EXTINCTION OF HABIT MEMORY:

BEHAVIORAL AND NEUROBIOLOGICAL MECHANISMS

A Dissertation

by

JARID GOODMAN

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Committee Chair,	Mark G. Packard
Committee Members,	Stephen Maren
	Darrell Worthy
	Michael Smotherman
Head of Department,	C. Jane Welsh

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ABSTRACT

A defining symptom of numerous human psychopathologies is the inability to control maladaptive behaviors—or "habits." Extensive research using animal learning paradigms has led to exciting developments regarding the neurobiological bases of how habits are acquired and retrieved. However, little progress has been made in clarifying the neurobiological mechanisms through which habits might be suppressed. The present dissertation experiments explored novel behavioral and neurobiological mechanisms underlying suppression or, in experimental terms, *extinction* of habit memory, using a response learning task.

In the response learning task, animals are released from opposite starting positions in a plus-maze and are reinforced to make a consistent body-turn at the maze intersection in order to retrieve food reinforcement. Response learning critically depends on function of the dorsolateral striatum and is considered by many an exemplar of habit memory. Following initial acquisition of response learning, memory performance may be suppressed using an extinction procedure in which the food reinforcement is removed from the maze. Extinction learning becomes evident when the animal suppresses the original running body-turn response that was reinforced during initial acquisition.

The present dissertation project consisted of multiple experiments grouped into three distinct aims. Experiments in the first aim indicated that in order for extinction of response learning to occur the animal must be given the opportunity to perform the original running body-turn response. In contrast, place learning in the plus-maze, which

represents a different kind of memory dependent on the hippocampus, may be extinguished with or without overt performance of the previous response.

Experiments in the second aim indicated that the brain region engrossed in initial acquisition of response learning—the dorsolateral striatum—is also critically implicated in extinction of response learning. In fact, inactivation of the dorsolateral striatum with the sodium channel blocker bupivacaine blocked extinction of response learning altogether. The dorsolateral striatum, however, is not needed for extinction of place learning. To the contrary, some of the evidence in the present dissertation indicated that inactivation of the dorsolateral striatum actually *enhanced* extinction of place learning.

Experiments in the third aim indicated that the role of the DLS in extinction of response learning could be more specifically attributed to NMDA receptor activity. Blocking NMDA receptor activity in the DLS with the NMDA receptor antagonist AP5 impaired extinction of response learning, whereas increasing NMDA receptor activity in this brain region with the NMDA receptor agonist d-cycloserine *enhanced* extinction of response learning.

The present findings are discussed within the context of extensive previous evidence on the neurobiology of place and response learning in the plus-maze, as well as emerging evidence indicating a role for multiple memory systems in extinction. In addition, the possibility that the present findings may be relevant to suppression of maladaptive memory in some human psychopathologies, in particular those characterized by intractable habit-like symptoms (e.g. drug addiction), receives extensive attention.

Dedicated to

Mom who told me to follow my dreams,

Dad who told me to do what makes me happy,

and Sharron who told me to stop and smell the roses.

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An old African proverb asserts that it takes a village to raise a child. Less popular, however, is the notion that it also takes a village to raise a brainchild. Although I parented this dissertation with the crazed, impassioned fervor of a soccer mom, I am deeply indebted to a village of brilliant hands, hearts, and minds, all of whom played a critical part in maturing this project into dissertationhood.

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CHAPTER I

INTRODUCTION

Repeating a behavior over multiple iterations typically increases the ease with which a behavior is performed. This leads to a terminal point whereby a behavior may be executed with little attention, intention, or cognitive effort, constituting a "habit." In most cases, habits are good, serving to automate everyday behaviors such as driving a car or riding a bicycle. In other cases, habits are bad, hampering the intentional or motivational control of behavior. At their worst, habits can produce self-destructive and pernicious behaviors, such as those evident in drug addiction whereby recreational drug use shifts to harmful drug abuse. These less-than-propitious habits underscore the usefulness in examining mechanisms through which habits might be suppressed.

In experimental situations, memory may be suppressed through extinction procedures. Extinction may be broadly defined as the learned suppression of a previously acquired memory. When an animal is returned to a situation in which some memory had been acquired, but without the original reinforcer that had motivated initial acquisition of the memory, extinction learning typically follows. This extinction learning becomes evident when the behaviors that had manifested during initial acquisition of the memory begin to decline. For instance, a rat that had acquired a running approach response down a straight alley to retrieve food reward at the opposite end of the maze will demonstrate extinction learning when the food reward is suddenly withdrawn, and extinction learning will be expressed behaviorally as a suppression of

the original running approach response. Decrements of the original behavior constitute the most commonly cited outcome of extinction training and serve as the dominant measure of extinction learning and memory in most studies. Research into extinction is believed to be clinically relevant in that animal models of extinction learning may be adapted to alleviate maladaptive memory formation in some human psychopathologies (e.g. drug addiction and relapse). In view of its potential clinical applications, the behavioral and neurobiological mechanisms of extinction have received substantial attention.

Although extinction remains a popular topic, the scope of contemporary research proves to be, in some aspects, quite narrow. Whereas in the past, investigators had examined extinction across a range of animal learning paradigms, including instrumental and maze learning tasks, contemporary investigators study extinction primarily within the context of Pavlovian fear conditioning. Thus, while knowledge of Pavlovian fear extinction continues to grow, investigation into other types of extinction has more or less reached a standstill. Examining extinction across a variety of learning paradigms remains important, given that the behavioral and neurobiological mechanisms underlying extinction may depend partly on the type of memory being extinguished. The mechanisms underlying extinction of habits, especially in the maze, have received limited attention, and in view of its potential clinical applications extinction of habit memory warrants considerable investigation.

The present dissertation project examines the behavioral and neurobiological mechanisms underlying extinction of habit memory. However, the hypotheses driving

these experiments are drawn from extensive prior evidence regarding multiple memory systems. Thus, it will be necessary to place this dissertation project in the appropriate historical context by first reviewing the multiple memory systems view of learning and memory with a particular emphasis on extinction. In addition, given that the present dissertation experiments also focus on the potential role of the dorsolateral striatum (DLS) in extinction of habit memory, it may also be useful to provide a brief summary of DLS anatomy and physiology, as well as a critical assessment of the type(s) of memory believed to be mediated by the DLS. Finally, the present dissertation project examines extinction of habit memory using a response learning version of the plus-maze task. For this reason, it will be necessary to provide a thorough description of the response learning protocol, as well as a consideration of the behavioral and neurobiological mechanisms supporting successful acquisition in this task.

CHAPTER II

THERE IS MORE THAN ONE KIND OF EXTINCTION LEARNING

2.1 The War between Stimulus-Response and Cognitive Views of Learning

Learning theory in the first half of the 20th century was dominated by two opposing views regarding *what* animals learn. One school adhered to a strict behaviorist point of view and suggested that animals acquire associations between stimuli and responses with the strength of the association depending on parametric factors, stimulus characteristics, and according to some adherents, intervening variables within the organism (e.g. drive and incentive value of the outcome). Given the emphasis of this view on stimulus-response (S-R) associations, this approach to learning was labeled the S-R view, and some of its noteworthy figureheads included Watson (1914), Skinner (1938), Hull (1943), and Spence (1956). In contrast to the S-R view, another approach regarded animals as intelligent, thinking beings that performed actions with cognition, expectation, and purpose. This approach, called "purposive behaviorism" or, more generally, the "cognitive view," was fathered by Tolman (1932) and espoused by many of Tolman's contemporaries.

