escape and, therefore, rapidly conditioned. Notably, in both our and Packard’s studies, learning was considerably more rapid in the submerged T-maze than in the food-rewarded T-maze.
Figure 2. (A) Mean latency to reach the escape platform and (B) mean number of errors during acquisition in the Frequent and Infrequent groups. Rats received two training trials per day. Error bars denote standard error of the mean. (C) Percentage of response learners. Rats in Group Single received a single probe trial after the second training session and rats in Groups Frequent and Infrequent received a single probe trial after every 2 or 4 training sessions, respectively. Trend line (equation shown) is based on Frequent data points.
Figure 3. (A) Mean latency to reach the reward cup and (B) mean number of errors during acquisition. Rats received two training trials per day. Error bars denote standard error of the mean. (C) Percentage of response learners. Rats in Groups Infrequent and Rare received a single probe trial after every 2 or 4 blocks of two training sessions, respectively. Trend line (equation shown) is based on Infrequent data points.
A series of maze experiments by Tolman and colleagues showed various types of strategies that animals may employ, including the aforementioned place and response strategies. Based on such observations, Tolman (1949) concluded that there are multiple forms of learning. This concept has infiltrated the memory field: different types of information processing are subserved by separate brain regions. Much of the earliest evidence for multiple memory systems came from patients with differing levels of brain damage. The most decisive investigations came from Patient H.M., Henry Gustav Molaison, who received a bilateral medial temporal lobectomy in early adulthood to control epileptic seizures (Milner et al., 1998; Squire, 2004; Squire & Wixted, 2011).

Although H.M. suffered from fewer seizures, he also showed retrograde amnesia, the loss of memory for past events. Interestingly, he had intact memory for remote events, such as those from his childhood, but worse memory for events leading up to his surgery, an observation termed temporally-graded retrograde amnesia, or Ribot’s Law.

It also became apparent that H.M. was unable to form new memories for facts or personal events, but was able to solve tasks requiring motor skill and dexterity. For example, he showed improved performance over repeated presentations on a task requiring him to trace an image based on its mirror reflection, despite reporting no knowledge of having encountered the task previously. This observation led to the conclusion that there may be a memory system dedicated to facts and events, with a separate system for skill-based performance. Because H.M. had portions of his medial temporal lobe (MTL) removed, it was suggested that the MTL might be implicated in the former, now called declarative memory, but not in the latter, or nondeclarative memory.
The hippocampus, a region in the MTL, and the striatum, a region in the basal ganglia, are commonly associated with declarative and nondeclarative memory, respectively. The aforementioned place vs. response strategy distinction has been profitably interpreted into an analogous hippocampus vs. striatal memory system dichotomy.

Several experiments have reported that not only are these strategies expressed during distinct phases of learning, they are also subserved by different brain systems (McDonald & White, 1994). Packard and McGaugh (1996) (Figure 4) confirmed that, early in training, rats expressed a place strategy, which required the dorsal hippocampus, but not the dorsolateral striatum (referred to as the caudate nucleus in Figure 4). When the dorsal hippocampus was inactivated bilaterally, rats did not exhibit evidence for either strategy. Thus, although hippocampal inactivation impaired expression of a place strategy, a robust response learning strategy had yet to form. By contrast, later in training, rats expressed a response strategy, which required the dorsolateral striatum, but not the dorsal hippocampus. Bilateral inactivation of the dorsolateral striatum resulted in reduced response learning, but a preserved place strategy.

*Figure 4 (Packard & McGaugh, 1996).* Number of animals expressing a place or response strategy early and late in training with either saline or lidocaine infused in the caudate or hippocampus.
preference. Therefore, acquisition of a response strategy over extended training did not accrue at the expense of the previously-established place representation.

SUBMERGED T-MAZE

In the dry T-maze, rats switch from a hippocampal-dependent place strategy to a striatal-dependent response strategy (Packard & McGaugh, 1996). Thus, in the spatial navigation literature, the place strategy has been discussed as spatial learning, cognitive in nature, whereas the response strategy is touted as habit learning, stimulus-response (S-R) in nature.

However, in the submerged T-maze, we observed the opposite behavioral pattern: rats produced a response strategy and gradually switched to a place strategy (Asem & Holland, 2013). Due to a complete inversion of previous behavioral results, the underlying neural circuitry warrants investigation, and the characterization of place and response learning as spatial and habit learning deserves reconsideration.

Neural systems often compete in the control of performance, such that the elimination of one system results in greater control by another. For example, inactivation of the hippocampus impairs acquisition of a spatial strategy, but facilitates learning of a response strategy (Schroeder et al., 2002).

There are a number of ways by which the hippocampal and striatal systems may be dissociated. One alternative is that they are dissociable by behavioral performance—the hippocampus subserves place learning (McDonald & White, 1993; McDonald & White, 1994; Packard & McGaugh, 1996), and the striatum subserves response learning (Packard & McGaugh, 1996), regardless of the stage of training. A second alternative is
that they are dissociable by the stage of training—the hippocampus is recruited for early or immediate learning (Cammarota et al., 2000; Izquierdo et al., 2000; Chang & Gold, 2004; Rutishauser et al., 2006), followed by the gradual recruitment of cortical and subcortical structures, regardless of the behavioral phenotype. Furthermore, it is possible that the implicated brain region is necessary for one, but not more, of the following processes: acquisition, consolidation, or expression of learning.

To address a few of these possibilities, we conducted a series of experiments using temporary inactivation, or reversible lesions. In particular, we infused lidocaine, a sodium channel blocker, either into the dorsal hippocampus or into the dorsolateral striatum. In Chapter 4, we infused lidocaine into the dorsal hippocampus prior to probe tests, assessing its role in the expression of either strategy. Based on surprising results, a subsequent experiment assessed immediate early gene (IEG), *c-fos*, expression in select subregions of the dorsal hippocampus. Chapter 5 discusses a plausible theory of hippocampal function in light of these results. In Chapter 6, we infused lidocaine into the dorsolateral striatum prior to probe tests, assessing its role in the expression of either strategy. We also infused lidocaine into the DLS prior to early training sessions, investigating its necessity in the acquisition of the response strategy. These results are discussed in a broader perspective in Chapter 7.
CHAPTER 4: THE DORSAL HIPPOCAMPUS IS UNNECESSARY FOR THE EXPRESSION OF EITHER STRATEGY

The hippocampus has been implicated many times over in spatial learning and navigation, as well as rapid, even single-trial, learning. Critically, these results provide conflicting predictions for the submerged T-maze. If the hippocampus is critical for rapid, initial learning (Cammarota et al., 2000; Izquierdo et al., 2000; Chang & Gold, 2004; Rutishauser et al., 2006), then it subserves the immediate response strategy observed in Experiment 1. If the hippocampus is critical for spatial and relational information (McDonald & White, 1993; McDonald & White, 1994; Packard & McGaugh, 1996; Kumaran & Maguire, 2005), its recruitment in the submerged T-maze is a gradual one.

We sought to investigate whether the role of the dorsal hippocampus is similar in the submerged T-maze as that in the dry T-maze. In Experiment 3, we examined performance of rats receiving either a lidocaine or saline infusion to the dorsal hippocampus immediately prior to probe tests. We observed no effect of drug condition on behavior exhibited during probe tests, suggesting that the dorsal hippocampus is not required for the expression of either strategy. In Experiment 4, we confirmed the effectiveness of the infusion protocol in a positive control task with the same subjects. In Experiment 5, we examined immediate early gene (IEG) expression in select subregions of the dorsal hippocampus in subjects expressing a place or response strategy after early or late training.
Experiment 3 Methods

Subjects

Male Long-Evans rats (275-325g; Charles River Laboratories; Raleigh, NC) were housed individually in a temperature-controlled room, with lights on 7 A.M. to 7 P.M. Rats (n = 36) had free access to food (Teklad Chow 2018, Harlan Laboratories; Madison, WI) and water. Weights of all subjects, immediately prior to any manipulation, were 350-415g.

A set of 24 rats received bilateral cannulations to the dorsal hippocampus (Packard & McGaugh, 1996; Paxinos & Watson, 1998). A set of 12 rats (Normal) did not receive surgical manipulation, but were handled alongside the cannulated rats during post-operative care. These subjects were compiled from two replications, which did not differ in any methods or procedures. Unfortunately, we experienced the attrition of one rat during one such replication.

Apparatus

The submerged T-maze apparatus was the same as that previously described (Experiment 1).

Surgery

Subjects were anesthetized with 2-3% isoflurane (Piramal Critical Care, Inc., Bethlehem, PA) mixed with oxygen, and placed into the stereotaxic apparatus (Model 902, Kopf; Tijunga, CA). After incision, two 1/8-inch self-tapping mounting screws were installed into the skull. The dura was punctured with a 27-gauge needle, and a 26-gauge
guide cannula (PlasticsOne; Roanoke, VA), with stainless steel tubing cut to extend 6mm below the 8.0mm-long pedestal, was bilaterally implanted into the dorsal hippocampus. Coordinates for the dorsal hippocampus placements were AP = -3.1mm, ML = ±2.5mm, and DV = -2.0mm from bregma.

Cannulae were anchored with dental acrylic and fitted with dummy injectors, to ensure cannulae patency, which were cut to match the length of the guide cannulae. The incision was closed with surgical staples, and topical antibiotic ointment was applied to the wound. A single subcutaneous injection (0.03 mg/kg) of sterile buprenorphine hydrochloride (Sigma; St. Louis, MO) was administered to ameliorate pain. Rats were weighed and handled during post-operative care, which included the removal and replacement of dummy injectors to reduce stress during later infusions. Behavioral procedures began approximately 10 days after surgery.

**Infusions**

Rats had their dummy injectors removed and reinserted before each training session, to familiarize them with manipulation of their headstages. Two 33-gauge injector cannulae, which extended 2mm below the tip of the guide cannulae, were connected by polyethylene (PE50) tubing to separate 10µl Hamilton microsyringes in a multi-syringe pump (KD Scientific; Holliston, MA). The pump simultaneously administered 0.5µl of drug or vehicle bilaterally into the dorsal hippocampus, over 1min. After infusion, the injector was left in place for an additional 1min to allow for diffusion of the solution away from the needle tip. The dummy injectors were reinserted after removal of the injectors.
A 2% lidocaine hydrochloride solution (Vedco, Inc.; St. Joseph, MO) was used to produce temporary inactivation of brain sites, with 0.9% saline infused as vehicle. The volume of 2% lidocaine solution used was chosen on the basis of previous evidence indicating that this volume induces functional inactivation of the dorsal hippocampus (Perez-Ruiz & Prado-Alcala, 1989; Packard & McGaugh, 1996), sufficient to cause memory impairment. Drug or vehicle infusions were administered immediately prior to maze exposure.

**Histology**

Subjects were perfused following completion of Experiment 4. Histological procedures are described in Experiment 4 Methods.

**Experiment 3 Procedures**

*Habituation.* Procedures were the same as those previously described (Experiment 1).

*Training.* Procedures were the same as those previously described (Experiment 1), except that rats were run in squads of six subjects. The interval between the two daily trials ranged from approximately 2min to 8min during early training (Sessions 1-4) and 1min to 3min during later training (Sessions 5-24). After two days of training, cannulated subjects were divided into two groups, determining the order of drug infusion for each pair of probes. These groups were matched for mean latency and number of errors over the first two training sessions (Group 1: n = 12, Group 2: n = 11).
Probe Tests. Rats were trained for 24 days, with pairs of probe tests given at early, intermediate, late, and final stages of training. We limited our probe tests to these samples to minimize the number of infusions needed, and to avoid potential mechanical damage to the dorsal hippocampus. Recall that, in Experiment 1, the frequency of probe testing did not affect rats’ strategy selection.

At each stage of training, rats received one probe test after lidocaine and one after saline infusion. Thus, each rat could serve as its own control for the evaluation of the effect of drug. Rats in Group 1 received lidocaine on the first probe test, within each pair of tests, and saline on the second probe test, whereas rats in Group 2 received the infusions in the opposite order within each probe pair. Rats in these two groups were matched for mean latency and number of errors over the first two training sessions. Probe tests were given after training sessions 2 and 4 (Early), 10 and 12 (Intermediate), 18 and 20 (Late), and 22 and 24 (Final).

Subjects were run in squads of six subjects, such that all rats in a squad were infused before maze exposure. Each squad contained rats in both Groups 1 and 2. The order in which the rats in a squad were infused was counterbalanced across squads, so that all rats received varying delays between infusion and test (approximately 2-12 min).

Each probe test consisted of a single trial. On this trial, no escape platform was available in either arm, and access to the South (Start) arm was blocked using a Plexiglas insert. Rats were placed in the North arm (opposite the Start arm) and were allowed to choose between the previously trained or untrained arm. We reported the first arm entered by each rat. Rats approaching the reinforced extra-maze cues and entering the previously trained arm were designated as “place learners,” whereas rats producing the
reinforced response and entering the previously untrained arm were designated as “response learners.” Upon reaching the end of the arm, rats were removed from the maze, towel-dried, and returned to their home cages.

**Data Analysis**

An overhead camera and computer-assisted tracking system recorded each rat’s position in the maze. During training, an experimenter recorded each rat’s latency to reach the platform, as well as errors accrued. These measures were analyzed with mixed-design $2 \times 2 \times 2 \times 3 \times 24$ ANOVAs, with between-subjects variables Replication (1, 2) and the two counterbalancing variables, Group (1, 2) and Goal Arm (East, West), with repeated measures on Drug (Normal, Saline, Lidocaine) and Training Day. All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

For each probe test, we determined the probability of observing the recorded number of response learners in each group, based on the binomial distribution. We also analyzed the change in probe test performance over pairs of probe tests using mixed-design $2 \times 2 \times 2 \times 3 \times 4$ ANOVAs, with between-subjects variables Replication, Group, and Goal Arm, with repeated measures on Drug and Probe Pair (Early, Intermediate, Late, Final).

**Experiment 3 Results**

One rat showed symptoms of ataxia and difficulty swimming; this subject was removed from all analyses, and their data are not reported. Figure 5 shows the cannulae tip locations; they ranged between -2.56 and -3.30 mm AP. Three subjects showed small
unilateral lesions near the site of the guide cannula; data from these rats were included in our data analysis.

**Acquisition.** During training, we observed rapid acquisition, evidenced by asymptotic mean latency and number of errors to the platform after only four training sessions (Figure 6). We observed a significant decline in the mean latency to the platform \( (F(23,529) = 17.855, p < .001) \) and mean number of errors across Training Day \( (F(23,529) = 18.015, p < .001) \). There were no main effects or interactions of any counterbalancing variable (Replication, Group, Goal Arm) on errors \( (ps > .358) \) or latency \( (ps > .187) \) across Training Day. There was a significant Replication x Group interaction on mean errors \( (F(2,23) = 5.244, p = .013) \), but no other between-subject effects or interactions on errors \( (ps > .085) \) or mean latency \( (ps > .081) \).

Following early, intermediate, late, and final training, rats received either saline or lidocaine infusions to the dorsal hippocampus immediately prior to pairs of probe tests. All subjects were tested within pairs of probes, providing a report of strategy selection of all subjects under both vehicle and drug exposure.

**Probe Tests (Strategy Selection).** Across the interspersed probe tests, we observed the behavioral pattern expected on the basis of Asem and Holland’s (2013) findings, with rats switching from response learning to place learning. However, this pattern was not affected by lidocaine infusions.