The S-R and cognitive views of learning offered different hypotheses regarding not only what information animals acquire during initial acquisition of memory, but also what animals acquire during *extinction* of memory. According to the stimulus-response (S-R) view, learned behavior may be likened to an acquired reflex, to the extent that stimuli (S) in the learning environment may gain the capacity to activate automatic

behavioral responses (R). Clark L. Hull, who provided the most complete iteration of the S-R view at this time, suggested that extinction may also be achieved through S-R learning mechanisms (Hull, 1943). However, instead of stimuli having an excitatory impact on the response, stimuli during extinction training may gain the capacity to activate a habit of not responding or a "no response." In opposition to the S-R view, the cognitive view championed by Edward C. Tolman (1932) suggested that animals acquire meaningful relationships between stimuli in the learning environment. These learned associations between stimuli culminate into a sign-gestalt expectation that guides behavior to the reinforcer (e.g. food reward). During extinction training, a change in expectation might occur in which the animal expects the *absence* of reinforcement. To the extent that the original behavior was guided by the expectation of reinforcement, subsequently expecting *absence* of reinforcement during extinction training should result in a decrement of the original behavior.

Although Tolman was a passionate advocate for the cognitive view of learning, he also offered the possibility that "there is more than one kind of learning" (Tolman, 1949). Tolman suggested that perhaps some of the debates between learning theorists could be resolved if we accepted that the distinct learning mechanisms being proposed by different groups are not mutually exclusive and that they instead co-exist and contribute uniquely to learning and memory function. Over the past few decades, this general contention has been extensively corroborated by lesion studies indicating that the acquisition and retrieval of different kinds of information might be mediated by different parts of the brain (White, Packard, and McDonald, 2013). That is, instead of a single

mechanism guiding learning and memory, learning and memory may be achieved through multiple memory systems. Although dissociations between memory systems have been made primarily during initial acquisition, consolidation, and retrieval of memory, some recent evidence suggests that multiple memory systems also differentially contribute to extinction.

2.2 Multiple Memory Systems: Acquisition, Consolidation, and Retrieval

Extensive research indicates that memory is not a unitary phenomenon, but rather it transpires through distinct systems. These "memory systems" differ in terms of not only the type(s) of memory they mediate, but also the brain regions that subserve them (Figure 1). Although a variety of memory systems have been dissociated in the mammalian brain (Squire, 2004; White, Packard, and McDonald, 2013), two memory systems have absorbed the bulk of attention: a spatial/cognitive memory system mediated by the hippocampus and an S-R/habit system mediated by the dorsolateral striatum (DLS).

An elegant dissociation between hippocampus- and DLS-dependent memory systems may be observed in the plus-maze. The plus-maze consists of four arms arranged in a cross (+) orientation. In a dual-solution version of the plus-maze (Blodgett and McCutchan, 1948), the experimenter places the rat in the same starting arm (e.g. south) across multiple trials. The reward also remains in a consistent goal arm (e.g. west) for these trials, requiring the rat to make the same turn (e.g. left) toward the same

spatial location for every trial. Thus, the rat can learn this task in at least two distinct ways. The rat can learn to make the same turning response (e.g. a response strategy), or the rat can learn the spatial location of the goal and thus make whatever response necessary—which happens to be the same turning response for each trial—to reach this spatial location (e.g. a place strategy). In a subsequent probe trial, an experimenter may examine which strategy the rat had acquired (or the strategy they will *retrieve*) by placing the rat on the opposite starting arm (e.g. north). If the rat continues to make the same turning response as

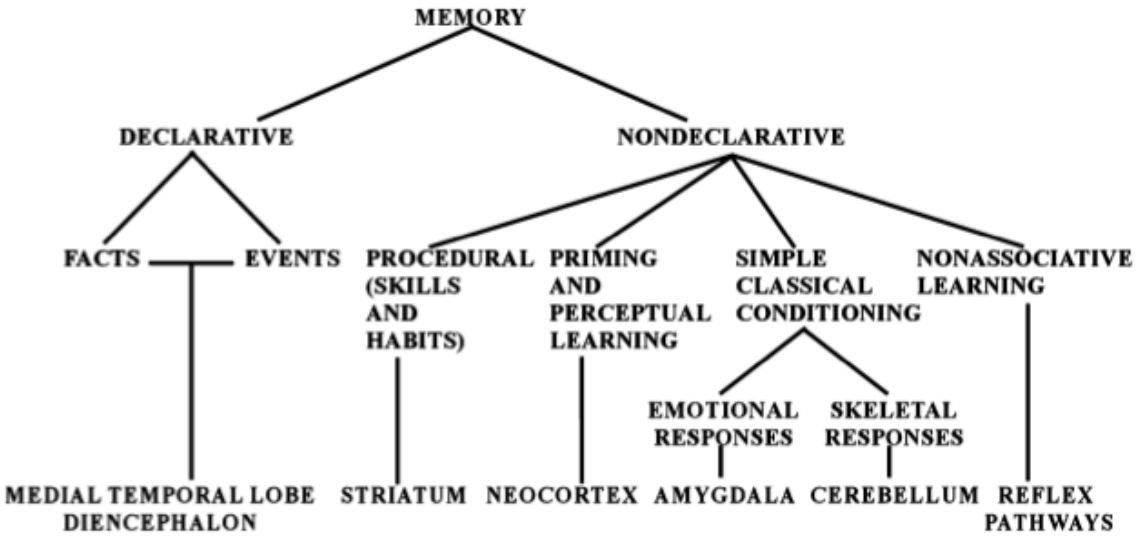

Figure 1. Taxonomy of memory systems in the mammalian brain.

during training (e.g. a left turn), the rat is believed to have acquired a response strategy. If the rat instead makes the opposite turning response (e.g. a right turn), thus running toward the same spatial location as during training, the rat is considered to be using a

place strategy. Response learning in this task, as well as other maze tasks, is regarded as an exemplar of habit learning, given that the turning response is not guided by cognitive spatial navigation nor the value of the outcome (Sage and Knowlton, 2000; Lin and Liao, 2003; De Leonibus et al., 2011; Smith et al., 2012; Smith and Graybiel, 2013).

Several studies indicate that after limited training in the dual-solution plus-maze task, rats display a place strategy, whereas after extended training rats display a response strategy (Ritchie, Aeschliman, and Pierce, 1950; Hicks, 1964; Packard and McGaugh, 1996). Moreover, inactivating the hippocampus with lidocaine disrupts expression of a place strategy, whereas inactivating the DLS disrupts expression of a response strategy (Packard and McGaugh, 1996). The place and response learning tasks will be discussed in greater detail later. For now, it is worth emphasizing that similar dissociations between the hippocampus and DLS have been made during acquisition and consolidation of memory using the radial maze (Packard, Hirsh, and White, 1989), water maze (Packard and McGaugh, 1992), and Barnes maze (Rueda-Orozco et al., 2008a), as well as across a variety of species including rats, mice, monkeys, and humans (Buffalo et al., 1999; Fernandez-Ruiz et al., 2001; Iaria et al, 2003; Lee, Duman, and Pittenger, 2008).

These dissociation experiments have been instrumental in identifying the unique kinds of information acquired by the hippocampus and DLS (White and McDonald, 2002). The hippocampus presumably mediates stimulus-stimulus associations, which can be employed to build cognitive maps of the learning environment or "sign-gestalt expectations" about the learning situation. This hippocampal information can be used to

guide purposive behavior toward a pleasurable state of affairs in the learning situation, such as directing running behavior toward a rewarded spatial location. In contrast, the DLS mediates associations between stimuli and responses (i.e. S-R learning), so that stimuli can automatically activate a behavioral response that might lead to reinforcement. However, reinforcement is only necessary to stamp in the S-R association. Following acquisition, the learned behaviors inherent in an S-R memory mediated by the DLS are retrieved without anticipation of reinforcement, but rather are activated by specific stimuli. Notably learning and memory functions of the DLS appear remarkably consistent with Hull's S-R habit view of learning (Hull, 1943), whereas the mnemonic functions of the hippocampus resemble Tolman's cognitive view of learning (Tolman, 1932).

2.3 Competition between Memory Systems

Research indicates that the DLS-dependent S-R memory system and the hippocampus-dependent cognitive memory system sometimes interact in a competitive fashion. A competitive interaction between these two systems may be observed when disrupting the function of one memory system enhances learning mediated by the other "intact" system. Lesioning the hippocampal formation, for instance, facilitates learning in tasks that involve DLS-dependent memory (Packard, Hirsh, and White, 1989; McDonald and White, 1993; Schroeder, Wingard, and Packard, 2002), and disrupting dorsal striatal function facilitates learning in tasks that involve hippocampus-dependent

cognitive memory (Mitchell and Hall, 1988; Lee, Duman, and Pittenger, 2008; Kosaki et al., 2015). Competitive interactions between memory systems may also be demonstrated when pharmacologically increasing the function of one memory system disrupts learning mediated by the other system. For example, intra-DLS infusion of glucose or CREB (a transcription factor that promotes plasticity) impairs hippocampus-dependent cognitive learning (Pych, Kim, and Gold, 2006; Kathirvelu and Colombo, 2013). Moreover, the hippocampus-dependent learning deficits produced by morphine administration may be reversed following CREB inhibition in the DLS (Baudonnat et al., 2011).

2.4 Multiple Memory Systems in Extinction

Recent evidence suggests that the hippocampus and DLS might not only be involved in distinct learning and memory processes guiding initial acquisition, consolidation, and retrieval, but that these neural systems also subserve different kinds of extinction learning. It is possible that consistent with the Hullian S-R view of extinction, the DLS is involved in acquiring inhibitory S-R associations during extinction training, so that cues in the learning environment directly inhibit the original behavior. The hippocampus, consistent with Tolman's cognitive view, might be involved in acquiring changes in expectation, such as learning that a previously rewarded goal location no longer contains reinforcement. Determining whether the DLS and hippocampus are involved in different kinds of extinction learning would require the

use of separate extinction protocols that presumably depend on different learning mechanisms.

Early experimental psychologists demonstrated by training rats in maze tasks that extinction learning can be achieved using a variety of protocols. In the straight alley maze (Figure 2), animals are initially trained to make a running approach response down a straight runway to retrieve food reward at the opposite end of the maze. Following initial acquisition of the straight alley maze, memory performance may be extinguished using two distinct protocols. In a typical "response extinction" protocol, a subject is given the opportunity to perform the original behavior, but without reinforcement. For example, response extinction in the straight alley maze involves releasing a rat from the original starting position, thus affording the animal the opportunity to execute the original running approach response toward the goal box at the opposite end of the maze, only now this goal box does not contain food.

On the other hand, a "latent extinction" protocol involves confining an animal to the previous goal location without reinforcement. Importantly, this protocol prevents the animal from having the opportunity to perform the original behavior. For example, latent extinction in the straight alley maze involves confining a rat to the goal box without food, thereby preventing the animal from performing the running approach response to the empty goal box. Even though the animal is not able to perform the original response, these goal box confinements remain effective at producing extinction by presumably "informing" the animal that the goal box is no longer baited (Seward and Levy, 1949). The effectiveness of latent extinction is revealed through subsequent probe

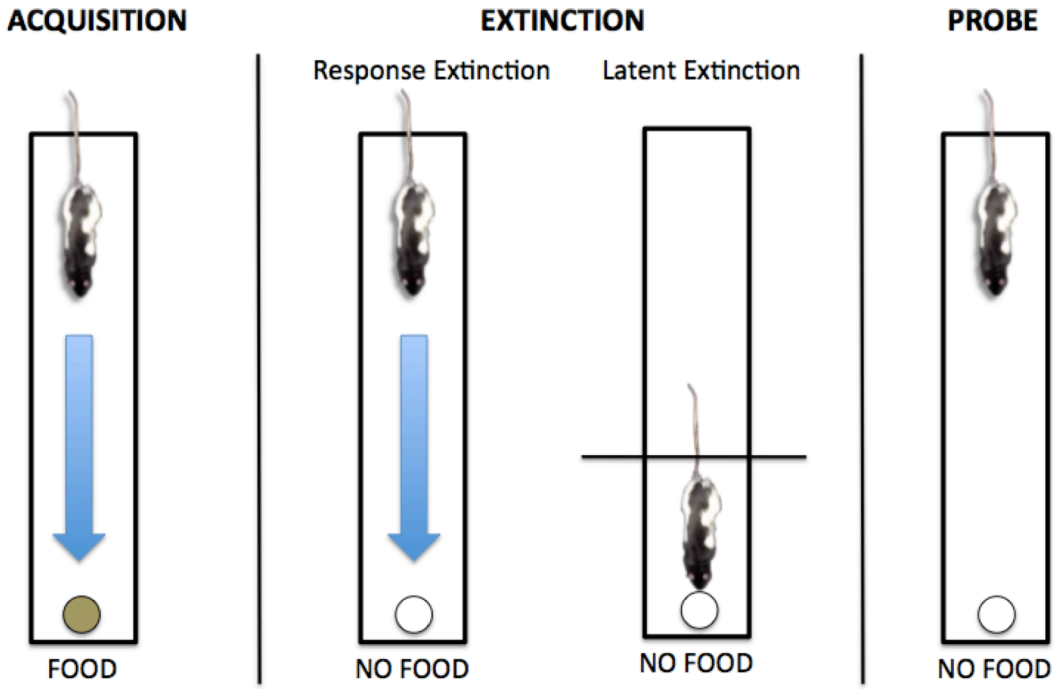

Figure 2. Extinction in the straight alley maze. The straight alley maze has been previously used to demonstrate a role for multiple memory systems in extinction learning. During initial acquisition in the straight alley maze, the subject acquires a quick running approach response down the straight runway to retrieve food at the other end. During response extinction, animals have the opportunity to perform the original running approach to an empty food well. In contrast, animals given latent extinction are simply confined to the original goal location without food. Thus, during latent extinction, subjects do not have the opportunity to make the original running approach response. During subsequent extinction probe trials, in which the animal is placed in the original starting position, animals previously given latent extinction training exhibit a suppression of the running approach response. This suggests that some extinction learning had occurred during the confinements to the unrewarded goal location.

trials, in which a rat is released from the original starting position, therefore having the opportunity to perform the running approach toward the unrewarded goal location. Animals previously given latent extinction demonstrate a greater suppression of the

running approach response during these probe trials, relative to control animals that were confined to another, neutral box (Seward and Levy, 1949). These observations suggest that some extinction learning occurs during the unreinforced goal box confinements, and this extinction memory becomes manifest during the probe trials. Importantly, the extinction learning that occurs during the goal box confinements is achieved without the animal having to perform the previously acquired running approach response.