On the first probe test, 25 of 35 rats \( (p = .008) \) entered the previously untrained arm and were designated as response learners. Of these response learners, 9 of 11 rats had received saline \( (p = .033) \). However, neither normal rats \( (8/12, p = .194) \) nor lidocaine rats \( (8/12, p = .194) \) were significantly response learners. The proportion of control
subjects (normal + saline) as response learners was not significantly different from the proportion observed in lidocaine animals (17/23 vs. 8/12, $\chi^2(1, n = 35) = .203, p = .652$). However, within the Early probe pair, 18 of 23 rats ($p = .005$) showed the response strategy on their saline test, and 12 of 23 rats ($p = .500$) showed that strategy on their lidocaine test. There was a marginally significant difference between these proportions ($\chi^2(1, n = 46) = 3.450, p = .063$).

On the final probe test, 26 of 35 rats entered the previously trained arm and were designated as place learners ($p = .003$). Of these, 11 of 12 normal animals were place learners ($p = .003$). However, neither saline rats (7 of 12, $p = .387$) nor lidocaine rats (8 of 11, $p = .113$) showed a significant place strategy on that test alone. Nevertheless, the proportion of control subjects (normal + saline) as place learners was not significantly different from the proportion observed in lidocaine animals (18/24 vs. 8/11, $\chi^2(1, n = 35) = .020, p = .886$). Similarly, within the Final probe pair, 17 of 23 rats ($p = .017$) showed the place strategy on their saline test, and 15 of 23 rats ($p = .105$) showed that strategy on their lidocaine test. There was no significant difference between these proportions ($\chi^2(1, n = 46) = .411, p = .522$).

This gradual switch from a response strategy to a place strategy (Figure 7A) was confirmed by a repeated measures ANOVA. This ANOVA showed a significant effect of Probe Pair ($F(3,69) = 7.155, p = .001$) and a significant linear trend over Probe Pair ($F(1,23) = 14.590, p = .001$) (Figure 7A). Furthermore, there was no effect of Drug ($F(1,69) = .165, p = .688$), but a marginally significant Drug x Probe Pair interaction ($F(3,69) = 2.453, p = .093$). This effect is likely driven by the marginally significant difference between saline and lidocaine subjects in the Early probe pair.
Additional analyses showed that the simple main effects of Probe Pair were significant for both lidocaine ($F(3,69) = 3.014$, $p = .043$) and saline ($F(3,69) = 7.919$, $p = .001$) tests (Figure 7A). Likewise, there was a significant linear trend over Probe Pair for both lidocaine ($F(1,23) = 4.037$, $p = .056$) and saline ($F(1,23) = 19.734$, $p < .001$) tests, suggesting a similar decrease in the use of a response strategy when tested under either condition. There were no between-subject effects or interactions involving Replication or any counterbalancing variables (Group, Goal Arm) on performance ($ps > .106$).

**Probe Tests (Latency).** As mentioned, there was no effect of drug on mean errors or latency to the platform on training trials, nor on the strategy selection when tested under lidocaine compared to saline (Figure 7B). Furthermore, there was no drug effect on the latency to the goal location on probe tests when tested under lidocaine ($F(3,69) = .799$, $p = .470$) or saline ($F(3,69) = .570$, $p = .580$).

There was a significant Replication x Group x Probe Pair interaction for saline probe tests ($F(6,69) = 2.525$, $p = .049$), but no other interactions with Probe Pair were significant ($ps > .117$). The lack of drug effect on latency during probe tests suggests that lidocaine infusions do not affect general motor movement.

There was a significant effect of our Group counterbalancing variable on probe test latency under saline conditions ($F(2,23) = 4.635$, $p = .020$), such that animals receiving saline first in each probe pair were slightly slower than those receiving lidocaine first in each probe pair.

Finally, there was a significant effect of Goal Arm on probe test latency under saline conditions ($F(1,23) = 9.880$, $p = .005$), such that rats swimming toward the East
(right) arm were slightly slower than those swimming to the West (left) arm. There was no effect of Replication or any other between-subject interactions (ps > .143).

Infusions of lidocaine to the dorsal hippocampus did not affect strategy selection or progression on probe tests compared to normal subjects or when those same subjects received infusions of saline. However, we observed a behavioral replication of Asem and Holland (2013), such that, when combined for analysis, rats exhibited an immediate response strategy, which shifted to a place strategy with extended training.

**Discussion**

In conclusion, despite observing a behavioral replication, infusing lidocaine into the dorsal hippocampus immediately prior to probe tests had no effect on behavior. Although several experiments suggest that the dorsal hippocampus is necessary for a spatial strategy in the Morris water maze (McDonald & White, 1994) and for a place strategy in the dry T-maze (Packard & McGaugh, 1996), data presented here suggest that the dorsal hippocampus is not required for the expression of either strategy in the submerged T-maze.

Because we observed no effect of drug, we sought to confirm the effectiveness of the lidocaine infusions to the dorsal hippocampus by testing the same subjects in a positive control task, a paradigm previously observed to require that region (Experiment 4).
Figure 5. Dorsal hippocampus cannulae placements showing the anterior/posterior extent of needle tip locations at 0.4mm sections. Placements ranged from -2.56 to -3.30mm from bregma. Plates adapted from atlas of Paxinos and Watson (1998), used by permission of Elsevier.
Figure 6 (A) Mean number of errors and (B) mean latency (s) to reach the escape platform during acquisition. Rats received two training trials per day. Error bars denote standard error of the mean.
Figure 7. (A) Percentage of response learners. Rats received an infusion prior to each single-trial probe test after Early (Days 2 and 4), Intermediate (Days 10 and 12), Late (Days 18 and 20), and Final (Days 22 and 24) training. Trend lines are based on relevant data points. (B) Mean latency (s) to the goal location. Error bars denote standard error of the mean.
Although we observed no effect of drug in the submerged T-maze, it is possible that the lidocaine infusions were ineffective in altering hippocampal activity. To assess these concerns, we sought to confirm a drug effect in the same subjects in a task known to require the dorsal hippocampus. A multitude of studies suggest the necessity for this region in appetitively motivated dry mazes (e.g., radial arm maze, plus maze) and in the Morris water maze. However, these paradigms closely resemble the apparatus and procedures of the submerged T-maze, which would likely result in a transfer of learning from previous experimentation.

Instead, we turned to an unrelated paradigm, a modification of the immediate shock effect, a task known to require an intact, active dorsal hippocampus. In the typical immediate shock protocol, normal animals are exposed to an operant chamber context, but are immediately shocked and removed from it. Under these conditions, subjects do not show fear to that context upon later presentation.

However, when normal animals are pre-exposed to the context and subsequently undergo the immediate shock, they show fear (i.e., freezing response) in that context. This context pre-exposure facilitation effect (CPFE) suggests that animals require some amount of time to form a contextual representation that allows subsequent conditioning to it. Animals with muscimol infusions to the dorsal hippocampus prior to pre-exposure

*Figure 8 (Matus-Amat et al., 2004). Average percent freezing observed in muscimol and vehicle rats during Test.*
(Matus-Amat et al., 2004; Figure 8) or prior to test do not show this freezing behavior, suggesting that both the encoding and retrieval of a contextual representation require an online dorsal hippocampus.

**Experiment 4 Methods**

**Subjects**

Subjects were the same male Long-Evans rats (Charles River Laboratories; Raleigh, NC) as in Experiment 3 and were matched for previous maze conditions and performance (Normal: n = 12, Saline: n = 11, Lidocaine: n = 12). Weights of all subjects (n = 35), immediately prior to Experiment 4, were 350-415g.

**Apparatus**

The behavioral training apparatus consisted of a set of four identical behavioral chambers (20.5cm x 22.0cm x 22.5cm) with stainless steel front and back walls, clear acrylic sides, and a floor made of 0.48cm stainless steel rods spaced 1.9cm apart. A dimly illuminated food cup was recessed in the center of the front wall. An infrared photocell, placed just inside the food cup, was polled (1 kHz) by computer circuitry to record the time each rat spent with its head in the food cup (not reported here). Each chamber was enclosed inside a sound attenuating shell.

Constant dim illumination was provided by a 6W lamp behind a dense red lens, mounted 10cm above the experimental chamber and even with the front wall of the chamber. A television camera was mounted within each shell to provide a view of the chamber; the output from each camera was digitized, merged into a single image of all
four chambers, and recorded on videotape to allow for viewing and behavioral scoring (not reported here).

**Infusions**

Procedures were the same as those previously described (Experiment 3).

**Histology**

After the completion of behavioral training and testing, animals without cannulae were sacrificed using carbon dioxide exposure. Cannulated animals were deeply anesthetized with isoflurane and perfused intracardially with 0.9% saline, followed by a 3.7% formalin solution. After removal of the headstages, brains were removed and stored at 4°C in 3.7% formalin containing 12% sucrose. Brains were sliced on a freezing microtome, and 40µm coronal sections were taken in series.

To confirm cannulae tip placements in the bilateral dorsal hippocampus, every third section was mounted on glass slides, dehydrated in ascending concentrations of alcohol, defatted in xylene, and stained with thionin. Slides were coverslipped, using Permount thinned with xylene, and examined with a light microscope. Cannulae placements were examined for verification of needle tip location using the atlas of Paxinos and Watson (1998).
Experiment 4 Procedures

Pre-Exposure. Rats were run in squads of four subjects. Subjects received either lidocaine or saline infusions, matched for previous performance and drug condition, immediately prior to 6min pre-exposure to the chamber.

Immediate Shock. On the next day, rats were placed in the same chamber as during pre-exposure and received immediate delivery of electric shock (2 mA, 2s; model H13-15; Coulbourn Instruments; Holliston, MA) through the metal bars comprising the training chamber floor. Rats were immediately removed from the chamber, within 1s of shock termination.

Test. On the final day, rats were placed in the same chamber as the previous two days for 6min and monitored for freezing behavior.

Data Analysis

To allow for convenient data sampling, the 6min test session was divided into ten trials with equal duration and ITIs. During these trials, the computer program recorded the activity counts of each rat. Activity counts were subjected to mixed-design 2 x 3 x 10 ANOVAs, with between-subjects variables Replication (1, 2) and Drug (Normal, Saline, Lidocaine), with repeated measures on Trial. All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

Additionally, a series of univariate analyses assessed these between-subject effects on the mean activity of the two five-trial halves of the session, as well as the mean activity of all ten trials.
Experiment 4 Results

Test. Although a repeated measures ANOVA did not report a significant change in activity counts across trials (F(9,198) = 2.830, p = .076), there was a significant Drug x Trial interaction (F(72,198) = 3.236, p = .002), such that lidocaine rats showed greater activity across trials than normal or saline subjects (Figure 9A). This observation was confirmed by a main effect of Drug (F(8,22) = 18.039, p < .001). All other interactions involving Trial were nonsignificant (F(18,198) < .753, p > .551), as were all effects involving Replication (ps > .519).

A series of univariate ANOVAs assessed the effects of Drug and Replication: there was a significant effect of Drug on the first half of trials (Trials 1-5) (F(8,22) = 2.925, p = .022), on the second half of trials (Trials 6-10) (F(2,22) = 3.865, p = .006), and on the mean activity of all trials (Trials 1-10) (F(8,22) = 21.443, p < .001) (Figure 9B). However, all effects involving Replication were nonsignificant (ps > .562).

Discussion

In the same subjects from Experiment 3, in which we found no evidence that dorsal hippocampal lidocaine infusions affected behavior in the submerged T-maze, similar infusions produced substantial deficits on the context pre-exposure facilitation effect, a modification of the immediate shock effect, known to require the dorsal hippocampus. These data suggest that the lidocaine infusions themselves are effective at reducing hippocampal activity; from this, we can infer that, unlike in Packard and
McGaugh’s (1996) dry maze task, performance in our submerged T-maze task may not require an intact, active dorsal hippocampus.
Figure 9. (A) Mean activity/min across trials of Test. (B) Mean activity/min of all ten trials of Test.
The literature has accepted the critical role of the dorsal hippocampus and the dorsolateral striatum in the acquisition and expression of a place and response strategy, respectively, and in broader spatial learning and habit formation. However, our data suggest that the expression of a place strategy, at least in this task, may be independent of the hippocampus.

Notably, our infusions targeted the CA1 subregion of the dorsal hippocampus. It is possible that hippocampal subregions are differentially active in our task. To assess this possibility, we examined expression of a common marker of neural activity, Fos, the protein product of the immediate early gene (IEG) c-fos, in the dentate gyrus (DG), CA1, and CA3 subregions of the dorsal hippocampus.

We hypothesized that an approach targeting several subregions of the hippocampus may elucidate its role in the submerged T-maze. To explore this possibility, we investigated Fos expression in the dorsal hippocampus immediately following early- and late-training probe tests, which provided a cursory survey of hippocampal subregions that may show alterations in activity as strategy selection changes. Examining the expression of IEGs, such as c-fos, has been a useful tool in associating region-specific neuronal activity with particular functions.

Subjects (n = 24) were habituated and trained as previously described (Experiment 1). After two days of training, rats were divided into two groups, matched for training performance, determining whether they would be tested and sacrificed early or late in training. Group Early (n = 12) was sacrificed immediately following their first probe test, after two days of training. Group Late (n = 12) did not receive the early probe test, but was given extended training and sacrificed immediately following their first
probe test, after 24 days of training. One rat in each group, matched for performance on the first two training sessions, was designated as a control subject for baseline comparison of Fos expression measures. These control rats received identical training, but no probe tests.

Experiment 5 Methods

Subjects

Male Long-Evans rats (275-325g; Charles River Laboratories; Raleigh, NC) were housed individually in a temperature-controlled room, with lights on 7 A.M. to 7 P.M. Rats (n = 24) were experimentally naïve and had free access to food (Teklad Chow 2018, Harlan Laboratories; Madison, WI) and water. Weights of all subjects, immediately prior to any manipulation, were 350-415g.

Apparatus

The submerged T-maze apparatus was the same as that previously described (Experiment 1).

Histology

Rats were perfused 90 minutes after the start of the probe test in order to detect Fos protein expression associated with the observed strategy solution. Rats were deeply anesthetized with isoflurane and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in 0.1M phosphate buffer (PB).
Brains were removed, post-fixed, and cryoprotected overnight in 4% paraformaldehyde in 0.1M PB containing 12% sucrose, frozen with powdered dry ice, and stored at -80°C. Brains were sliced on a freezing microtome, and 40µm coronal sections were collected in series; these slices were stored in cryoprotectant at 4°C.

_Fos Immunohistochemistry_

Slices were divided into several immunohistochemistry runs; each run included subjects from various conditions and always included at least one Early and Late subject or at least one Place and Response subject. Every effort was made to match each run for diversity of represented conditions.

After several rinses in 0.1M phosphate buffer containing 0.9% saline (PBS), tissue was incubated in PBS containing 0.3% Triton X-100 (PBST) (Sigma-Aldrich; St. Louis, MO) and 6% normal goat serum (S-1000; Vector Laboratories; Burlingame, CA) for 2 hours. Sections were incubated in rabbit polyclonal IgG (1:2000 dilution, sc-52-G; Santa Cruz Biotechnology; Santa Cruz, CA) in PBST containing 3% goat serum for 72 hours at 4°C.

After the primary antibody incubation, sections were rinsed in PBS, incubated in biotinylated goat anti-rabbit IgG (1:250 dilution, BA-1000; Vector Laboratories) for 1 hour, rinsed in PBS, and then incubated in Avidin-Biotin horseradish-peroxidase complex (PK-6100, Vectastain ABC Elite Kit; Vector Laboratories) for 1 hour. After several rinses in PBS, tissue was reacted using a 3,3’-diaminobenzidine (DAB) peroxidase (HRP) substrate kit (SK-4100; Vector Laboratories). Tissue was mounted and dried overnight on
glass slides, dehydrated in ascending concentrations of alcohol, defatted in xylene, and coverslipped with Permount thinned in xylene.