The original demonstration of latent extinction (Seward and Levy, 1949) proved historically significant in learning theory by providing powerful evidence against the S-R view. An important component of the S-R view of extinction was that subjects needed to perform the previously acquired response for extinction to occur (Hull, 1943), and in latent extinction animals demonstrated learned suppression of responding (i.e. extinction) after only being confined to the empty goal box. Latent extinction instead provided evidence in favor of the cognitive view. The cognitive view did not require that animals perform the original response for extinction to occur, but only that a change in expectation takes place, which can occur by simply pairing the goal box with the absence of food. S-R learning theorists struggled to make latent extinction fit within their framework using novel S-R principles (Moltz, 1955), however research into latent extinction, in the end, contributed to the downfall of the S-R view and the rise of the cognitive view of extinction. Hulse, Deese, and Egerth (1975) wrote:

> In the light of phenomena like latent extinction, for example, there appears to be little question that the [S-R theory of extinction] faces a

> formidable task if it is to extricate itself from the serious trouble in which the data place it. For the present, this does not seem worth attempting, and it is perhaps for this reason that little attention has been devoted to the theory in recent years (p. 118).

An alternative approach based on the multiple memory systems hypothesis, however, is that latent extinction might only tap into one kind of extinction learning that does not depend on S-R mechanisms. Response extinction, on the other hand, might tap into another kind of extinction learning that partially depends on S-R mechanisms.

Regarding what mechanisms underlie latent extinction, unreinforced confinements to the goal box during latent extinction training could allow animals to acquire a new association in which the original rewarded location is associated with the absence of reinforcement. This new memory may effectively compete with the original memory (i.e. that the goal location contains food), thereby producing a response decrement. Consistent with this hypothesis that the effectiveness of latent extinction involves a new association between the original spatial location and the absence of reinforcement, latent extinction is only effective when conducted in the presence of extra-maze cues that are conducive to spatial memory processing, and latent extinction remains ineffective when it is conducted in the absence of allocentric spatial cues that prevent spatial memory processing (Seward and Levy, 1949; Bugelski, Coyer, and Rogers, 1952; Scharlock, 1954; Denny and Ratner, 1959; Dyal, 1962). In addition, being confined to a neutral goal box in a different room or a distinct spatial location in

the same room does not result in a response decrement (Iwahara, Asami, Okano, and Shibuya, 1953; Clifford, 1964). Notably the contention that the kind of extinction learning underlying latent extinction involves an association between the original spatial location and absence of reinforcement is consistent with Tolman's cognitive view of extinction, in that this proposed mechanism involves changes in expectation. The animal *expects* that the goal location does not contain food.

In contrast to latent extinction, response extinction remains effective in the absence of allocentric spatial cues (e.g. Scharlock, 1954), suggesting that response extinction might depend on a distinct learning mechanism. Animals given response extinction have the opportunity of performing the original behavior, now unreinforced, which could produce inhibitory S-R associations that suppress the original behavior. This proposed mechanism is consistent with the Hullian S-R view of extinction.

It should be emphasized that whether response extinction specifically relies on Hullian S-R mechanisms and latent extinction relies on Tolmanian cognitive mechanisms remains unexamined. However, given the differences in the protocols and the observation that experimental factors such as extra-maze cues differentially influence latent and response extinction, it is reasonable to suggest that these types of extinction training depend on distinct learning mechanisms. In addition, behavioral evidence is at least consistent with the S-R and cognitive mechanisms proposed to underlie response and latent extinction, and regardless of their absolute veracity these proposed mechanisms are useful in generating hypotheses about what brain regions could be involved in these protocols.

2.5 Hippocampus and DLS Mediate Different Kinds of Extinction Learning

Based on the multiple memory systems approach to extinction learning, latent and response extinction protocols might invoke different kinds of extinction learning. Considering that acquisition, consolidation, and retrieval of different kinds of memory have been associated with anatomically dissociable neural systems, different kinds of extinction learning could also be associated with distinct neurobiological substrates. Consistent with the potential role of inhibitory S-R mechanisms, response extinction could depend on function of the DLS. In contrast, given the potential role of cognitive/spatial memory mechanisms, latent extinction could depend on function of the hippocampus.

The role of the DLS and hippocampus in response and latent extinction was examined in a series of experiments conducted in the straight alley maze (Gabriele and Packard, 2006; Gabriele, 2008). To examine the role of the DLS in response extinction, rats were implanted with guide cannulas targeting the DLS and were subsequently trained in a straight alley maze task. During initial acquisition of this task, animals were placed in a consistent starting position of a straight runway, and food reward was consistently located in a recessed food well at the opposite end of the runway. Over the course of initial acquisition, no drugs were administered, and mean latency to reach the food well decreased dramatically for all rats. Following initial acquisition, animals received response extinction training, in which they were placed in the original starting position and had the opportunity to make the original running approach response to the empty goal location. Immediately before response extinction training, animals received

bilateral intra-DLS injections of bupivacaine (a sodium channel blocker which effectively shuts down neural activity) or saline solution for control rats. Over the course of response extinction training, animals receiving DLS inactivation with bupivacaine demonstrated lower latencies to reach the empty goal location, relative to animals given saline infusions. These findings suggest that the kind(s) of extinction learning invoked by the response extinction protocol partially depend on DLS activity. However, it is worth noting that during response extinction training, DLS inactivation did not produce a complete blockade of extinction, but rather an attenuation. It is possible that response extinction involves one dominant learning mechanism that depends on DLS function, but that other kinds of extinction learning might partially compensate when the DLS goes offline. These alternative mechanisms likely depend on separate neural systems.

In contrast to the DLS, the hippocampus does not appear to be required for the learning mechanisms underlying response extinction (Gabriele and Packard, 2006). In a separate experiment, rats with cannulas in the hippocampus were trained in the straight alley maze task and given response extinction training using identical parameters to the above study. Animals that received hippocampal inactivation before response extinction training demonstrated a comparable increase in extinction latencies relative to control animals receiving saline, suggesting that hippocampal inactivation failed to perturb the effectiveness of response extinction.

On the other hand, the hippocampus does seem to be required for latent extinction (Gabriele and Packard, 2006). A separate group of animals with cannulas in

the hippocampus were trained in the straight alley maze task and then received latent extinction training. For latent extinction training, animals were confined to the goal box without food reward. Immediately before latent extinction training, animals received hippocampal inactivation with bupivacaine or control injections of saline. Following latent extinction training, both groups received drug-free probe trials in which animals were returned to the original starting position, and mean latency to reach the empty goal location was recorded. Animals that previously received hippocampal inactivations during latent extinction training demonstrated lower extinction latencies than saline control animals during the probe trials, indicating an impairment in extinction learning. Additional analyses indicated that hippocampal inactivation completely blocked the effectiveness of latent extinction. Moreover, it was demonstrated in a separate experiment that the DLS, in contrast, is not needed for the learning mechanisms underlying latent extinction (Gabriele, 2008). Animals having received DLS inactivation during latent extinction training displayed comparable extinction latencies to saline-treated control animals during subsequent drug-free probe trials.

The findings from these experiments demonstrate a double dissociation regarding the role of multiple memory systems in extinction learning. The DLS, but not the hippocampus, is needed for response extinction, whereas the hippocampus, but not the DLS, is needed for latent extinction. One interpretation of these findings is that response and latent extinction protocols tapped into different kinds of extinction learning, which are mediated by dissociable neural systems. This is consistent with the multiple memory systems view of extinction learning.