Images of selected Fos-stained sections were acquired using an AxioCam camera. Using an image analysis system (ImageJ), Fos positive cells were counted in dorsal DG, CA1, and CA3 subregions in the left and right hemispheres of the hippocampus in two brain slices for each subject (Figure 12). These slices were selected approximately -3.14 mm from bregma (Figure 12), and ranged between -2.80 mm and -3.30 mm from bregma. A series of paired-sample t-tests showed no significant difference between the number of Fos positive nuclei counted in the left and right hemispheres for any subregion (ps > .647), so all analyses used the mean of these counts.

Experiment 5 Procedures

Habituation. Procedures were the same as those previously described (Experiment 1).

Training. Procedures were the same as those previously described (Experiment 1). Rats were divided into two subgroups, matched by performance over the first two training sessions, determining whether they would be tested and sacrificed early or late in training (Early: n = 12, Late: n = 12). At this juncture, two control subjects were designated, matched by performance.

Probe Tests. Procedures were the same as those previously described (Experiment 1). Rats were run in squads of four subjects. Subjects in Group Early received two days of training, followed by one probe test. Late animals were not tested on the first probe test, but began extended training on the following day. Subjects in Group Late received
24 days of training, followed by one probe test. Control subjects were given identical treatment during the probe test without exposure to the maze. To be explicit, they were brought to the experimental room, but were not placed in the maze apparatus itself. All rats were returned to their home cage after testing for 90 minutes before perfusion.

Data Analysis

An overhead camera and computer-assisted tracking system recorded each rat’s position in the maze. During training, an experimenter recorded each rat’s latency to reach the platform, as well as errors accrued. These measures were analyzed with mixed-design 2 x 2 x 2 ANOVAs, with between-subject counterbalancing variables of Group (Early, Late) and Goal Arm (East, West), with repeated measures on Training Day for the first two sessions, to confirm matched performance.

Extended training measures were analyzed in the Late group with mixed-design 2 x 24 ANOVAs, with counterbalancing variable of Goal Arm, with repeated measures on Training Day. All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

For each probe test, we determined the probability of observing the recorded number of response learners in each group, based on the binomial distribution.

To assess Fos positive nuclei in hippocampal subregions, the mean cell counts from both hemispheres of each experimental subject were normalized by the selected area, averaged, and analyzed using a series of 2 x 2 x 2 x 2 univariate analyses, with between-subject variables of Group (Early, Late) and Strategy (Place, Response), with
counterbalancing variable of Goal Arm (East, West) and nuisance variable of Stain (Light, Dark) to account for observable variation.

**Experiment 5 Results**

*Acquisition.* During the first two days of training, we observed rapid acquisition, confirmed by a significant effect of Training Day on mean latency (F(1,20) = 6.874, p = .016) and a marginally significant effect on mean number of errors (F(1,20) = 4.287, p = .052), with no significant interactions (ps > .373). Additionally, our ANOVA confirmed matched performance: there was no significant difference between Groups Early and Late in early training performance, by either mean latency (F(1,20) = .024, p = .879) or mean number of errors (F(1,20) = .057, p = .813), nor any other between-subjects effects or interactions (ps > .109).

During extended training in Group Late, this rapid acquisition was evidenced by asymptotic mean latency and number of errors to the platform after Day 6 (Figures 9A and 9B). We observed a significant decline in the mean latency to the platform (F(23,230) = 5.307, p = .011) and a marginally significant decline in the mean number of errors (F(23,230) = 2.425, p = .062) across Training Day. There were no effects or interactions of Goal Arm (ps > .388), except a main effect on mean number of errors (F(1,10) = 5.284, p = .044).

*Probe Tests (Strategy Selection).* Probe test data include one fewer animal per group due to two control subjects (Group Early: n = 11, Group Late: n = 11). After two days of training, Group Early received a probe test, in which 8 of 11 rats entered the previously untrained arm and were designated as response learners (p = .113). Critically,
this proportion is not significantly different from that reported in Experiment 1’s Single
group, which received identical procedures (8/11 vs. 8/10, $\chi^2(1, n = 21) = .153, p = .696$);
thus, we argue that the lack of a significant strategy choice is not crucial (Figure 10A).

After 24 days of training, Group Late received a probe test, in which 4 of 11 rats
entered the previously trained arm and were designated as place learners ($p = .887$). This
proportion is significantly different from that reported in Experiment 1’s final probe test
(7/11 vs. 21/24, $\chi^2(1, n = 38) = .11.120, p < .001$), but is not significantly different from
the proportion of place learners observed in Group Early’s probe test (8/11 vs. 7/11, $\chi^2(1,
n = 22) = .210, p = .647$).

**Probe Tests (Latency).** Latencies observed on probe tests were comparable after
early and late training, confirmed by an ANOVA reporting no significant effect of Group
($F(1,18) = .737, p = .402$) (Figure 11B) and no effect or interaction of Goal Arm ($p > .217$). These latencies were comparable to those observed on contemporaneous training
sessions (Figure 10B).

**Fos Expression.** We examined Fos expression in the dorsal hippocampus (Figures
12 and 13). Using immunohistochemical procedures used extensively by others in the
laboratory (e.g., Lee et al., 2010), we compared the number of Fos positive labeled cells
in subregions of the dorsal hippocampus in rats entering the originally trained arm versus
those entering the originally untrained arm (inferring place and response strategies,
respectively), and assessed the difference between those counts observed early and late in
training.

Due to degraded tissue, four subjects were removed from all analyses involving
Fos counts. Furthermore, observable differences in staining density resulted in
questionable counts in a few subjects. Thus, clear outliers were eliminated, resulting in relatively matched sample sizes for each of the four experimental conditions (Early Response: n = 3, Early Place: n = 3, Late Response: n = 3, Late Place: n = 4). Because of the low sample size (n = 13) and missing data, ANOVAs reported here used adjusted dfs and p-values, as provided by SPSS, formerly known as Statistical Package for the Social Sciences (International Business Machines (IBM) Corporation; Armonk, NY).

Dentate Gyrus. A Group x Strategy x Goal Arm x Stain analysis on Fos positive nuclei in the dentate gyrus (DG) yielded no main effects (ps > .192) or interactions (ps > .225), except for a marginally significant Strategy x Stain interaction (F(1,2) = 16.102, p = .057). Separate Strategy x Goal Arm x Stain analyses for Groups Early and Late also yielded no significant effects (ps > .202, ps > .488, respectively), except for a marginally significant main effect of Strategy in Group Late (F(1,2) = 13.411, p = .067), such that Late place learners had more Fos positive nuclei than Late response learners.

CA1. A Group x Strategy x Goal Arm x Stain analysis on CA1 resulted in significant main effects of Group and Strategy (F(1,2) = 59.090, p = .017; F(1,2) = 40.207, p = .024, respectively), such that Early subjects and place learners showed more Fos positive nuclei.

There was also a significant main effect of our counterbalancing variable, Goal Arm (F(1,2) = 75.481, p = .013), such that subjects swimming to the East (right) arm showed more Fos positive nuclei. Furthermore, all interactions involving the Goal Arm variable were significant: Group x Goal Arm, Strategy x Goal arm, and Goal Arm x Stain (ps < .047). We also observed Goal Arm effects in Experiment 3, in which subjects
swimming to the East (right) arm had significantly slower latency. These results suggest that the maze cues and/or quadrants may not be equally salient.

Separately analyzing Group Early maintained the main effect of Goal Arm (F(1,2) = 142.533, p = .053), but there was no significant effect of Strategy (F(1,1) = 3.132, p = .327). On the other hand, separately analyzing Group Late maintained the significant main effect of Strategy (F(1,2) = 32.101, p = .030), such that place learners yielded more Fos positive nuclei. Although the main effect of Goal Arm was no longer significant (F(1,2) = 4.194, p = .177), both interactions involving the variable were (ps < .047).

Finally, the observable variation in staining density (light vs. dark) resulted in cell counting differences. An ANOVA on all subjects yielded a significant main effect of our nuisance variable, Stain (F(1,2) = 315.525, p = .003), such that lighter-stained sections had more (countable) Fos positive nuclei. This effect was maintained in separate analyses on Groups Early and Late (F(1,2) = 327.449, p = .035; F(1,2) = 130.116, p = .008, respectively); however, the Strategy x Stain interaction was nonsignificant (F(1,2) = 7.899, p = .107). This result is consonant with other findings of stain visualization and particle-counting procedures (Rieux et al., 2002).

CA3. A Group x Strategy x Goal Arm x Stain analysis on CA3 showed no significant main effects or interactions (ps > .096). Separate Strategy x Stain x Goal Arm analyses for Groups Early and Late also yielded no significant effects (ps > .384, ps > .155, respectively).
Discussion

Our hypothesis that hippocampal subregions play more selective roles in behavior in the submerged T-maze was supported by our preliminary assessment of immediate early gene (IEG) expression in the dorsal hippocampus. Although the DG and CA3 are not significantly differentially active in behavior, CA1 may be.

CA1 was significantly more active early in training regardless of spatial strategy, supporting a role for CA1 in initial learning about mazes. Nevertheless, CA1 was also significantly more active among place learners than among response learners; this effect was driven mostly by rats tested later in training and is consistent with common views of hippocampal involvement in the use of place strategies. Although no other significant differences were observed, CA3 also appeared to be more active early in training, and its activity seemed to be confined to place learners. However, our inactivation results cannot be forgotten: although these regions may play a role in behavioral selection, the necessity of each subregion is far from certain.

Indeed, several issues prevent us from making any strong conclusions. Although we infer a strategy selection in other experiments based on each subject’s entrance into one arm or the other, this inference is less tenable here due to nonsignificant behavioral results. Our decision to treat our control subjects similarly to experimental subjects—i.e., transporting them to the experimental room, but not exposing them to the maze itself—may have contributed to their high Fos counts.

Furthermore, our sample size is far lower that used in similar experiments presented here, which underlies the more obvious point that we did not observe a significant number of place or response learners either early or late in training. Finally,
this issue of low sample size is exacerbated due to a limited sample of Fos sections per subject. Based on these issues, it is clear that the results presented here are preliminary in nature, meant to suggest future directions.
Figure 10. (A) Mean number of errors and (B) mean latency to reach the escape platform during acquisition of all subjects. Training Days 1 and 2 contain 24 subjects, whereas the remaining days contain 12 subjects. Rats received two training trials per day. Error bars denote standard error of the mean.
Figure 11. (A) Percentage of response learners. Rats in Group Early (n = 11) received a single probe trial after the 2nd training session and Group Late (n = 11) received a single probe trial after the 24th training session. (B) Mean latency (s) to the goal location. Error bars denote standard error of the mean.
Figure 12. Analyzed Fos protein expression from (A) noted areas, and (B) sample high-resolution image from CA1. This sample and analyzed images were taken from -2.80 mm to -3.30mm from bregma (Paxinos & Watson, 1998), used by permission of Elsevier.
Figure 13. (A) Mean Fos positive nuclei in dorsal hippocampal subregions in place and response learners compared to relevant Control subjects after early or late training. Error bars denote standard error of the mean. There are no error bars for Controls (n = 1). Number of Fos positive nuclei for individual subjects (circles) and mean number of nuclei (squares) in (B) DG, (C) CA1, and (D) CA3 subregions of the dorsal hippocampus.
CHAPTER 5: THEORIES OF HIPPOCAMPAL FUNCTION

The hippocampus has been implicated, many times over, in the acquisition, consolidation, storage, retrieval, and expression of declarative memory (Scoville & Milner, 1957; Marr, 1971; O’Keefe & Nadel, 1978; Eichenbaum, 2000; Leutgeb et al., 2004; Squire et al., 2004; Vazdarjanova & Guzowski, 2004; Davachi, 2006; Leutgeb & Moser, 2007; McHugh et al., 2007; Eichenbaum & Cohen, 2014). Although the earliest hypothesis of hippocampal function primarily revolved around its role in spatial learning and used rodent maze paradigms for elucidation, the data presented in this dissertation suggest that the hippocampus is unnecessary for the expression of a place (spatial) strategy in the rodent submerged T-maze.

To account for these results, this chapter reviews a few of the most popular theories of hippocampal function and its elusive role in spatial, relational, and declarative memory. We suggest that the hippocampus subserves episodic memory in humans and episodic-like memory in nonhuman animals. Such autobiographical memory is often discussed via the dissection into its necessary components: the what, where, and when of one’s personal experiences. Indeed, the hippocampus is critical in spatial learning and navigation, binding such representations to nonspatial information, and providing an order or timestamp to such events.

To begin, we visit the earliest conceptualization of the hippocampus as a cognitive map, then consider its broader role as a convergence zone for spatial and nonspatial information in relational memory. A more computational theory is then summarized, based on intrahippocampal microcircuitry: pattern separation, pattern completion, and the hippocampus as an auto-associator. After such a consideration, we
propose that the primary role of the hippocampus is to bind information into episodes, allowing for flexible predictions and novel inferences.

SPATIAL LEARNING

As discussed in Chapter 1, mazes are common paradigms used in the investigation of animal learning. In the early 1900s, behaviorists attributed maze acquisition to stimulus-response (S-R) learning, in which animals primarily solved the task by making a series of motor movements. Controversial at the time, Tolman (1948) hypothesized that animals used a more cognitive “field map,” supported by several pieces of empirical experiments, such as the “starbust” experiment (Tolman et al., 1946). In this task, rats were placed in the entrance arena of a maze (Figure 14), and trained to receive a food reward in the goal box. In a critical test of the animals’ knowledge beyond the appropriate set of turns, subjects were placed in a revised maze (Figure 14), with the identical starting arena. Subjects were unable to use the typical route, but preferentially entered arms that provided a shortcut to the goal location.

Figure 14 (Tolman et al., 1946). Training (left) and testing (right) apparatus.
Our acceptance and understanding of such a “field map” representation expanded with the addition of the Morris water maze paradigm (Morris, 1984; Vorhees & Williams, 2006) and the rotation/transposition manipulations previously discussed in Chapter 1. However, pertinent to our current discussion is the recognition of the hippocampus as one of the critical regions of interest, which came from an entirely different source.

This consideration began with the impairments observed in Henry Gustav Molaison (Patient H.M.) after a bilateral lobectomy intended to control severe and frequent epileptic seizures. For such a goal, the surgery was a success; however, Patient H.M. also showed temporally-graded retrograde amnesia—forgetting the more recent events prior to his surgery, but remembering more long-term ones—as well as anterograde amnesia, an inability to form new memories (Scoville & Milner, 1957). Structural imaging during his life (Corkin et al., 1997) and histology after his death (Annese et al., 2014) showed medial temporal lobe tissue (MTL) damage, centering on the hippocampus. The stark deficits observed after such hippocampal damage in the human Patient H.M. produced a flurry of data, particularly after the addition of comparable animal models in the rodent (Clark & Squire, 2010) and non-human primate (Zola-Morgan & Squire, 1986).

A COGNITIVE MAP

This burst of activity, combined with the aforementioned spatial learning literature, spurred one of the earliest formalized theories of hippocampal function as a cognitive map (O’Keefe & Nadel, 1978). This theory is based on two major lines of
evidence: first, lesions of the hippocampus lead to impairments in spatial learning and memory; second, there are cells in the hippocampus and general medial temporal lobe that respond to the components necessary to form such a representation.

Indeed, early investigations on the function of the hippocampus noted the necessity for the region in the expression of correct performance in maze tasks (e.g., McDonald & White, 1993; McDonald & White, 1994; Packard & McGaugh, 1996). In one of the earliest examples, a newly-developed water maze protocol showed that hippocampal lesions impaired place navigation (Morris et al., 1982).

However, there are exceptions to this first line of evidence: damage to the hippocampus does not always result in impairments in spatial learning. Indeed, the results presented in this dissertation appear to be one of many examples. In one such case, rats with fornix lesions were impaired in the conventional radial arm maze, in which subjects actively visit several arms. However, subjects were spared of impairments in a variant task, which required memory for distal cues when confined (McDonald & White, 1993).

Not only are there spatial tasks that do not require the hippocampus, there are also nonspatial tasks that do. For example, in simple object discrimination, increasing the delay interval can result in impairments in hippocampal-lesioned rats compared to shorter delays (Vnek et al., 1995). Indeed, increasing the delay in eyelid conditioning between the offset of the signaling stimulus and the onset of the offending stimulus results in impaired memory in rabbits with hippocampal lesions (Kim et al., 1995).

Together, these results suggest that a variety of factors affect whether memory performance is hippocampal-dependent or –independent, such as active exploration, the presence of a foreground signal, and variations in the timing of events (Eichenbaum,
1996). Furthermore, hippocampal-dependent spatial learning can be affected by stress factors; this consideration is discussed in Chapter 8.

The potential role of the hippocampus in spatial learning was corroborated by the discovery of place cells, pyramidal cells in the hippocampus that preferentially respond to specific places in particular environments (O’Keefe & Dostrovsky, 1971). Critically, place representations alone are not enough to build a map; a sense of direction and a metric for distance are also necessary. We see these necessary cell types in the hippocampus and surrounding cortices: boundary cells in the hippocampus, grid cells in the entorhinal cortex, as well as landmark/vector and head direction cells in the subiculum.

However, this second line of evidence is not without issue. Further experimentation showed that place cells represent both spatial and nonspatial information. Moreover, they exhibit directionality; that is, they show the same signal when direction does not matter in the task, as in an open-field foraging task, but these same nondirectional cells will become directional, producing a different pattern of activity in a task in which direction matters, e.g., radial arm maze, or even when the same environment is used in a task in which direction has become relevant. The directionality of place cells suggests that they code the behavioral significance of specific locations.

The ability of the hippocampus to provide a cognitive map depends on two major sources of information: external stimuli as well as an internal navigational (e.g., movement-based) system that is independent of the current environmental stimuli. This information is provided by two separate inputs. Cortical input via neocortex association regions provide sensory and environmental information to the parahippocampal gyrus
and then to the hippocampus. On the other hand, the modulatory input of the septum via the fornix fiber bundle provides organization, body state, and movement information.

Critically, our task, with its current manipulations, cannot speak to the cognitive map theory. Subjects in our task were never probed to confirm such a “field map” spatial representation: that their flexible knowledge of the environment would react to manipulations of transposition but not rotation, addition, or subtraction of cues. However, other results, from literature using maze paradigms, provide the inference that several factors, other than spatial stimuli denoting a “place,” determine the encoding of an environment. When both spatial and nonspatial cues are available, hippocampal cells encode both types of information, providing that they are salient and consistent, suggesting that hippocampal representations include several types of relationships that are relevant to task performance (Eichenbaum, 1996).

TIME MATTERS

The cognitive map theory of the hippocampus focuses on the spatial relationship between items in the environment. However, such a physical arrangement is likely just a prime example of configuring information. Although space is one of the most reasonable ways that we might expect to organize information, time is another. Indeed, our very existence is inextricably tied to time. Recent data pinpoint the hippocampus in its role in chronological, or temporal, information such as sequence memory and elapsed time.

The ability to encode and utilize a temporal pattern in item presentation seems to depend on the hippocampus, assessed through the choosing of the earlier or later odor
presented in a sequence of various lengths (Fortin et al., 2002; Kesner et al., 2002), or in avoiding previously visited arms in a radial arm maze (Chiba et al., 1994).

A more recent discovery was that of time cells in the hippocampus, cells that respond to specific epochs in an experience, even when location, speed/velocity, and other such variables are controlled. These cells behave similarly to place cells, showing re-mapping in response to changes in the environment or timing of the event (McDonald et al., 2011; Kraus et al., 2013).

Moreover, subregions of the hippocampus are sensitive to specific episodes of time. For example, Mankin et al. (2012) trained rats to forage for food in an open-field arena in a morning and afternoon session each day. Cells in CA1 were preferentially active for either the morning or afternoon session, whereas cells in CA3 responded similarly during both sessions. These results suggest that the hippocampus has the ability to use time as an organizing variable, and that it is necessary for putting items in an ordered sequence.

RELATIONAL MEMORY

Accumulating evidence indicates that the hippocampus organizes memories by both spatial and temporal properties, suggesting a primary role in forming relational representations. Although the role of the hippocampus in spatial memory is of special interest in this dissertation, this role extends to a broader one in relational memory, in which items and events are integrated into a coherent, unified representation.

However, as with spatial memory, the evidence can be controversial—perhaps a spatial organization is too limiting, but not all relationships are mapped in the
hippocampus. One human neuroimaging study showed hippocampal activation in a spatial relational task, but not in nonspatial (social) one (Kumaran & Maguire, 2005). However, another experiment, testing memory for three different types of relationships (i.e., temporal, spatial, and item co-occurrence) showed hippocampal activation for all types (Konkel & Cohen, 2009).

One elegant paradigm, utilizing ethological relevance, takes advantage of animals’ natural foraging strategies, requiring rats to dig in scented sand to receive food reward (Bunsey & Eichenbaum, 1993). Here, lesions to the hippocampus produce impaired performance. Importantly, some form of stimulus-stimulus representations can be acquired without the hippocampus; however, they are hyperspecific, and not flexible (Eichenbaum, 1999).

Another ethologically relevant paradigm used to examine relational memory is the social transmission of food preferences. This form of social learning falls under the adaptive heuristic that a food item that is consumed by a conspecific is safe for personal consumption. Rats with hippocampal lesions showed intact

![A Memory space](image1.png)

![B Spatial memory](image2.png)

*Figure 15 (Eichenbaum & Cohen, 2014).* A conceptual illustration of memory space, designating the three key types of relational processing, as they apply generally and to spatial memory specifically.
short-term memory, but showed no evidence of the association one day later (Bunsey & Eichenbaum, 1995), similar to the sparing of immediate memory, but impairment in delayed memory in human amnesics (Eichenbaum, 1999).

It is clear that the hippocampus is not only important in cognitive maps of spatial representations; more accurately, it may be important in the mapping of relationships between relevant cues (Eichenbaum, 1996). Indeed, a reconciliation of the hippocampus’ role in spatial and relational memory can be achieved if we consider our memory system as having organizing principles similar to that of spatial memory, but with broader components (Eichenbaum & Cohen, 2014; Figure 15).

A critical piece of evidence for this “memory space” theory comes from observations that the hippocampus treats events within a context similarly to objects within a space. For example, rats with hippocampal damage are able to recognize objects when presented in their original context, but not when misplaced (Eacott & Norman, 2004; Langston & Wood, 2010), and object information can be represented in place cell firing activity (e.g., Moita et al., 2003; Komorowski et al., 2009; Itskov et al., 2011; Itskov et al., 2012).

A CONVERGENCE ZONE

It appears that the hippocampus supports a more general function of flexible, relational memory that can be used for spatial and nonspatial information. The critical issue, determining hippocampal involvement, is the representational demands of the task, and not whether or not the task should be considered spatial (Eichenbaum, 1996). Thus, we can consider the role of the hippocampus as a convergence site for content and
organization: binding contextual information and organizing memories in both space and time—first, based on its extrahippocampal inputs and, second, based on its intrahippocampal circuitry and likely computations.

Evidence suggests that spatial information from the parahippocampal cortex and nonspatial, object or item, information from the perirhinal cortex feeds into the hippocampus via the medial and lateral entorhinal cortex, respectively, to be bound into one coherent representation with both components (Davachi, 2006).

The parahippocampal cortex contains an expanse that has been called the parahippocampal place area (PPA) for obvious reasons: in neuroimaging tasks, this region is selectively active when participants view scenes (Epstein et al., 1999). In rhesus monkeys, lesions of the TH/TF (parahippocampal cortex) lead to selective impairments in the discrimination of spatial locations (Bachevalier & Nemanic, 2008).

The perirhinal cortex has been implicated in object recognition and discrimination. In neuroimaging tasks, this region is active when participants make accurate face recognition judgments (O’Neil et al., 2009), and lesions of this region lead to deficits in object discrimination in rhesus monkeys (Bachevalier & Nemanic, 2008) and odor recognition in rodents (Young et al., 1997). However, its role in object identity is not limited to simple factors; rather, it is involved in the binding of identity-related information including color, size, complexity, and shape.

The hippocampal formation and related cortices are critically involved in the binding of complex information. Another form of binding can be discussed within the concept of a schema, an organized collection of related information within a comprehensive category. Consolidation forms a schema of the environment, within which
new information can be quickly integrated with little effect on the previously stored information. We see that rapid systems consolidation can occur if a schema is in place, allowing new information to be incorporated quickly (Tse et al., 2007). This consolidation is hippocampal-dependent for at least three hours, but is no longer so after one day (see Squire, 2007, and Rudy & Sutherland, 2008, for commentary and criticism, respectively). Thus, the hippocampus is considered to play a role in spatial and relational memory, as well as rapid, even single-trial, learning.

AN AUTO-ASSOCIATOR

To understand the mechanisms by which the hippocampus might subserve rapid, single-trial learning, as well as maintain such broad relational representations, the intrahippocampal microcircuitry should be considered. To begin, the hippocampal formation has mostly unidirectional projections. The entorhinal cortex (EC) provides the major input to the dentate gyrus (DG), via the perforant path, but the DG does not return a projection. The EC receives cortical sensory information and, thus, the DG is thought to be the first step of processing, but it only projects to CA3.

Two mechanisms that play a critical role in learning and memory are pattern separation and pattern completion, involving the DG (Gilbert et al., 2001; Neunuebel & Knierim, 2014; Newman & Hasselmo, 2014) and CA3 (Gold & Kesner, 2005; Bakker et al., 2008), respectively. CA1 appears to produce both under different circumstances (pattern separation: Gilbert et al., 2001; pattern completion: Bakker et al., 2008).

Pattern separation involves the amplification of small differences into large ones; in other words, it distinguishes two similar representations by re-mapping hippocampal
population codes. We see the necessity for the DG in behaviors requiring such resolution in that lesions of the DG affect the ability to discriminate spatial similarity, operationalized in terms of degrees of separation in space by using a radial arm maze paradigm (Kesner, 2007). Indeed, lesioned animals were more impaired with smaller separations, and this deficit decreased as the amount of separation increased.

In early considerations, pattern separation was considered to be supported by characteristics of the mossy fiber projection system. The unmyelinated axons of the granule cells, in the granule layer of the DG, are called mossy fibers, which project to the dendritic spines on the mossy cells of the polymorphic layer. These mossy fibers also project to the pyramidal cells in CA3. The sparse coding of these fibers is one physiological signature by which small changes in input to the DG may result in very different population activation.

However, a recent report provides clearer evidence that DG granule cells themselves provide the processing power for pattern separation. Because pattern separation is defined as the orthogonalizing of inputs, one must assess the (EC) inputs to DG. Neunuebel and Knierim (2014) recorded in both DG and CA3 in awake, behaving animals, while local and global cues were either congruent or incongruent with previous experience. Based on earlier results showing a coherent representation by MEC neurons (Neunuebel et al., 2013), the lack of such coherence in the DG (Neunuebel & Knierim, 2014) suggests a pattern separation process by those neurons.

On the other hand, pattern completion is not driven by small differences. CA3’s recurrent collaterals allows it to autoassociate. Synaptic plasticity strengthens the connections among constellations of coactive neurons. This association later allows a
subset of that constellation to provide excitatory drive to the remaining portion of the original set of coactive neurons and reactive, or complete, the full original constellation. Thus, a hippocampal representation will persist, despite the removal or modification of subsets of the original input. Critically, the pattern observed in CA3 more closely resembles the original EC input pattern than the subsequently degraded DG input, indicating a pattern completion process (Neunuebel et al., 2013; Neunuebel & Kierim, 2014).

These results complement previous reports on CA1 and CA3. Using a “morphing” enclosure, in which the environment can slowly change from one shape to another, Leutgeb et al. (2005) showed that parametrically varying the change or similarity in input resulted in changes in the population activity of neurons in CA1 in a graded fashion, but in a step-wise transfer function in CA3, suggesting that CA3 is highly sensitive to small changes in input, whereas CA1 is not. Human neuroimaging later provided corroborating evidence by varying pairs of words with mnemonic similarity (Lacy et al., 2011).

These results support the theory of the hippocampus as an attractor network. The step-wise function observed in CA3 suggests that representations of different, but familiar, environments are represented by distinct attractor states. A defining property of an attractor network is its ability to undergo distinct transitions between previously established representations when the inputs are varied (Leutgeb et al., 2005a). To support this observation, the authors recorded from CA1 and CA3 while a familiar square environment was systematically morphed into a familiar circular environment. If distinct attractors exist, which represent different environments, then there would be a sharp transition as the network switches between them. Because this experiment used
systematic changes in the environment, the inputs varied progressively from one
environment, or state of attraction, to another (Leutgeb et al., 2005b).

One seminal paper attempted a similar hypothesis by morphing two
environments, but presented the systematically changed environments in a scrambled
order (Wills et al., 2005). Wills et al. (2005) reported a sharp function in CA3, which
conflicts with the results just mentioned. However, Leutgeb et al. (2005a) progressively
morphed the environments in the “correct” order, which is more similar to what might be
encountered in the natural world when changes in environmental inputs are more gradual.

The results contradict the idea of discrete attractors, but do not rule out the
possibility of more continuous local attractor states (Leutgeb et al., 2005a). In practice,
we tend to consider sequential processes of learning (memory states are established and
stored) and recall (plasticity has concluded). However, in reality and in the brain, learning
and recall are intermixed; new information may be assimilated into the existing
representations (Leutgeb et al., 2005a). And so, pattern separation and pattern completion
accompany consolidation and reconsolidation in memory formation and schema
modification (Tse et al., 2007; Squire, 2007; Rudy & Sutherland, 2008; Tse et al., 2011;
Zeithamova et al., 2012).

ADAPTIVE SIGNIFICANCE

Although the role of the hippocampus may be to form and maintain relational
memories in a memory space, the benefit of such a process is manifested in the flexibility
and novelty of subsequent behaviors. For example, in the radial arm maze, hippocampal-
lesioned subjects perform similarly to sham subjects when trained to locate a visible
platform (McDonald & White, 1994) or a hidden platform from the same starting point (Eichenbaum et al., 1990). However, lesioned animals are impaired when the platform is hidden as well as when starting points differ (Eichenbaum et al., 1990; McDonald & White, 1994), and must be slowly shaped to correct performance (Eichenbaum et al., 1990). Similarly, subjects with hippocampal lesions are able to approach a particular location in a Y-maze, but are unable to adequately perform during reversal training, suggesting a disposition to perseverate (Kimble & Kimble, 1965). These results combine to suggest that a critical benefit of an intact hippocampus is behavioral flexibility.

An emergent property of such relational memories is the ability to make novel predictions in the face of information that has not been experienced previously. For example, the ability to form a hierarchical association between items is observed in a test of transitive inference. In this task, the animal must choose between items that have never been paired together before, but which have been hierarchically ordered based on previous experience: A is reinforced when presented with B, but B is reinforced when presented with C; the animal should choose A when presented with A and C. In both human neuroimaging studies (Heckers et al., 2004; Preston et al., 2004) and rodent disconnection experiments (Dusek & Eichenbaum, 1997), the hippocampus is implicated in the production of a correct response in such a circumstance.