2.6 Does the Type of Memory Being Extinguished Matter?

Compared to other mazes, the straight alley remains unrivaled in its elegant simplicity, but with this simplicity come a few disadvantages. Most notably, it is difficult to determine what kind of memory was initially acquired in the task, as several different types of learning could have contributed to successful acquisition. According to the Hullian S-R view, stimuli in the learning environment may have acquired the ability to activate the running approach response. On the other hand, according to Tolman's cognitive view, animals may have acquired the spatial location of the food reward, and the running approach response was purposefully directed toward this location. Whether animals acquired an S-R memory or cognitive/spatial memory in this task is difficult to determine, because acquisition of either type of memory would result in the same behavior: a running approach response. On a neural level, DLS and hippocampus have both been implicated in initial acquisition in the straight alley maze (Kirkby, Polgar, and Coyle, 1981; Dunnett and Iversen, 1981; Rawlins, Feldon, Ursin, and Gray, 1985), suggesting that both S-R and cognitive mechanisms could be involved.

Because it remains unclear precisely what kind of memory was initially acquired in the straight alley maze, we also do not know what kind of memory was being extinguished. Consideration of the initially acquired memory leads to a couple empirical questions:

1. Are the kinds of learning and memory promoted by latent and response extinction protocols effective at extinguishing all kinds of memory, or in contrast is each protocol only effective at targeting specific kinds of memory?

2. In addition, are the DLS and hippocampus still critically implicated in latent and response extinction when different kinds of memory are being extinguished?

These questions will be partially addressed by the dissertation experiments described below. Thus, the discussion of the multiple memory systems view of extinction will be suspended for the time being and continued in the discussion of the present dissertation. Specifically I will consider how the dissertation findings fit into the multiple memory systems view of extinction. For now, it is important to review other topics that have led to the present dissertation hypotheses and experiments.

One of the central hypotheses motivating the present dissertation experiments is that the DLS might be implicated in *extinction* of habit memory. Thus, the subsequent section briefly reviews the anatomy of the DLS and also critically considers current theories regarding DLS function.

CHAPTER III

A FORCE OF HABIT

3.1 Anatomy of the DLS: A Summary

Although investigators have studied the DLS and its surrounding structures across multiple species, most of what we know about this brain region comes from the rat brain. Therefore, the present anatomical summary focuses on the rat. However, anatomical evidence suggests that the striatum has remained relatively unchanged across avian and mammalian evolution, and therefore major features of the rat striatum have also been observed in the brains of other species (Reiner, 2010).

The DLS is part of a constellation of midbrain structures called the basal ganglia. The basal ganglia include the dorsal striatum (medial and lateral regions), the globus pallidus (internal and external segments), and ventral striatum (core and shell of the nucleus accumbens). Often included in this group is the substantia nigra (pars compacta and pars reticularis). The dorsal striatum, along with the ventral striatum, is the major input structure of the basal ganglia. The striatum receives glutamatergic input from most areas of the cerebral cortex (Gerfen and Bolam, 2010). This input is topographically organized, in that the spatial organization of the cortical areas is maintained in the cortical projections to the striatum. The somatosensory and motor areas of the cortex predominantly innervate the lateral portion of the dorsal striatum (DLS), whereas visual and auditory areas of the cortex innervate the medial portion of the dorsal striatum

(DMS). The striatum also receives glutamatergic input from limbic regions. These limbic regions, including the hippocampus and the amygdala, terminate in the ventral striatum and the more ventral portions of the DMS, making little to no contact with the DLS. Conversely, the substantia nigra releases dopamine into all regions of the striatum; however, this input too maintains a topographical organization (Gerfen et al., 1987a,b; Jimenez-Castellanos and Graybiel, 1987; Langer and Graybiel, 1989). Based on their afferent inputs—as well as their functional differences, which I will mention later—the DMS and DLS are often considered sovereign systems. The DMS is often dubbed the "associative" striatum, whereas the DLS is dubbed the "sensorimotor" striatum. Aside from disparate afferents, however, the DMS and DLS—in addition to the ventral striatum—of the rat appear anatomically similar. Thus, the remainder of this anatomical synopsis will describe the striatum as a single unit.

The striatum, as a whole, is predominantly comprised of GABAergic medium spiny neurons (MSNs). These account for about 95% of the neurons in the striatum, whereas the other 5% are believed to be mostly interneurons, many of which are GABAergic or cholinergic. The exact function of these interneurons remains undetermined, but investigators have set forth multiple hypotheses, which the interested reader may learn about elsewhere (see Oorschot, 2000). Of course, MSNs have attracted greater attention, and for good reason. Not only are MSNs greater in number, but they also serve as both the main targets of cortical input to the basal ganglia (Somogyi et al., 1981) and the major projection neurons from the striatum (Grofova, 1975). These medium spiny projection neurons may be divided into two subtypes based on a neuron's

differential expression of certain proteins or whether the neuron predominantly contains one type of dopamine receptor over another. Based on relative receptor expression, investigators have divided these neurons into "D1-expressing" and "D2-expressing" MSNs. These neuron subtypes are part of different projection pathways. D1-expressing MSNs are part of the "direct" pathway. It is called the direct pathway, because the projection neurons release GABA directly into the substantia nigra and external segment of the globus pallidus, i.e. the major output nuclei of the basal ganglia. On the other hand, D2-expressing neurons are part of the "indirect" pathway. These neurons project to the internal segment of the globus pallidus; however, in contrast to the external segment, the internal segment of the globus pallidus does not project outside the basal ganglia. Rather, the GABAergic neurons of this region innervate the subthalamic nuclei, which in turn release glutamate into the substantia nigra pars compacta and internal segment of the globus pallidus. In other words, indirect pathway neurons of the striatum only *indirectly* contact the output nuclei of the basal ganglia.

Due to their different routes, the direct and indirect pathways render different effects on basal ganglia output nuclei. Activation of the direct pathway leads to a greater release of GABA, thus reducing activation of the output nuclei, whereas activation of the indirect pathway leads to a smaller release of GABA, thus indirectly increasing activation of the output nuclei. Put simply, the direct pathway dampens, whereas the indirect pathway stimulates, activity of the basal ganglia output nuclei. Next in the sequence, the GABAergic output nuclei (i.e. the substantia nigra pars compacta and internal segment of the globus pallidus) innervate glutamatergic thalamic nuclei, which

in turn stimulate motor areas of the cortex. This brings the prominent motor loop of the basal ganglia full circle.

3.2 Motor Function of the DLS

After the above synopsis, it should come as no surprise to the reader that early research on the dorsal striatum focused on its role in controlling movement (for historical review, see White, 2009). Indeed, the dorsal striatum resides in a prime location to fulfill such a role. It is a nexus through which multiple cortical inputs—carrying sensory, emotional, and executive information—may affect motor output. In a classic model of basal ganglia function that still receives widespread support, the basal ganglia receive input from limbic and thalamic nuclei, as well as multiples areas of the neocortex. The basal ganglia, after processing this information, send messages back to motor areas of the cortex, and in this way the basal ganglia may be viewed as a structure that uses converging input to select the appropriate motor behaviors for a given situation (Albin et al., 1989; Mink et al., 1996; Redgrave et al., 1999). Given that input to the DLS remains primarily sensorimotor in nature—that is, limbic and executive areas do not innervate this region of the striatum—the DLS might only subserve sensorimotor modulation of basal ganglia motor output. The DMS and ventral striatum, which receive limbic and executive inputs from cortical and subcortical efferents, might confer emotional and cognitive modulation of motor behavior (Figure 3).

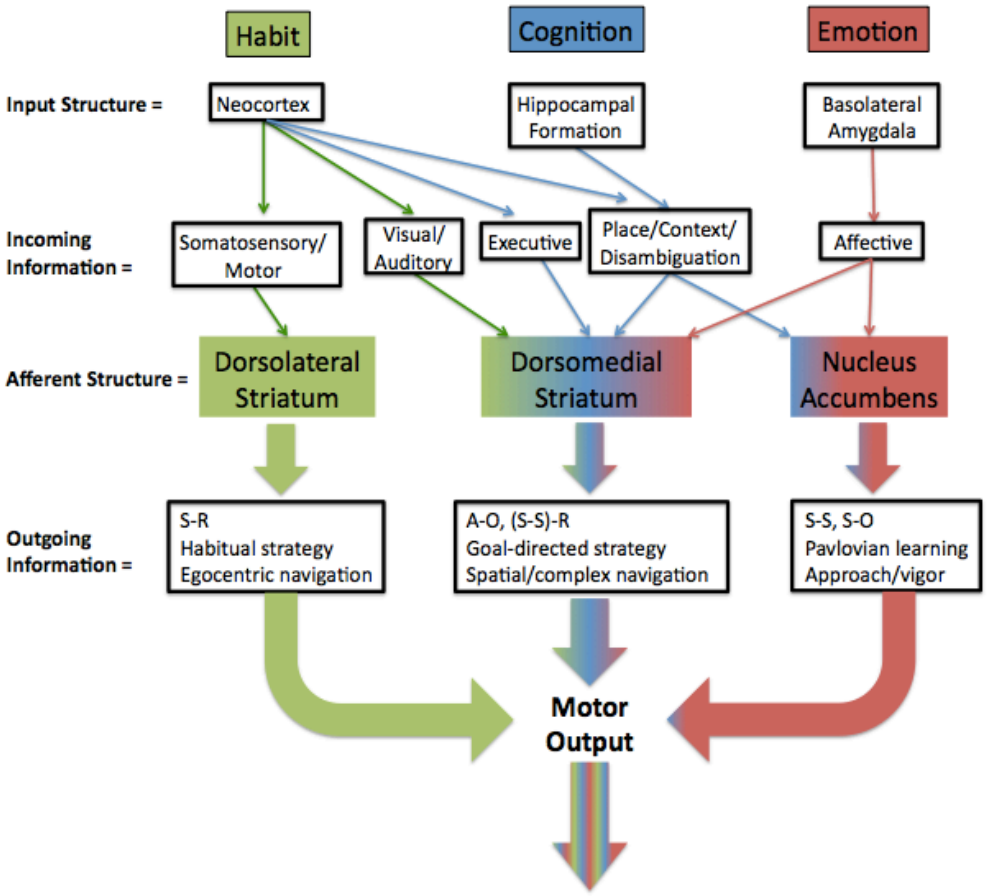

Figure 3. Memory systems of the striatum. Diagram depicts simplified version of basal ganglia circuits important for learning and memory. The striatum receives inputs from cortical and subcortical structures carrying sensory, cognitive, and affective information from which the basal ganglia may fashion learning strategies and influence motor output accordingly. Inputs to the striatum remain largely segregated, creating three anatomically distinct memory systems. The dorsolateral striatum (DLS) receives sensorimotor information from the neocortex and mediates stimulus-response (S-R) associations, habitual responding, and egocentric navigation. The dorsomedial striatum (DMS) receives sensory, executive, and affective information from the neocortex, hippocampal formation, and amygdala, respectively. Through these inputs, the DMS mediates action-outcome (A-O) associations as well as spatial and higher-order habit learning strategies (e.g. [S-S]-R) characterized by complexity, behavioral flexibility, and sensitivity to outcome devaluation. The nucleus accumbens receives affective information from the basolateral amygdala, allowing for the formation of Pavlovian CS-US or stimulus-outcome (S-O) associations, which may manifest as conditioned approach or enhanced response vigor. The nucleus accumbens also receives spatial information from the hippocampal formation, which may allow for conditioned approach to rewarding spatial locations.

As reviewed above, the striatum contains two subtypes of MSNs that have different effects on basal ganglia output nuclei. These are the direct and indirect pathways. Considering the anatomical differences between these pathways, investigators have suggested that the direct and indirect pathways render different neteffects on motor behavior. Activating the direct pathway may result in initiation of behavior, whereas activating the indirect pathway may result in inhibition of behavior (Albin, Young, and Penney, 1989; Delong, 1990). This hypothesis has received both criticism and support (Calabresi et al., 2014). Recent evidence remains largely consistent with this model, yet offers some important refinements. Indeed, optogenetic stimulation of the direct pathway increases locomotor activity, and stimulation of the indirect pathways decreases locomotor activity (Kravitz et al., 2010). However, electrophysiological recordings using optogenetics-aided cell identification indicate that both direct and indirect pathway MSNs display increased neural activity during action initiation and termination. However, during performance of the action sequence indirect pathway MSNs decrease neural activity, while direct pathway MSNs display sustained activity throughout performance (Jin et al., 2014). Thus, contemporary models of striatal function suggest that balanced activation of these pathways is important for proper action selection. The direct pathway may activate the desired action sequences, while the indirect pathway may inhibit the alternative, undesired action sequences. These functions have been proposed for the direct and indirect pathways arising in all regions of the striatum, including the DLS.

3.3 Mnemonic Function of the DLS

In the latter half of the 20th century, interest in the dorsal striatum as a brain region that also mediates memory blossomed (for reviews, see Packard, 2001; White, 2009). Although investigators searching for the engram, i.e. the "locus of memory" in the brain, focused on the hippocampus, it soon became clear that only some types of memory depended on the hippocampus, whereas others did not. Such findings led to several dual-memory hypotheses (Hirsh, 1974; O'Keefe and Nadel, 1978; Olton, Becker, and Handelmann, 1979; Cohen and Squire, 1980; Zola-Morgan, Squire, and Mishkin, 1982; Mahut and Moss, 1984; Mishkin and Petri, 1984; Graf and Schacter, 1985; Tulving, 1987; Sutherland and Rudy, 1989; Cohen and Eichenbaum, 1993). In many of these proposed models, one form of memory was considered hippocampus-dependent and another hippocampus-independent. Based on some early findings in the monkey literature (Buerger, Gross, and Rocha-Miranda, 1974), the dorsal striatum was introduced as a candidate brain structure that mediates some non-hippocampal memories (Mishkin and Petri, 1984). A large body of evidence has since corroborated this assertion. By making selective brain lesions, investigators demonstrated dissociations whereby one type of memory was associated with the hippocampus and not the dorsal striatum, whereas another type was associated with the dorsal striatum and not the hippocampus (Packard, Hirsh, and White, 1989; Packard and McGaugh, 1992; McDonald and White, 1993; for review, see White, Packard, and McDonald, 2013). These findings were critical in bolstering the once controversial, but now well accepted "multiple memory systems" approach to learning and memory described in Chapter II.

Importantly, later studies have indicated that the dorsal striatum-dependent memories being investigated in these non-hippocampal tasks may be more precisely linked to DLS function, whereas the DMS—a product of its limbic and executive inputs—was associated with a type of flexible/cognitive/goal-directed learning more closely related to hippocampus-dependent learning and memory function (for review, see Devan, Hong, and McDonald, 2011).