From its earliest conception, as a region responsible for spatial learning, through the subsequent decades, the clearest conclusion is that the hippocampus subserves declarative memory by utilizing relational representations for flexible expression. Interestingly, our results failed to support the claim that the hippocampus is critical for such a function in the submerged T-maze; we return to this issue in Chapter 8, discussing
the modulation of behavior by several parameters, including both motivation and behavioral constraints.
CHAPTER 6: THE DORSOLATERAL STRIATUM IS NECESSARY FOR THE ACQUISITION, BUT NOT THE EXPRESSION, OF THE IMMEDIATE RESPONSE STRATEGY

The striatum is implicated in the production of automatic motor movements as well as gradual learning. Critically, the results presented in Chapter 2 provide conflicting predictions for the submerged T-maze. If the striatum is critical for response-based strategies, then it is recruited immediately. On the other hand, if the striatum is critical for gradual, habit-based, learning, its recruitment in the submerged T-maze will be observed in later, place strategy, expression. Furthermore, it is possible that the implicated brain region is necessary for one, but not more, of the following processes: acquisition, consolidation, or expression of learning.

We sought to investigate whether the role of the dorsolateral striatum (DLS) is similar in the submerged T-maze as that in the dry T-maze. In Experiment 6, we examined performance of rats receiving either a lidocaine or saline infusion to the dorsolateral striatum immediately prior to probe tests. We observed no effect of drug condition on behavior exhibited during probe trials, suggesting that the dorsolateral striatum is not required for the expression of behavior. In Experiment 7, we confirmed the effectiveness of the infusion protocol in a positive control with the same subjects. In Experiment 8, rats received either lidocaine or saline immediately prior to training sessions. We observed early retardation of learning accompanied by a lack of response strategy solution on early probe trials, suggesting that the dorsolateral striatum is necessary for the acquisition of the immediate response strategy, but not the expression of it.
Experiment 6 Methods

Subjects

Male Long-Evans rats (275-325g; Charles River Laboratories; Raleigh, NC) were housed individually in a temperature-controlled room, with lights on 7 A.M. to 7 P.M. Rats (n = 29) had free access to food (Teklad Chow 2018, Harlan Laboratories; Madison, WI) and water.Weights of all subjects, immediately prior to any manipulation, were 350-415g.

These subjects received bilateral cannulations of the dorsolateral striatum (Packard & McGaugh, 1996; Paxinos & Watson, 1998). Unfortunately, we experienced the attrition of five rats during the experiment; weights of the remaining subjects, prior to behavioral experimentation, were 350-415g.

Apparatus

The submerged T-maze apparatus was the same as that previously described (Experiment 1).

Surgery

Procedures were the same as those previously described (Experiment 3), except that subjects received bilateral cannulations of the dorsolateral striatum (DLS). Coordinates for the dorsolateral striatum placements were AP = -.26mm, ML = ±4.2mm, DV = -4.0 mm.
Infusions

Procedures were the same as those previously described (Experiment 3).

Histology

Subjects were perfused following completion of Experiment 7. Histological procedures are described in Experiment 7 Methods.

Experiment 6 Procedures

Habituation. Procedures were the same as those previously described (Experiment 1).

Training. Procedures were the same as those previously described (Experiment 3). After two days of training, subjects were divided into two groups, determining the order of drug infusion for each pair of probes. These groups were matched for mean latency and number of errors over the first two training sessions (Group 1: n = 12, Group 2: n = 12). The interval between the two daily trials ranged from approximately 2min to 8min during early training (Sessions 1-4) and 1min to 3min during later training (Sessions 5-24).

Probe Tests. Procedures were the same as those previously described (Experiment 3). Subjects were run in squads of six subjects, such that all rats in a squad were infused before maze exposure. Each squad contained rats in both Groups 1 and 2. The order in which the rats in a squad were infused was counterbalanced across squads so that all rats received varying delays between infusion and test (approximately 2-12min). In addition, in the first replication of this experiment, rats were exposed to the maze immediately
following infusions of the relevant squad. In the second replication, rats received a 10min delay, resulting in 12-22min prior to maze exposure. This delay was introduced in the second replication to better match delays introduced in Experiment 7 and to explore the infusion parameter space.

Data Analysis

An overhead camera and computer-assisted tracking system recorded each rat’s position in the maze. During training, an experimenter recorded each rat’s latency to reach the platform, as well as errors accrued. These measures were analyzed with mixed-design 2 x 2 x 2 x 24 ANOVAs, with between-subjects variables Replication (no delay, delay) and the two counterbalancing variables, Group (1, 2) and Goal Arm (East, West), with repeated measures on Training Day. All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

For each probe test, we determined the probability of observing the recorded number of response learners in each group, based on the binomial distribution. We also analyzed the change in probe test performance over training using mixed-design 2 x 2 x 2 x 2 x 4 ANOVAs as described previously, but with repeated measures on Drug (Saline, Lidocaine) and Probe Pair (Early, Intermediate, Late, Final).

Experiment 6 Results

Five of the rats lost their headsets prior to completing the experiment and were dropped from the study, leaving 12 rats in each of Groups 1 and 2, equal representation of the two goal arm locations in each group, as well as 16 rats with no delay and 8 rats with
a delay. Figure 16 shows the cannulae tip locations; they ranged between +0.20mm and -0.40mm AP. Five subjects showed small unilateral lesions near the site of the guide cannula; data from these rats were included in our data analysis.

_Acquisition._ During training, we observed rapid acquisition, with substantial improvement over the first three sessions and asymptotic mean number of errors and latency to the platform after Day 6 (Figures 17A and 17B). A repeated measures ANOVA across Training Day reported a significant decline in the mean number of errors (F(23,368) = 5.304, p = .002) and mean latency to reach the platform (F(23,368) = 3.926, p = .023).

There was no effect or interaction of Replication or any counterbalancing variable (Group, Goal Arm) across Training Day or with each other for either mean number of errors (F(23,368) < 2.039, p > .114, F(1,16) < 1.979, p > .179) or mean latency (F(23,368) < 2.234, p > .114, F(1,16) < 2.563, p > .129), except a Replication x Goal Arm interaction on mean number of errors (F(1,16) = 7.137, p = .017).

_Probe Tests (Strategy Selection)._ Across the interspersed probe tests, we observed the behavioral pattern expected on the basis of Asem and Holland’s (2013) findings, with rats switching from response learning to place learning. However, this pattern was not affected by lidocaine infusions.

On the first probe test, 18 of 24 rats (p = .011) entered the previously untrained arm and were designated as response learners. Of these 24 rats, 10 of 12 rats had received lidocaine (p = .019) and 8 of 12 rats had received saline (p = .194) and were response learners. There was no significant difference between these proportions ($\chi^2(1, n = 24) = .889, p = .346$). Similarly, within the Early probe pair, 18 of 24 rats (p = .011) showed the
response strategy on their saline test and 17 of 24 rats (p = .032) showed this strategy on their lidocaine test. There was no significant difference between these proportions (sign test, z = 0, p = 1).

On the final probe test, 16 of 24 rats (p = .076) entered the previously trained arm and were designated as place learners. Eight of 12 rats in each drug condition (ps = .194) were place learners. There was no significant difference between these proportions ($\chi^2 (1, n = 24) = 0, p = 1$). Similarly, within the Final probe pair, 12 of 24 rats (p = .581) showed the place strategy on their saline test and 16 of 24 rats (p = .076) showed this strategy on their lidocaine test. There was no significant difference between these proportions (sign test, z = .617, p = .537).

This gradual switch from a response strategy to an (albeit nonsignificant) place strategy was confirmed by a repeated measures ANOVA. This ANOVA showed a significant effect of Probe Pair ($F(3,48) = 6.276, p = .003$), but no effect of Drug ($F(1,48) = .574, p = .460$), nor a Drug x Probe Pair interaction ($F(3,48) = .992, p = .399$). Furthermore, there was a significant linear trend over Probe Pair ($F(1,16) = 10.070, p = .006$) (Figure 18A). Additional analyses showed that the simple main effects of Probe Pair were significant for both lidocaine ($F(3,48) = 3.636, p = .028$) and saline ($F(3,42) = 5.920, p = .002$) tests (Figure 18A). Likewise, there was a significant linear trend over Probe Pair for both lidocaine ($F(1,16) = 7.376, p = .015$) and saline ($F(1,16) = 8.540, p = .010$) tests, suggesting a similar decrease in the use of a response strategy when tested under either condition. There were no effects or interactions involving Replication or any counterbalancing variables (Group, Goal Arm) on performance (ps > .079).
Probe Tests (Latency). Not only was there no effect of Drug on strategy choice during probe tests, a repeated measures ANOVA reported no effect of Drug on latency to reach the goal location \( (F(1,48) = .262, p = .616) \), no effect of Probe Pair \( (F(3, 48) = 1.530, p = .236) \), and no Drug x Probe Pair interaction \( (F(3,48) = .359, p = .700) \) (Figure 18B). Likewise, there was no significant linear trend across Probe Pair \( (F(1,16) = 2.196, p = .158) \), nor of the effect of Drug \( (F(1,16) = .262, p = .616) \), nor of the Drug x Probe Pair interaction \( (F(1,16) = .011, p = .919) \). Additional separate analyses confirmed that there was no change in latency to the goal location by Probe Pair when tested with either lidocaine \( (F(3,48) = 1.399, p = .262) \) or saline \( (F(3,48) = .758, p = .457) \). Finally, there were no significant effects or interactions involving any counterbalancing variables \( (ps > .235) \).

These probe test latencies were comparable to those observed on contemporaneous training sessions (Figure 17B). The lack of a drug effect on the mean latency to the goal location during probe tests indicates that the lidocaine infusions did not affect general motor movement.

Discussion

The probe test data are consistent with Asem and Holland’s (2013) observation that, in the submerged T-maze, rats exhibit a response strategy at the beginning of training and switch to a place strategy with extended training. Importantly, we found that, unlike previous results from appetitive tasks (McDonald & White, 1994; Packard & McGaugh, 1996), the expression of a response strategy was unaffected by temporary bilateral DLS inactivation by lidocaine infusion.
Figure 16. Dorsolateral striatum cannulae placements showing the anterior/posterior extent of needle tip locations at 0.4mm sections. Placements ranged from +0.20 to -0.40 mm from bregma. Plates adapted from atlas of Paxinos and Watson (1998), used by permission of Elsevier.
Figure 17. (A) Mean number of errors and (B) mean latency (s) to the escape platform on correct trials during acquisition. Rats received two training trials per day. Error bars denote standard error of the mean.
Figure 18. (A) Percentage of response learners. Rats received an infusion prior to each single-trial probe test after Early (Days 2 and 4), Intermediate (Days 10 and 12), Late (Days 18 and 20), and Final (Days 22 and 24) training. Trend lines are based on relevant data points. (B) Mean latency (s) to the goal location. Error bars denote standard error of the mean.
Because we observed no effect of lidocaine infusions in Experiment 6, proper interpretation of the results requires demonstrating disruption by the same drug in a task known to depend on DLS function. In principle, we could have simply replicated Packard and McGaugh’s (1996) experiment with the rats of Experiment 6; however, this approach is unreasonable because we might expect substantial transfer between submerged and dry versions of the task used in Experiment 6. Instead, we examined the effects of identical infusions on performance of these rats in another spatial task previously found to require the DLS for acquisition of a response strategy (Chang & Gold, 2004; Figure 19).

In this task, rats placed in a plus maze are given food reward for making the same response (i.e., turning right or turning left, counterbalanced) when started from various maze arms. Rats appear to be intrinsically biased toward using spatial cues: even control rats in Chang and Gold’s (2004) study were extremely poor at solving this task unless the environment had minimal spatial cues and dim lighting (cue-available vs. cue-poor conditions). However, rats that received DLS lidocaine infusions were much slower to acquire correct performance, even in the cue-poor condition, in which control subjects acquired the task (Chang & Gold, 2004).

Figure 19 (Chang & Gold, 2004). (A) Percent correct across training blocks. (B) Trials to criterion for all conditions.
Experiment 7 Methods

Subjects

The same rats were used from Experiment 6. Subjects were given 2-3 days to recover from the previous experiment and were deprived to 85% of their maximum weight over the course of 3-5 days. Prior to subsequent experimentation, rats were pre-exposed to sucrose pellets in their home cage until consumption was verified. Weights of subjects prior to Experiment 7 were 350-415g.

Apparatus

The plus maze was removed from the tank, and a Plexiglas floor was added, giving it walls that were 12cm high. An aluminum reward cup (h = 2cm, d = 6cm) was secured at the ends of all arms. The Plexiglas maze was placed in a different experimental room, stripped of as many extra-maze cues as possible. Due to poor acquisition in the first replication, the second replication included black cloth hung from the ceiling to obscure any immovable cues present in the room, producing a more “cue-poor” environment as suggested by Chang and Gold (2004).

The room was dimly lit, illuminated only by a small lamp with a single red lightbulb, which was initially located behind the North arm. In the first replication, this lamp was moved to the South arm after every block of 10 training trials, so as to not provide a predictive cue. In the second replication, the lamp was moved from the SouthWest quadrant to the SouthEast quadrant after every block of 10 trials, due to space accommodation. The experimenter and cart, containing the holding cage and sucrose
pellets, were located directly behind the Start arm for each trial (first replication) or moved behind North and South arms after every block of 10 trials (second replication).

**Infusions**

Infusions were administered as in Experiment 6, except that rats were trained one at a time, with the single training session beginning immediately after the infusion. Training sessions lasted 50-75 min, depending on the rat’s performance in attaining criterion.

**Histology**

Procedures were the same as those previously described (Experiment 4).

**Experiment 7 Procedures**

**Habituation.** All rats were habituated on the same day. For habituation, rats were started from the South arm and exposed to the maze for 2 min. Four sucrose pellets were spaced apart in each arm, as well as four pellets in each aluminum cup, secured at the ends of each arm. The experimenter recorded the order of explored arms. Matched by previous trained arm and drug experience from Experiment 6, as well as their Experiment 7 habituation performance, rats were divided into four conditions, determining their rewarded response (turning left or right) and new drug condition (lidocaine or saline).

**Training.** Training began 24 hours after habituation. Each rat received an infusion (lidocaine or saline) immediately prior to its single maze training session. Rats were started from each of the four arms, in a random order, for 60 trials, divided into six 10-
trial blocks. The rewarded arm was always the left or right (counterbalanced) arm from the Start arm, and was baited with four sucrose pellets, placed inside the aluminum cup. Approximately 2-4 rats were run each day until all rats were run (~7 days).

There was no correction procedure: rats were removed from the maze after producing an incorrect response. If correct, rats were allowed 10s to consume the reward; any remaining pellets were placed in the holding cage for the rat to consume during the ITI, which was 30-60s. If a rat “timed out” by failing to leave the Start arm after 2min, it was removed, without reward, and was not assigned a latency. Similar procedures have been shown to produce striatal-dependent learning (Chang & Gold, 2004).

Data Analysis

An experimenter recorded the number of correct trials, as well as the rat’s latency to reach the reward cup on correct trials. These measures were analyzed with mixed-design 2 x 2 x 2 x 6 ANOVAs, with between-subject variables Replication (curtains, no curtains), Drug (Saline, Lidocaine), and one counterbalancing variable, Response (right, left), with repeated measures on Block (1-6, consisting of 10 trials each). All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

Analyses involving latency to reach the reward cup on correct trials included fewer subjects (n = 13) because of subject elimination due to incomplete data. Several subjects lacked a value for mean latency on correct trials in one or more blocks due to the lack of any correct trials in that block. In these analyses, there are lidocaine rats from the second replication (n = 4) and saline rats from both replications (n = 5, n = 4,
respectively). Although unfortunate, these eliminations provided a conservative comparison between drug conditions.

**Experiment 7 Results**

Lidocaine rats showed significantly impaired acquisition of the response strategy compared to saline controls. Notably, even the control (saline) subjects performed poorly, supporting the contention that rats are predisposed to spatial solutions.

**Acquisition (Trials to Criterion).** A simple approach to determine the acquisition of the task is to compare the subject’s performance to a standard, such as the number of successful trials performed consecutively. We selected a liberal criterion of 3 or more consecutive trials performed correctly in a block of 10 trials.

By this standard, 3 of 12 lidocaine rats and 11 of 12 saline rats acquired the task. These proportions were significantly different from each other ($\chi^2 (1, n = 24) = 10.971, p < .001$), suggesting that, even with a liberal standard for acquisition, rats receiving lidocaine infusions to the DLS were severely impaired in solving the task. This conclusion was corroborated by a univariate analysis, showing a significant effect of Drug on the highest number of correct consecutive trials per subject across all blocks ($F(1,17) = 39.099, p < .001$) (lidocaine: mean = 1.988, SEM = .207; saline: mean = 3.842, SEM = .213) and on the total number of correct trials ($F(1,17) = 28.106, p < .001$) (lidocaine: mean = 10.363, SEM = 1.287; saline: mean = 20.150, SEM = 1.324).

**Acquisition (Number of Correct Trials).** There was no effect of Drug on the mean number of correct trials in Block 1 ($F(1,17) = .023, p = .882$), suggesting that all rats were matched for performance at the beginning of training. A repeated measures
ANOVA showed that, although the main effect of Block was not significant (F(5,85) = 1.502, p = .227), there was a significant main effect of Drug (F(1,17) = 28.106, p < .001) and a Drug x Block interaction (F(5,85) = 3.217, p = .033), such that saline rats showed an increase in the number of correct trials per block, whereas lidocaine rats showed no such improvement (Figure 20A).

*Acquisition (Mean Latency on Correct Trials).* A repeated measures ANOVA showed a decrease in the mean latency on correct trials across Blocks (F(5,30) = 28.562, p < .001), but there was no main effect of Drug (F(1,6) = 1.141, p = .327), and the Drug x Block interaction was only marginally significant (F(5,30) = 3.181, p = .055) (Figure 20B).

*Acquisition (Replication Effects).* The poor acquisition observed in the first replication prompted the addition of black curtains in the second replication, further reducing the salience of potential spatial cues. The addition of the curtains in the second replication is confounded by the added infusion-test delay that these rats received in Experiment 6, but the latter is unlikely to be critical in the present experiment.

Consistent with Chang and Gold’s (2004) observation of better acquisition by control rats in cue-poor vs. cue-available conditions, the addition of curtains, presumably reducing the salience of spatial cues, resulted in better acquisition overall, but did not significantly alter the drug effect. In particular, for the number of correct trials, we observed a main effect of Replication (F(1,17) = 53.373, p < .001) and a Replication x Block interaction (F(5,85) = 2.856, p = .049), but no Replication x Drug (F(1,17) = .431, p = .520) nor Replication x Drug x Block (F(5,85) = 2.285, p = .093) interaction.
Likewise, ANOVAs of the number of consecutive correct trials and the mean latency on all correct trials revealed significant effects of Replication ($F(1,16) = 5.852, p = .028$; $F(1,17) = 15.591, p = .001$, respectively), such that there were more consecutive correct trials and shorter latencies when curtains were introduced. Finally, there were no significant effects or interactions involving the Response counterbalancing variable ($ps > .134$).

The addition of curtains in the second replication primarily affected learning: performance in the initial block of trials was unaffected. ANOVAs of performance in Block 1 alone showed there was no effect of Replication on either the mean number of correct trials ($F(1,17) = 2.434, p = .137$) or the mean latency on those trials ($F(1,12) = 1.152, p = .304$).

**Discussion**

In summary, we used this paradigm to verify the effectiveness of the infusions used in the submerged T-maze task in Experiment 6. Although lidocaine infusions produced no effect in the submerged T-maze, the same lidocaine infusion protocol in the same rats produced a significant learning impairment in another task requiring the use of a response strategy. Notably, the learning deficit in Experiment 7 was observed as early as the second block of trials—that is, 10-20min post-infusion—comparable to the infusion-test intervals used in Experiment 6 (2-22 min).
Figure 20. (A) Mean number of correct trials and (B) mean latency (s) to Goal Arm on correct trials across trial blocks (each block consisted of 10 trials). Rats received infusions immediately prior to the first training block. Error bars denote standard error of the mean.
Although the DLS appears to be unnecessary for the expression of a previously developed response strategy (Experiment 6), it is possible that it is essential for the acquisition of it. In Experiment 8, we examined the effects of DLS lidocaine infusions on the acquisition of performance in the submerged T-maze: rats received infusions before training sessions, but not before probe tests. Because, in the previous experiments, the response strategy was evident only during initial acquisition, we only trained subjects for eight sessions and performed only four probe tests, which permitted a reasonably small number of infusions per animal, and reduced potential mechanical damage.

Experiment 8 Methods

Subjects

Male Long-Evans rats (275-325g; Charles River Laboratories; Raleigh, NC) were housed individually in a temperature-controlled room, with lights on 7 A.M. to 7 P.M. Rats (n = 46) had free access to food (Teklad Chow 2018, Harlan Laboratories; Madison, WI) and water.

These subjects received bilateral cannulations of the dorsolateral striatum (DLS) (Packard & McGaugh, 1996; Paxinos & Watson, 1998). Weights of all subjects (n = 46), immediately prior to any manipulation, were 350-415g.

Apparatus

The submerged T-maze apparatus was the same as that previously described (Experiment 1).
Surgery

Procedures were the same as those previously described (Experiment 3).

Infusions

Procedures were the same as those described in Experiment 3, except that infusions were given before training sessions rather than probe tests, and the Drug variable was evaluated between separate groups of subjects. That is, as a between-subject drug manipulation, each rat received either lidocaine or saline infusions throughout the entire experiment.

Histology

Procedures were the same as those previously described (Experiment 4).

Experiment 8 Procedures

Habituation. Procedures were the same as those previously described (Experiment 1).

Training. Procedures were the same as those previously described (Experiment 3), except that rats received infusions prior to training sessions and not probe tests.

Subjects were trained for eight days and received infusions of either lidocaine (n = 23) or saline (n = 22) prior to each training session, with one drug-free probe test after every two training sessions, for a total of four probe tests. Rats were constant in their drug condition throughout the experiment, which included three replications.
Subjects were run in squads of 3-5 subjects, such that all rats in a squad were infused before maze exposure. Each squad contained both lidocaine and saline rats. The order of infusions (lidocaine or saline) was counterbalanced across squads. The intervals between infusion and the first daily training trial ranged from 2min to 10min in the first replication, and between 12min to 20min in the second and third replications (similar to Experiment 6). The interval between the two daily trials ranged from approximately 2min to 8min during early training (Sessions 1-4) and 1min to 3min during later training (Sessions 5-8) (similar to Experiment 6).

Probe Tests. Procedures were the same as those previously described (Experiment 1); rats were drug-free for probe testing.

Data Analysis

An overhead camera and computer-assisted tracking system recorded each rat’s position in the maze. During training, an experimenter recorded each rat’s latency to reach the platform, as well as errors accrued. These measures were analyzed with mixed-design 3 x 2 x 2 x 8 ANOVAs, with between-subjects variables Replication (no delay, delay, delay), Drug (Saline, Lidocaine), and one counterbalancing variable of Goal Arm (East, West), with repeated measures on Training Day. Another mixed-design ANOVA investigated the effects of the aforementioned between-subject variables with repeated measures on the first four trials of training (Training Days 1 and 2). All ANOVAs used the Greenhouse-Geisser correction for violations of sphericity.

For each probe test, we determined the probability of observing the recorded number of “response learners” in each group, based on the binominal distribution. We also
analyzed the change in probe test performance over training using repeated measures ANOVAs, as described above.

Experiment 8 Results

Two rats lost their headsets after the first probe test. Because they showed no adverse symptoms prior to the loss, these subjects were included in analyses involving the first two training days and the first probe day (Lidocaine: n = 24, Saline: n = 22). However, we removed their data from all analyses involving extended training and testing (Lidocaine: n = 23, Saline: n = 21). Figure 21 shows the cannulae tip locations; they ranged between +0.20mm and -0.40mm AP. Four subjects showed small unilateral lesions near the site of the guide cannula; data from these rats were included in our data analysis.

Acquisition (All Training Days). Prior to each training session, rats received either saline or lidocaine infusions to the DLS. As in Experiment 6, we observed rapid acquisition of task performance. However, acquisition of rats that received lidocaine infusions was initially impaired.

A repeated measures ANOVA that included all training sessions confirmed a significant effect of Training Day for both mean number of errors (F(7,224) = 30.305, p < .001) and mean latency to the platform (F(7,224) = 42.837, p < .001). Importantly, the main effect of Drug on mean number of errors was also significant (F(1,32) = 6.306, p = .017), although the main effect of Drug on mean latency to the platform was not (F(1,32) = 2.383, p = .132).
There were no main effects of Replication or of the counterbalancing variable (Goal Arm) for either the mean number of errors or mean latency measures. For mean number of errors, only the Replication x Training Day interaction was significant (F(7,224) = 2.508, p = .042), and, for mean latency, only the Replication x Drug x Goal Arm (F(2,32) = 12.553, p > .001) and the Replication x Drug x Goal Arm x Training Day (F(7,224) = 7.350, p < .001) interactions were significant.

*Acquisition (Early Training Trials).* To further investigate the main effect of Drug on training performance, we examined the mean number of errors and mean latency on a trial-by-trial basis over the first two training days (Trials 1-4). All subjects were included in these analyses. A repeated measures ANOVA reported a significant decline in the number of errors (F(3,102) = 11.850, p < .001) and latency (F(3,102) = 11.709, p < .001) across trials (Figures 22A and 22B).

Critically, there was a significant main effect of Drug on errors (F(1,34) = 5.412, p = .026), although that effect was not significant for latency (F(1,34) = 2.457, p = .126). There were no significant interactions for either errors (F(3 or 6, 102) < 2.093, p > .090) or latency (F(3 or 6) < 1.685, p > .197) across Trials 1-4, except a Replication x Drug x Goal Arm x Trial interaction for latency (F(6,102) = 2.802, p = .039). Additionally, we observed a marginally significant main effect of our Goal Arm counterbalancing variable on errors (F(1,34) = 3.973, p = .054), such that rats swimming to the West (left) arm had more errors than those swimming to the East (right) arm.

Because performance of lidocaine and saline rats started at the same level and reached similar asymptotes on the fourth training trial, we contrasted the quadratic trends of these two groups of rats over the first four trials. This contrast was significant for
errors (F(1,34) = 6.778, p = .014) and marginally significant for latency (F(1,34) = 3.989, p = .054), supporting our assertion that lidocaine infusions slowed the initial acquisition of maze performance.

*Probe Tests (Strategy Selection).* All rats were trained for two days before receiving the first probe test. On the first probe, the majority of saline rats entered the previously untrained arm and were designated as response learners (19/22, p < .001), marginally greater ($\chi^2(1, n = 46) = 3.390, p = .066$) than the chance performance observed in the lidocaine group (15/24, p = .154).

Both saline and lidocaine rats displayed a marginally significant response strategy on Probe 2 (15/22, p = .067, and 16/24, p = .076, respectively). However, saline rats continued to display a significant response strategy on Probes 3 and 4 (16/22, ps = .026), whereas lidocaine rats did not (13/24, p = .419, and 11/24, p = .729, respectively). The proportion of rats exhibiting a response strategy did not differ between lidocaine- and saline-trained rats on Probe 2 ($\chi^2(1, n = 46) = .012, p = .913$) or 3 ($\chi^2(1, n = 46) = 1.697, p = .193$), but there was a marginally significant superiority of saline rats on Probe 4 ($\chi^2(1, n = 46) = 3.424, p = .064$) (Figure 23A).

*Probe Tests (Latency).* Unlike in Experiment 6, a Replication x Drug x Goal Arm x Test ANOVA showed that response latency differed across probe days (F(3,96) = 3.532, p = .029) (Figure 23B). Furthermore, there was a significant main effect of Drug (F(1,32) = 11.528, p = .002), such that lidocaine rats were faster to the goal location. This difference was observed on the first probe day and did not change; there was no Drug x Test interaction (F(3,96) = 1.428, p = .246). Although we have no account for this
apparent speed-accuracy trade-off, it at least supports our contention that infusions of lidocaine did not generally impair motor performance.

Finally, latency during probe tests was affected by a number of the counterbalancing variables. There were significant main effects of both Replication and Goal Arm (F(1 or 2, 32) > 7.237, p < .011), such that rats in the second replication and rats swimming to the West (left) arm were slower to reach the goal location. In addition, the Replication x Test interaction (F(6, 96) = 2.963, p = .021) and all between-subject interactions (Replication x Goal Arm, Replication x Drug, Drug x Goal Arm, Replication x Drug x Goal Arm) were significant (F(1 or 2, 32) > 3.993, p < .028).

Discussion

We successfully replicated our previous observations that, in the submerged T-maze, control rats exhibit a response strategy at the beginning of training. Additionally, we observed that early acquisition of maze performance was impaired by bilateral DLS inactivation by lidocaine infusion. This effect was transient: the impairment observed in lidocaine rats dissipated after two training sessions, but resulted in the disruption of response strategy selection on subsequent drug-free probe tests.

Our observations regarding the behavior and role of the DLS in the rodent submerged T-maze have important implications across the broader framework of habit formation in animal behavior. Although the response strategy might be symptomatic of habit learning in appetitive tasks, this assumption may be deceptive for aversive ones. Furthermore, the role of the DLS in such motor-based movements is not universal and may be modified by task-based parameters, such as motivation and reinforcement, via
amygdalar modulation. These issues will be discussed in greater detail in the remaining chapters.
Figure 21. Dorsolateral striatum cannulae placements showing the anterior/posterior extent of needle tip locations at 0.4mm sections. Placements ranged from +0.20 to -0.30 mm from bregma. Plates adapted from atlas of Paxinos and Watson (1998), used by permission of Elsevier.
Figure 22. (A) Mean number of errors and (B) mean latency (s) to the escape platform on correct trials during acquisition. Rats received infusions immediately prior to the first of two training trials per day. Error bars denote standard error of the mean.
Figure 23. (A) Percentage of response learners. Trend lines are based on relevant data points. (B) Mean latency (sec) to the goal location. Error bars denote standard error of the mean.
CHAPTER 7: THEORIES OF STRIATAL FUNCTION

Many discussions of animal learning and behavior stipulate that, over extended use, animals switch to automatic, inflexible responses, termed “habits.” These habits are fixed routines—acquired as a result of repeated presentations to static task demands—thought to accrue slowly, over the course of many iterations (Graybiel, 2008; Lally et al., 2010). In spatial tasks, the response strategy has often been characterized as a habit, based on the above attributes. However, previous observations (Asem & Holland, 2013; Asem & Holland, 2015) suggest that this division may not always be a useful classification. In the aversive maze, this same strategy is not inflexible; instead, subjects later switch to a different (place) strategy. Nor is this strategy acquired gradually; it is exhibited immediately, after as few as two days of training, with two trials per day.