The type of memory mediated by the DLS is often branded as S-R or habit memory. That is, the DLS is believed to mediate associations between stimuli and responses in the learning environment so that stimuli may acquire the capacity to activate a behavioral response. Thus, the type of learning and memory mediated by the DLS may be consistent with the Hullian S-R view of learning. As noted above, the DLS receives input from cortical sensorimotor areas (McGeorge & Faull, 1989) and sends efferent projections to the basal ganglia output nuclei so that sensory information might influence motor behavior. In this way, the DLS is well positioned to foster novel connections between stimuli and motor behavior and, thus, subserve S-R/habit learning. In addition, the DLS receives little to no input from cortical executive areas or subcortical limbic structures, suggesting that the learning mechanisms subserved by the DLS occur independently of executive control or anticipation of the rewarding properties of the outcome. This is consistent with the automatic, cue-evoked nature of S-R/habit memory (Knowlton, 2014).

3.3.1 Maze Learning

Early evidence implicating a role for the DLS in S-R/habit learning and memory employed a "win-stay" radial maze task. For each session in this task, rats are placed in the center of an eight-arm radial maze in which four randomly selected arms have been illuminated. The animal could retrieve a palatable food reward by entering these illuminated arms twice within a daily training session, whereas entries into the other four, unlit arms are counted as errors. Thus, this task may involve S-R learning mechanisms to the extent that the animal may acquire an association between the light (i.e. the stimulus) and running approach (i.e. the response). Several studies employing this task have indicated that pre-training lesions of the DLS impair acquisition in the S-R win-stay radial maze, while sparing acquisition of the cognitive spatial version of the radial maze (Packard, Hirsh, and White, 1989; McDonald and White, 1993; McDonald and Hong, 2004).

Another popular and historically significant task used to examine the mnemonic function of the DLS involves response learning in the plus-maze. In a dual-solution version of the task (also discussed in Chapter II), animals are released from a consistent starting arm in a plus-maze (e.g. the South arm) and have the opportunity to retrieve a palatable food reward in a consistent goal arm (e.g. the West arm). Thus, animals may learn to make a consistent body-turn response (e.g. a left turn) at the intersection—or choice point—of the maze, or they may learn that the food reward is located in a consistent spatial location (e.g. the West arm). In order to determine what strategy guided learning in this task, an animal may be given a subsequent probe trial in which

the animal is released from the opposite starting arm (e.g. the North arm). If the animal makes the opposite turn (e.g. a right turn) at the choice point and goes to the original goal arm, the animal is believed to be using a place learning strategy. If the animal makes the same body-turn response (e.g. a left turn) at the choice point, the animal is believed to be using a response learning strategy.

Whether an animal acquires/retrieves a place learning strategy or response learning strategy depends on a variety of factors (Packard and Goodman, 2013; see also Chapter IV). For instance, following limited training in this task, animals typically show place learning during the probe trial, whereas following extended training (presumably after a habit has formed) an animal displays response learning during the probe trial. Following extended training, inactivation of the DLS immediately before the probe trial blocks retrieval of response learning (Packard and McGaugh, 1996). In addition, reversible or irreversible pre-training lesion of the DLS impairs acquisition of the response learning strategy (Yin and Knowlton, 2004; Asem and Holland, 2015), and post-training intra-DLS administration of glutamate leads to greater use of the response learning strategy during the probe trial, even after limited training (Packard, 1999).

In a single-solution response learning task, animals are released from opposite start arms of the plus-maze and are reinforced to make a consistent body-turn response at the choice point, regardless of the starting position (Tolman, Ritchie, and Kalish, 1946a). Similar to observations in the dual-solution version of the plus-maze, reversible or irreversible lesions of the DLS impair acquisition of response learning in this single-solution version of the task (Chang and Gold, 2004; Compton, 2004; Palencia and

Ragozzino, 2005; Asem and Holland, 2015). The critical role of the DLS in response learning is consistent with early evidence indicating that the dorsal striatum may be involved in egocentric navigation (e.g. Potegal, 1972). The behavioral and neurobiological mechanisms of response learning will be discussed at length in Chapter IV.

3.3.2 Instrumental Learning

Although a role for the DLS in S-R/habit learning was originally demonstrated in maze learning tasks (Packard et al., 1989; Packard and McGaugh, 1996), more recent evidence indicates a prominent role for the DLS in mediating S-R/habit learning in instrumental tasks as well (for review see Yin & Knowlton, 2006; Balleine & O'Doherty, 2010). In instrumental learning, animals learn to press a lever that results in the delivery of a food reward, and extensive research indicates that at least two different learned associations may guide behavior in this task (Dickinson, 1985). Consistent with an S-R view, the animal may associate a stimulus (e.g. the lever) with a response (i.e. pressing the lever). Receiving a food reward is not important for expression of this S-R association after extended training, i.e. the animal presses the lever automatically regardless of whether the lever press results in reward delivery. In contrast to the S-R view, the action-outcome (A-O) view of learning suggests that the animal learns to associate pressing the lever (i.e. the action) with delivery of food reward (i.e. the outcome). Thus, the animal presses the lever for the purpose of gaining the food reward.

Whether animals acquire a habitual/S-R or goal-directed/A-O association in this task has been classically demonstrated using outcome devaluation procedures (Adams and Dickinson, 1981a,b; Adams, 1982; Dickinson and Nicholas, 1983; Dickinson, Nicholas, and Adams, 1983). Outcome devaluation may be achieved by pairing the food reward with lithium chloride injections that cause illness, or by pre-feeding the animal with the food reward until the animal reaches satiety. Continuing to press the lever for the devalued outcome indicates habitual/S-R responding, whereas pressing the lever less frequently for the devalued outcome indicates goal-directed/A-O responding. Whether an animal displays S-R or A-O learning depends in part on the reinforcement parameters. Thus, similar to what has been observed in the plus-maze (Ritchie, Aeschliman, and Pierce, 1950; Hicks, 1964), moderate instrumental training may be associated with goal-directed responding, whereas extended training in this task may be associated with habitual responding (Adams, 1982).

Evidence indicates that, using reinforcement parameters that favor habitual responding in control animals, rats or mice with DLS lesions in contrast display goal-directed responding (Yin, Knowlton, & Balleine, 2004; Corbit, Nie, & Janak, 2012; Quinn et al., 2013). Likewise, using a procedure in which, following instrumental learning, pressing the lever now causes a delay in receiving reward, animals with DLS inactivation display enhanced sensitivity to the change, i.e. pressing the lever less frequently than controls (Yin, Knowlton, & Balleine, 2006). In addition to outcome devaluation, DLS lesions also restore goal-directed learning following outcome *inflation*, i.e. the enhanced instrumental responding produced by increasing hunger (Quinn et al.,

2013). Thus, the DLS may be required for S-R/habit learning not only in maze tasks as originally demonstrated (Packard, Hirsh and White, 1989; Sage and Knowlton, 2000), but also instrumental learning tasks, in that lesion or inactivation of the DLS disrupts habit formation and reinstates goal-directed behavior.

Multiple neurotransmitter systems have been implicated in DLS-dependent habit learning and memory in both maze tasks (Packard and White, 1991; Packard and Teather, 1997; Chang and Gold 2003; Goodman and Packard, 2014), and in the operant chamber (Faure et al., 2005; Hilário et al., 2009; Corbit, Nie, & Janak, 2014), including dopamine, glutamate, acetylcholine, and cannabinoid mechanisms.

3.3.3 Novel Views of DLS Memory Function

The literature cited above has led to the view that the DLS may subserve S-R habit memory. Implicit in this view is that DLS-dependent memory promotes behavior that is insensitive to reward devaluation. In other words, the DLS links stimuli with responses (i.e. S-R), but does not encode the rewarding properties of the outcome (White and McDonald, 2002). This description, though complete in its own right, does not account for all findings. The DLS is required for quick changes in strategy selection following the omission of reward (Skelin et al., 2014), and electrophysiological evidence indicates that some DLS MSNs may be sensitive to the presence or absence of reward (Smith & Graybiel, 2016a). In addition, the DLS is critically implicated in learned habit-like behaviors that are acquired long before memory performance becomes

insensitive to reward devaluation (Smith and Graybiel, 2016b). Thus, the DLS may be involved in goal-directed forms of habit memory.