Furthermore, the response strategy specifically and habit formation generally are thought to require the basal ganglia, i.e., striatum (Yin et al., 2004; Barnes et al., 2005; Faure et al., 2005). In particular, striatal projection neurons and interneurons show activity patterns that modify gradually during the slow acquisition of stimulus-response (S-R) learning, producing “action chunks” for future efficient performance (Graybiel, 1998). However, the role of the DLS in the production of a response strategy may not be universal.

In Experiment 6, bilateral lidocaine inactivation of the dorsolateral striatum (DLS) prior to probe tests had no effect on the expression of an immediate response strategy, nor that of a later place strategy. Nevertheless, in Experiment 7, similar inactivation in the same rats disrupted performance in a dry maze task that required use of a response strategy. Notably, in Experiment 8, bilateral lidocaine inactivation prior to
training sessions resulted in disrupted early acquisition of the task and prevented the subsequent display of any solution strategy on drug-free probe tests. Thus, these data suggest differences in the role of striatal systems in spatial strategy selection in various maze tasks, depending on the motivational system involved in that task (Asem & Holland, 2015).

THE BASAL GANGLIA

Habit formation is thought to be subserved by the basal ganglia (BG) (Smith et al., 2004; Yin & Knowlton, 2006; Graybiel, 2008), a region consisting of a collection of subnuclei. The main components are the dorsal striatum (caudate and putamen), ventral striatum (nucleus accumbens: core and shell), and pallidum (globus pallidus and ventral pallidum), as well as the substantia nigra (pars compacta and pars reticulata), ventral tegmental area, and subthalamic nucleus due to reciprocal connections with the aforementioned core structures (Packard & Knowlton, 2002; Jarvis et al., 2005). For the sake of completeness, the globus pallidus is sometimes called the entopeduncular nucleus (EP) in non-primates (Ashby et al., 2010). The aforementioned limbic regions that form the basal ganglia also play an important role in reward learning via dopamine.

The largest subregion in the basal ganglia is the

Figure 24 (Gerfen, 1992). Schematic representation of the major connections of the basal ganglia.
striatum, which is the main region of input, but only sends output to other regions of the basal ganglia. The striatum receives most of its input from cortical—mostly frontal and parietal—lobes, forming the corticostriatal pathway. These cortical inputs are received by dendrites of medium spiny neurons (MSNs); as many as 10,000 cortical inputs may converge on a single projection neuron (Wilson, 1995). These GABAergic projection cells make up roughly 95% of the striatum (Paton & Louie, 2012), and project to the globus pallidus and substantia nigra pars reticulata, the two main output subregions of the basal ganglia. These output subregions are mostly oriented toward action systems of the frontal cortex and brainstem premotor regions (i.e., superior colliculus) (Graybiel, 1998).

As mentioned, the globus pallidus is a major output of the basal ganglia, whose projections loop back into the same cortical regions that provide substantial basal ganglia input via thalamic nuclei (Aosaki et al., 1995). The prominent thalamic projections primarily originate from intralaminar thalamic nuclei, although others exist (Mengual et al., 1999; Packard & Knowlton, 2002). It is possible that these 5+ parallel loops “train” the cortex to produce motor responses in the presence of particular stimuli (e.g., S-R habits) (Wise et al., 1996), whereas other pallidal and nigral output that directly projects to downstream brainstem structures allow for rapid, possibly automatic and unlearned, motor responses.

It is also likely that clusters of nuclei within the striatum are differentially critical for various components of behavior. For example, the medium spiny neurons of the corticostriatal pathway can be divided into two subtypes, based on their dopamine receptor types and projections (Paton & Louie, 2012). The direct pathway striatonigral MSNs (dMSNs) express D1 dopamine receptors and project directly to the basal ganglia.
output nuclei: the internal segment of the globus pallidus and/or the substantia nigra pars reticulata. The indirect pathway striatopallidal MSNs (iMSNs) express D2 dopamine receptors and project indirectly to basal ganglia output; they initially terminate in the external segment of the globus pallidus.

Furthermore, these pathways facilitate entirely different responses. Although both the globus pallidus and the substantia nigra pars reticulata receive inhibitory projections from the striatum and send inhibitory projections to the thalamus, the direct and indirect pathways have disparate effects on downstream activity: the direct pathway inhibits firing of GABAergic BG output nuclei (e.g., substantia nigra pars reticulata), which disinhibits thalamocortical and brainstem circuitry, promoting movement. On the other hand, the indirect pathway increases this firing via subthalamic nucleus (STN) excitation, which inhibits downstream circuitry, suppressing movement (Freeze et al., 2013).

Interestingly, these two subtypes of MSNs appear to differentially code reward and punishment (Kravitz & Kreitzer, 2012): optogenetic stimulation of dMSNs acted as a reward, but stimulation of iMSNs elicited escape responses and served as a punisher of operant behavior (Kravitz et al., 2012). This division is one clear example that volitional actions, based on the type of reinforcement, can be mediated by different pathways within the same brain regions. This reinforcement-based modulation might be similar to the mediation that we suggest might occur from task-based motivation, which also includes differing reinforcers. Thus, a more directed, specific approach might be fruitful in discerning the role of striatal subregions.
STRIATAL FUNCTION

The role of the striatum might be dissociated based on the type of learning (place vs. response strategy), amount of learning (early vs. late in training), or stage of learning (acquisition vs. expression). Our results suggest that the DLS is implicated in the response strategy, but its role may be specific to acquisition during early training in the dual-solution submerged T-maze. Supporting evidence comes from Experiment 7, in which subjects receiving lidocaine to the DLS were impaired in the acquisition of a response strategy, compared to matched controls, in a single-solution dry plus maze, even after extended training. This result corroborates our conclusion that the DLS is involved during acquisition.

On the other hand, interestingly, the DLS is necessary in the expression of the response strategy after extended training in the dual-solution dry T-maze (Packard & McGaugh, 1996). Indeed, several experiments using food reward suggest a role of the DLS in the performance of learned S-R associations, but not in relational “place” learning. For example, post-acquisition lesions of the DLS affect simple discrimination learning (Adams et al., 2001; Featherstone et al., 2005; Broadbent et al., 2007) and egocentric spatial learning (Cook & Kesner, 1988), but do not affect performance in a conditioned place preference task (Featherstone et al., 2005) or allocentric spatial learning (Cook & Kesner, 1988). Furthermore, using a two-choice discrimination task, Broadbent et al. (2007) showed that the olfactory condition was acquired more quickly than either objects or patterns and was also most affected by post-acquisition DLS lesions.
This role in simple S-R associations extends to habitual performance, which is insensitive to changes in reward. This imperviousness is commonly assessed by using outcome devaluation. In operant tasks, reducing the value of the reinforcer yields predictable behavioral changes. Habitual performance, on the other hand, shows less variation after outcome devaluation. This characteristic has proved fruitful in discerning the profile of several substances. For example, consumption of a high-fat diet and drugs of abuse, e.g., ethanol, yields S-R performance that is insensitive to outcome devaluation (Corbit et al., 2012; Corbit et al., 2014; Furlong et al., 2014). This insensitivity is related to DLS activity, such as increases in Fos positive nuclei (Furlong et al., 2014) and mediation by AMPA and D2 receptor signaling (Corbit et al., 2014).

A popular framework suggests that the dorsomedial striatum (DMS) is critical for flexible learning, whereas the DLS is critical for performance (e.g., Balleine et al., 2007, Liljeholm & O’Doherty, 2012). Indeed, many distinctions have been proposed between the control of initial learning and later—supposedly striatal-based—automaticity (Figure 25; see Ashby et al., 2010, for a review). For example, neurons in the sensorimotor (dorsolateral) striatum tend to respond strongly.
after over-learning of a motor sequence, and temporary inactivation does not interfere with the learning of new motor sequences, but disrupts the execution of previously acquired ones (Miyachi et al., 2002), whereas the opposite effect was observed after temporarily inactivating the associative (dorsomedial) striatum (Miyachi et al., 1997).

However, this effect of extended training may be task-dependent, mediated by requiring a single action or sequenced responding. For example, the DLS is active in monkeys when learning a sequence of motor responses, such as a series of successive button presses (Miyachi et al., 2002), and shows task-related ensemble firing that correlate with performance in the dry T-maze (Barnes et al., 2005). On the other hand, after extended instrumental training with reinforced vertical head movements, head movement-related neurons in the DLS decreased their firing rate (Tang et al., 2007). Similarly, lateral striatal neurons decreased responding to reinforced lever-pressing after extended training (Carelli et al., 1997).

As mentioned, our observations are in stark contrast to others (i.e., Packard & McGaugh, 1996), in which extended training in the dual-solution dry T-maze produced a switch to a response strategy, the expression of which required the DLS. It is worth noting that Packard and McGaugh’s (1996) coordinates were significantly more ventral to those targeted here, raising the possibility that the differences in the effects of lidocaine inactivation in the submerged and dry T-mazes reflect variance in subregion functions, rather than task differences. Recently, Jonkman et al. (2012) separately targeted the dorsolateral striatum, using coordinates comparable to ours, and a region they described as the midlateral striatum, comparable to Packard and McGaugh’s (1996) DLS coordinates. They found different effects of inactivating these two regions on
punished and unpunished drug-seeking behavior. Although inactivation of the midlateral striatum affected responding throughout training, DLS inactivation only interfered with rigid responding after overtraining. However, it is notable that, in Experiment 7, we found that our DLS inactivation indeed interfered with the ongoing acquisition of a food-based maze task that required a response strategy. Critically, these results replicate Chang and Gold’s (2004) observation, whose cannulae placements are intermediate to ours and Packard and McGaugh’s (1996).

Our observations regarding the behavior and role of the DLS in the rodent submerged T-maze have important implications across the broader framework of habit formation in animal behavior. Although the response strategy might be symptomatic of habit learning in appetitive tasks, this assumption may be deceptive for aversive ones. Furthermore, the role of the DLS in such motor-based movements is not universal and may be modified by task-based parameters such as motivation and reinforcement, via amygdalar modulation. These possibilities are further discussed in Chapters 8 and 9.
Motivation and reinforcement have an observable effect on performance, the expression of previous learning. A classic investigation is Tolman’s latent learning experiment (Tolman & Honzik, 1930). In a complex maze task, three groups of rats are given different reinforcement schedules. Animals that are consistently food-rewarded after correct performance show a decrease in the number of errors to the goal location across sessions, whereas subjects that are unrewarded show much slower improvement.

Figure 26 (Tolman & Honzik, 1930). Mean number of errors in groups experiencing different reinforcement schedules across training.
The critical condition, however, is the group that is not rewarded for several days (i.e., until Day #10), but is food-rewarded for correct performance on subsequent days. Rats in this group show very little learning, or change in behavior, when unrewarded; however, they exhibit a dramatic improvement after the first day of reward. Their progress continues beyond the group of rats that had been consistently rewarded from the beginning of training. Latent learning, and other phenomena, suggest that animals may acquire information at the time of experience, but may not express it without adequate motivation or reinforcement.

ROLE OF STRESS

Stress—any factor that causes deviation from a state of equilibrium, or, homeostasis—is a constant component of the daily life of all organisms. Not surprisingly, it affects attentional, mnemonic, and motivational processing. Glucocorticoids, such as cortisol and corticosterone, are stress hormones that are released in response to a stressful stimulus or environment. Cortisol is released in higher concentrations than corticosterone in humans and the opposite is the case in rodents.

Corticosteroids affect both the peripheral and central nervous system. Encountering a stressful stimulus or situation results in a fight-or-flight response, and these glucocorticoids are released into the bloodstream. This autonomic reaction primes the body to escape or confront the stressor. In order to provide the necessary energy, glucose is released into the blood, which is diverted from other bodily functions that are unrelated to immediate survival requirements and redirected to the brain and major somatic muscles.
Additionally, glucocorticoids affect a number of brain regions implicated in complex behavioral tasks, including the hippocampus, amygdala, and prefrontal cortex. First, the hippocampus and surrounding cortices are heavily implicated in memory and relational information (O’Keefe & Nadel, 1978; Eichenbaum et al., 1999). However, hippocampal plasticity and NMDA receptors are adversely affected by stress (McEwen, 1999; Baker, 2002; Harvey et al., 2004; Mirescu & Gould, 2006). Second, the amygdala has a well-known role in motivational and emotional learning and memory (LeDoux, 2003). Interestingly, the basolateral amygdala may be more significant for negative stimuli than for positive ones (Parkinson et al., 2000), suggesting that the role of the amygdala may be imperative in an aversive environment. Third, the prefrontal cortex is involved in top-down, goal-directed modulation of behavior, and these neuromodulatory systems are degraded following chronic stress (Arnsten, 2009). The potential role of the amygdala and prefrontal cortex are discussed in greater detail in Chapter 9.

Evidence indicates that rodents show increased switching from spatial to response-related strategies after chronic stress (Schwabe et al., 2008), after acute stress induced by restraint or exogenous administration of corticosterone (Schwabe et al., 2010), or by the reactivation of an adverse memory (Hawley et al., 2013). Similarly, chronic (Schwabe et al., 2008) or acute (Schwabe et al., 2007) stress increased the use of stimulus-response over spatial strategies in humans. Furthermore, in devaluation experiments, stress has been shown to modulate the nature of instrumental learning, prompting use of a stimulus-response, or habit, strategy over more cognitive goal-directed action (e.g., Schwabe & Wolf, 2009; Schwabe et al., 2011).
THE SUBMERGED T-MAZE

The shift from a place to a response strategy in the food-rewarded maze has been described as a shift from an initial tendency to use relatively complex allocentric information to locate food resources, which are naturally distributed widely in space, to a cognitively more economical, and perhaps automatic, strategy of using egocentric response information to obtain reward efficiently.

On the other hand, the submerged T-maze is inherently an escape-avoidance task, in which subjects are motivated to escape a negative experience via learned behaviors. Schwabe and colleagues have examined various effects of stress on instrumental behavior in rodents and declarative memory in humans (see Schwabe et al., 2012, for a review). Stress disrupts spatial learning, but not early stimulus-response learning (Schwabe et al., 2010), and appears to modulate the use of each in humans (Schwabe et al., 2007; Schwabe et al., 2008; Schwabe et al., 2009) and rodents (Schwabe et al., 2010). Notably, stress affects instrumental behavior during both goal-directed and habitual control (Schwabe & Wolf, 2011) and prompts habit behavior (Schwabe & Wolf, 2009). Additionally, we see that the effects of stress are pervasive, disrupting not only spatial learning, but both context-dependent (Schwabe et al., 2009) and declarative memory.

These effects on declarative memory in humans are widespread, affecting learning of emotional information (Schwabe et al., 2008; Schwabe & Wolf, 2010) and the reconsolidation of autobiographical memories (Schwabe & Wolf, 2010). These adverse effects are rescued by administration of a β-adrenoceptor antagonist, an anxiolytic agent, in both rodents (Schwabe et al., 2011) and humans (Schwabe et al., 2009). The effects of stress on spatial, contextual, and emotional memories, as well as on instrumental and
habitual behavior, are also relevant for the study of addiction behavior (Schwabe et al., 2011). Critically, these effects were examined in appetitive tasks, manipulating stress via restraint and corticosterone injections in rodents and psychosocial stress and self-report questionnaires in humans.

Additionally, stress contributes to the rapid extraction of gist information and impairs memory for specific details (Loftus et al., 1987; Kensinger, 2009; Mather & Sutherland, 2011), which may extend to a low-resolution representation of the extra-maze distal cues, or landmarks. Such a biasing effect is observed at encoding, as well as after consolidation (Hamann, 2001; McGaugh, 2004).

We might imagine similar effects in an aversive task in which the environment and task demands themselves elicit a stress response. It is likely that the aversive task results in a fight-or-flight response, producing automatic motor movements and the release of stress hormones. These actions are subsequently reinforced by escape and, therefore, conditioned (Asem & Holland, 2013; Asem & Holland, 2015). Packard and colleagues found that administration of anxiogenic drugs biased rats towards using a response strategy in the dual-solution submerged T-maze: after training that yielded a place strategy in control rats, drug-treated rats showed significantly greater use of a response strategy (Packard & Wingard, 2004; Elliott & Packard, 2008).

Reinforcement of escape responses and their exacerbation by stress might easily account for the immediate expression of a response strategy in the submerged T-maze, but does not address the subsequent shift to a place strategy. However, repeated exposure to the maze and the availability of a coping (escape) response might lead to habituation of
the maze’s anxiogenic properties, hence reducing expression of a response strategy and favoring expression of a place strategy.

Importantly, this possible reduction in stress is not accompanied by a complete lack of motivation; we continue to observe asymptotic performance during training sessions, measured by latency to the platform, as well as the number of errors accrued. Indeed, in more conventional escape-avoidance settings, rats often show reduced stress levels as learning continues, despite sustained performance on the task (e.g., Seligman & Johnston, 1973).

Critically, there appears to be a difference between the dry T-maze and the submerged T-maze, such that the aversive motivation results in the opposite behavioral pattern (Asem & Holland, 2013). However, this alteration in motivation alone cannot explain our present results. For example, Hamilton et al. (2009) found a place-to-response strategy switch in the Morris water maze, using water that was colder (22°C) than ours (27°C), and, hence, likely to have been even more aversively motivating. Notably, the submerged T-maze differs from the Morris water maze in its constraint upon behavior; subjects are forced to make a discrete left or right turn, whereas subjects in the Morris water maze are free to take more flexible routes to the goal location.

These constraints might bias the production of more response-based strategies, compared to arbitrary paths, and might have an additional effect in altering the perception of the extra-maze cues. The discrete turn results in all-or-none exposure to the cue at the end of the goal arm, possibly making it akin to a salient cue-based landmark, as opposed to broader relational information. A more parametric experiment may be necessary to
evaluate the dissimilarities between these paradigms that result in such variances in behavioral strategy selection and neural recruitment.

It is clear that the submerged T-maze is different from other maze tasks in a variety of ways. In general, its aversive motivation, coupled with discrete and finite possible responses, seems to result in entirely different behavioral selection and neural recruitment. In particular, stress, which compromises hippocampal function (Kim & Diamond, 2002), might encourage hippocampal-independent control over behavior in the submerged T-maze. Thus, under some circumstances, perhaps spatial learning performance is mediated by gradual hippocampal-independent learning, which occurs after rapid engagement of amygdalar, prefrontal, or other learning systems. Based on the aforementioned observations, the final chapter will discuss potential modulatory roles of the amygdala and prefrontal cortex.
The results of our inactivation experiments suggest that other regions play a role in behavioral performance; such a conclusion reiterates the concept of multiple memory systems, which operate via parallel processing in either a cooperative or competitive way (Mizumori et al., 2004). In a dual-solution maze task, which can be solved by either a hippocampal or striatal strategy, recorded activity from both the hippocampus and the striatum suggest parallel processing between the two that compete for expression in behavior. Notably, the observation that the response strategy had not yet formed when the hippocampus was inactivated (Packard & McGaugh, 1996) suggests a more gradual, if parallel, acquisition, or more gradual recruitment if processing at the same rate.

If one memory system is impaired, parallel processing allows another memory system to encode the information, albeit in a different way. We see that the content of learning is very different depending on which memory system is engaged, as well as the strategy implemented, since different strategies can solve the same learning problem (Squire, 2004). Clearly, other factors are involved that influence the expression of one system over the other. Some possibilities might be neurotransmitters, such as dopamine and acetycholine (Gold, 2004), as well as motivational factors or hormone status, as just discussed in Chapter 8.

AMYGDALAR MODULATION

Sustained activity in the basolateral amygdala (BLA) is thought to subserve systems consolidation for emotional memories, particularly fearful ones (Pelletier et al.,
2005). These negative experiences result in the release of glucocorticoids, which, provided the animal has an intact basolateral amygdala, enhance long-term memory for these negative events (Roozendaal et al., 1996; McGaugh, 2004). More specifically, this facilitation is mediated by noradrenergic activity in the BLA (Roozendaal et al., 2006). These results have been observed in spatial learning, as well as inhibitory avoidance learning (Roozendaal et al., 1999).

As discussed in the previous chapter, the additional stressful component of the submerged T-maze itself may result in entirely different processing. Packard and colleagues found that administration of anxiogenic drugs biased rats toward using a response strategy in the submerged T-maze. Although after 12 training trials, control rats tended to use a place strategy, drug-treated rats showed significantly greater use of a response strategy (Packard & Wingard, 2004; Elliott & Packard, 2008). Importantly, these effects were apparently mediated by the BLA: infusions of anxiogenic drugs directly into the BLA mimicked the effects of intraperitoneal injections, and, in dual-start versions of the task, inactivation of the BLA blocked the effects of intraperitoneal injections.

Additionally, the amygdala modulates the use of hippocampal and striatal neural systems (Packard & Teather, 1998). Importantly, the interaction between the amygdala and the medial temporal lobe has been implicated in this memory enhancement (Dolcos et al., 2004), and sustained noradrenergic activity in both the hippocampus and amygdala is critical (Quirarte et al., 1997; Roozendaal et al., 2004).

Amygdala lesions also block the memory enhancement gained by glucocorticoid administration to the hippocampus (Roozendaal & McGaugh, 1997). Moreover, we see
that stress enhances memory for gist information (Payne et al., 2006), and amygdala lesions disrupt memory for gist, but not details, of complex stimuli (Adolphs et al., 2005).

The amygdala is best known for its role in fear learning and memory; however, it also serves a role in appetitive tasks with positive reward, including spatial cue learning. There is a triple dissociation of the hippocampus, striatum, and amygdala for win-shift (“place”), win-stay (“response”), and conditioned cue-preference learning, respectively (McDonald & White, 1993).

It is important to note that, although lesions of these structures produce deficits specific to these tasks, damage to one system can have consequences for the performance of other tasks. For example, hippocampal lesions produce an enhancement in amygdala-dependent conditioned cue-preference learning (McDonald & White, 1993), reinforcing the understanding that multiple memory systems operate in a competitive parallel framework, with each neural system contributing to many types of learning.

PREFRONTAL MODULATION

Through top-down, goal-directed cognitive control, the prefrontal cortex modulates recruitment of these multiple neural systems via neuromodulators, such as noradrenergic, dopaminergic, serotonergic, and cholinergic systems (Arnsten & Li, 2005; Briand et al., 2007), which are adversely affected by stress (Arnsten, 2009).

Corticostriatal feedback loops have been associated with cognitive and motor functions (Chudasama & Robbins, 2006), but both the neocortex and striatum receive convergent inputs from the hippocampus and amygdala, and these connections are critical for solving the appetitive maze (Pennartz et al., 2009). Enhancing or suppressing these
signals will likely affect an animal’s ability to switch from a response strategy to a place strategy.

Unfortunately, although the major prefrontal-hippocampal connections are with ventral hippocampus (Verwer et al., 1997), most studies of hippocampal function in maze learning (McDonald & White, 1993; McDonald & White, 1994; Packard & McGaugh, 1996), including those in this dissertation, have manipulated or monitored dorsal hippocampal function. Future studies will need to examine prefrontal-hippocampal interactions in more detail.

The prefrontal cortex of the rat consists of two subregions—medial and lateral (orbitofrontal)—which are anatomically and functionally heterogenous (Uylings et al., 2003). However, within the medial prefrontal cortex, the ventromedial portion is the main recipient of hippocampal input (de Bruin et al., 1994). Paradoxically, thermal lesions of either of these regions had no effect on spatial learning, but rather on behavioral flexibility in the Morris water maze, suggesting that the medial prefrontal cortex may play a role in strategy switching (Rich & Shapiro, 2009), as opposed to acquisition. In particular, the anterior cingulate cortex (ACC), the rodent homologue of the human medial prefrontal cortex, may play a role. Recent evidence suggests that the ACC is important for set-shifting between strategies in a food exploration task (Karlsson et al., 2012; Caracheo et al., 2013).

The medial prefrontal cortex is activated by stress and modulates endocrine function; for example, medial prefrontal cortex lesions induced experimentally with the powerful mushroom-derived neurotoxin, ibotenic acid, resulted in increases or decreases in the magnitude of various stress reactions in response to acute or repeated restraint.
stress (Sullivan & Gratton, 1999), consistent with its role in fear reactivity (Morgan & LeDoux, 1995). However, these results are relevant mostly for coping with inescapable stress, which is treated and processed differently than controllable and escapable stress (Keay & Bandler, 2001). These reports highlight a lack of understanding of the role of the medial prefrontal cortex in spatial navigation under stressful, fearful circumstances.

CLOSING

Our observations regarding the behavior and role of the dorsal hippocampus and dorsolateral striatum (DLS) in the rodent submerged T-maze have important implications across the broader framework of relational memory and habit formation in animal behavior.

Although the place strategy is suggestive of hippocampal-dependent memory, and the response strategy might be symptomatic of habit learning in appetitive tasks, these assumptions may not hold in aversive ones. Indeed, the role of the hippocampus in spatial learning and the role of the DLS in motor-based movements may not be universal, and may be modified by task-based parameters, such as motivation and reinforcement, via amygdalar and prefrontal modulation.

The research presented in this dissertation develops and extends a novel observation regarding the motivational modulation of spatial strategy selection. Critically, beyond its implications for understanding spatial navigation, my data provide a powerful example of the role of motivational systems in cognition and behavior.
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LIST OF FIGURES AND CAPTIONS

Figure 1. Submerged T-maze used in all experiments. Here, the West (left) Goal arm is reinforced. This variable is counterbalanced in all experiments.

Figure 2. (A) Mean latency to reach the escape platform and (B) mean number of errors during acquisition in the Frequent and Infrequent groups. Rats received two training trials per day. Error bars denote standard error of the mean. (C) Percentage of response learners. Rats in Group Single received a single probe trial after the second training session and rats in Groups Frequent and Infrequent received a single probe trial after every 2 or 4 training sessions, respectively. Trend line (equation shown) is based on Frequent data points.

Figure 3. (A) Mean latency to reach the reward cup and (B) mean number of errors during acquisition. Rats received two training trials per day. Error bars denote standard error of the mean. (C) Percentage of response learners. Rats in Groups Infrequent and Rare received a single probe trial after every 2 or 4 blocks of two training sessions, respectively. Trend line (equation shown) is based on Infrequent data points.

Figure 4 (Packard & McGaugh, 1996). Number of animals expressing a place or response strategy early and late in training with either saline or lidocaine infused in the caudate or hippocampus.

Figure 5. Dorsal hippocampus cannulae placements showing the anterior/posterior extent of needle tip locations at 0.4mm sections. Placements ranged from -2.56 to -3.30mm from bregma. Plates adapted from atlas of Paxinos & Watson (1998), used by permission of Elsevier.

Figure 6. (A) Mean number of errors and (B) mean latency (s) to reach the escape platform during acquisition. Rats received two training trials per day. Error bars denote standard error of the mean.

Figure 7. (A) Percentage of response learners. Rats received an infusion prior to each single-trial probe test after Early (Days 2 and 4), Intermediate (Days 10 and 12), Late (Days 18 and 20), and Final (Days 22 and 24) training. Trend lines are based on relevant data points. (B) Mean latency (s) to the goal location. Error bars denote standard error of the mean.

Figure 8 (Matus-Amat et al., 2004). Average percent freezing observed in muscimol and vehicle rats during Test.

Figure 9. (A) Mean activity/min across trials of Test. (B) Mean activity/min of all ten trials of Test.
Figure 10. (A) Mean number of errors and (B) mean latency to reach the escape platform during acquisition of all subjects. Training Days 1 and 2 contain 24 subjects, whereas the remaining days contain 12 subjects. Rats received two training trials per day. Error bars denote standard error of the mean.

Figure 11. (A) Percentage of response learners. Rats in Group Early (n = 11) received a single probe trial after the 2nd training session and Group Late (n = 11) received a single probe trial after the 24th training session. (B) Mean latency (s) to the goal location. Error bars denote standard error of the mean.

Figure 12. Analyzed Fos protein expression from (A) noted areas, and (B) sample high-resolution image from CA1. This sample and analyzed images were taken from -2.80 mm to -3.30 mm from bregma (Paxinos & Watson, 1998).

Figure 13. (A) Mean Fos positive nuclei in dorsal hippocampal subregions in place and response learners compared to relevant Control subjects after early or late training. Error bars denote standard error of the mean. There are no error bars for Controls (n = 1). Number of Fos positive nuclei for individual subjects (circles) and mean number of nuclei (squares) in (B) DG, (C) CA1, and (D) CA3 subregions of the dorsal hippocampus.

Figure 14 (Tolman et al., 1946). Training (left) and testing (right) apparatus.

Figure 15 (Eichenbaum & Cohen, 2014). A conceptual illustration of memory space, designating the three key types of relational processing, as they apply generally and to spatial memory specifically.

Figure 16. Dorsolateral striatum cannulae placements showing the anterior/posterior extent of needle tip locations at 0.4 mm sections. Placements ranged from +0.20 to -0.40 mm from bregma. Plates adapted from atlas of Paxinos & Watson (1998), used by permission of Elsevier.

Figure 17. (A) Mean number of errors and (B) mean latency (s) to the escape platform on correct trials during acquisition. Rats received two training trials per day. Error bars denote standard error of the mean.

Figure 18. (A) Percentage of response learners. Rats received an infusion prior to each single-trial probe test after Early (Days 2 and 4), Intermediate (Days 10 and 12), Late (Days 18 and 20), and Final (Days 22 and 24) training. Trend lines are based on relevant data points. (B) Mean latency (s) to the goal location. Error bars denote standard error of the mean.

Figure 19 (Chang & Gold, 2004). (A) Percent correct across training blocks. (B) Trials to criterion for all conditions.
Figure 20. (A) Mean number of correct trials and (B) mean latency (s) to Goal Arm on correct trials across trial blocks (each block consisted of 10 trials). Rats received infusions immediately prior to the first training block. Error bars denote standard error of the mean.

Figure 21. Dorsolateral striatum cannulae placements showing the anterior/posterior extent of needle tip locations at 0.4mm sections. Placements ranged from +0.20 to -0.30 mm from bregma. Plates adapted from atlas of Paxinos & Watson (1998), used by permission of Elsevier.

Figure 22. (A) Mean number of errors and (B) mean latency (s) to the escape platform on correct trials during acquisition. Rats received infusions immediately prior to the first of two training trials per day. Error bars denote standard error of the mean.

Figure 23. (A) Percentage of response learners. Trend lines are based on relevant data points. (B) Mean latency (sec) to the goal location. Error bars denote standard error of the mean.

Figure 24 (Gerfen, 1992). Schematic representation of the major connections of the basal ganglia.

Figure 25 (Ashby et al., 2010). Schematic diagram of the basal ganglia and its afferents and efferents. Black lines (arrowheads) are excitatory, black lines (circles) are inhibitory, and gray lines (squares) are dopaminergic. Green shading indicates basal ganglia.

Figure 26 (Tolman & Honzik, 1930). Mean number of errors in groups experiencing different reinforcement schedules across training.
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Asem, J.S.A. (2012). Effects of High-Fat Diet on the Brain. Talk given to Advanced Placement Biology students, Baltimore Polytechnic Institute, Baltimore, MD.


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