At the same time another line of research impugns the popular labeling of DLS-dependent memory as strictly S-R. Electrophysiological studies uncover very few neurons that selectively respond to S-R combinations (Thorn et al., 2010; Smith and Graybiel, 2013). Moreover, the DLS electrophysiological pattern that emerges during extensive training remains relatively stable following changes in reward value and the ensuing changes in behavior (Smith and Graybiel, 2013). In other words, the pattern of DLS activity continues even though the stimulus-evoked behavior has ceased. This lies in contrast to what is expected from an S-R viewpoint.

Thus, the converging evidence begs for a reassessment of what the DLS encodes and, more to the point, what constitutes a DLS-dependent memory. One possibility is that the DLS may simply bind action sequences into "chunks" which, although formed and stored in the DLS, may require another brain region, such as the infralimbic prefrontal cortex, to express or "turn on" the DLS-dependent action chunk during task performance (Smith and Graybiel, 2014).

Another way to view the DLS is as a more broadly defined habit memory system. As mentioned previously, the DLS mediates habit-like memory performance that sometimes proves sensitive to reward devaluation. However, these DLS-dependent memories may remain insensitive to other types of higher-order cognitive processes. DLS-dependent memory has been variously described as implicit, motoric, egocentric, procedural, habitual, and S-R. Additional words used to describe DLS-dependent

memory include rigid, automatic, and inflexible. We may string these words together and subsume them under the banner: *doing without thinking*. In other words, the DLS may subserve the learning and retrieval of automatic, motoric responses to stimuli (i.e. *doing*), while at the same time actively disregarding intent, reward valuation, conscious deliberation, executive control, cognitive maps, or other higher-order cognitive processes (i.e. *thinking*) that would otherwise modulate behavior. In maze learning, this DLS-dependent *doing without thinking* may be operationalized as making the same egocentric or stimulus-approach response, while disregarding the spatial context of the learning environment (Packard et al., 1989; Packard & McGaugh, 1992, 1996). Also, DLS-dependent *doing without thinking* may be operationalized as persistent responding following devaluation of the outcome, i.e. S-R/habitual responding, in both maze-learning (Sage & Knowlton, 2000; De Leonibus et al., 2011) and instrumental lever-pressing tasks (Yin, Balleine, and Knowlton, 2004). The DLS may mediate not only the acquisition of *doing,* but also the suppression of *thinking.* Indeed, DLS lesions don't simply block retrieval of habit memory, but also lead to greater use of cognitive memory mechanisms in some learning situations (Mitchell and Hall, 1988; Packard and McGaugh, 1996; Yin and Knowlton, 2004; Kosaki et al., 2015).

Regardless of the precise type of memory that the DLS mediates, extensive evidence indicates that the DLS is needed for some learning and memory processes underlying tasks that are believed to contain S-R habit components. Therefore, these tasks may be used to examine the behavioral and neurobiological mechanisms of DLS-dependent memory. A series of tasks now commonly used to examine DLS-dependent

memory function, which also served as the historical battleground between the S-R and cognitive views of learning, was the place and response learning tasks conducted in the plus-maze. These tasks will be discussed at length in the subsequent chapter.

CHAPTER IV

LEARNING THEORY AT THE CHOICE POINT

4.1 Tolman at the Choice Point

In 1932, when Edward C. Tolman published his book titled *Purposive Behavior in Animals and Man*, he became, at once, the father of purposive behaviorism and the figurehead leading the rebellion against the S-R view of learning. *Purposive Behavior* was, in a way, the face that launched a thousand ships against the S-R view, inciting an academic civil war between the two learning theories, a war that lasted several decades. Not only were Tolman's salvos evident in his writings, but he was also wont to criticize the theory in his less official correspondences. James L. McGaugh, who was once a student of Tolman's and who has since made a name for himself investigating the emotional modulation of memory, provides an amusing anecdote:

> …in one lecture commenting on the limitations of S-R theory [Tolman] pointed out that, as there are important cognitive processes in the organism that intervene between the S and R, at the very least, an 'O' (for 'organism') must be inserted. Moreover, as it is behavioural acts that occur and not muscle-twitch responses, the 'R' should be changed to 'B' for 'behaviour.' He then, with a sly grin (and no doubt with some residual New England guilt), referred to S-R theory as the 'SOB' theory. (McGaugh, 2003, pg. 20)

The anecdote is amusing and informative, as it sheds light on Tolman's rather snarky distaste for the S-R view that in part motivated him to write *Purposive Behavior* and start the revolution.

Although Tolman became one of the most popular and energetic opponents of the S-R view, he did not always harbor a negative attitude toward the theory. As a graduate student at Harvard, Tolman took a course in comparative psychology, which was then taught by renowned psychologist Robert Yerkes. The textbook for the course was a seminal work by Yerkes' friend and colleague, John B. Watson, titled *Behavior: An Introduction to Comparative Psychology*. Tolman at the time regarded Watson's behaviorism as a "tremendous stimulus and relief" from the alternative introspective approach being employed by other psychologists around this time (Tolman, 1952, pg. 326). However, Tolman had some reservations about Watsonian behaviorism, and at the same time developed a penchant for the Gestalt view that had been emanating from Germany and gaining popularity in the US.

> I… did not like Watson's over-simplified notions of stimulus and of response. Nor did I like his treatment of each single stimulus and each single response as a quite insulated phenomenon which has practically no relation to any other stimuli or any other responses. That is, I was already becoming influenced by Gestalt psychology and conceived that a rat running a maze must be learning a lay-out or pattern and not just having

> connections between atom-like stimuli and atom-like responses "stamped in" or "stamped out," whether by exercise or by effect. (Tolman, 1952, pg. 329).

This retrospective account suggested that it was a lack of confidence in Watson's overly reductionist view of behavior that led Tolman to begin considering alternative Gestalt views of learning. It is tempting to imagine Tolman during these formative years as a rat at the critical intersection—or choice point—of a maze, in which he had the option to turn one way and continue in the spirit of Watsonian behaviorism or turn the opposite way and subscribe wholeheartedly to Gestalt psychology. However, contrary to what may be assumed by this false dichotomy, there was a third route that lay in between the Watsonian and Gestalt ways of thinking, a path that Tolman eventually took and made his own.

In *Purposive Behavior*, Tolman suggested that behavior is motivated by purpose, cognition, and expectation. That is, animals acquire meaningful relationships between stimulus objects in the environment, to the extent that an animal learns that "commerce" with one particular object will lead to the opportunity to make commerce with another object and so forth. Knowledge about how stimuli in the environment are related to each other is encoded through stimulus-stimulus (S-S) associations, which are encapsulated in what Tolman called a "sign-gestalt expectation" or, on a larger scale, a "field expectation." Stimuli in the environment may activate these expectations, which are employed by animals to purposefully guide behavior to a pleasurable state of affairs.

This idea was later expounded upon in Tolman's seminal paper on cognitive maps, in which Tolman suggested that animals (including people) acquire a variety of cognitive maps—including not only allocentric maps of space, but also more abstract interpersonal maps—that support behavior, thought, and (on a speculative note) psychopathology (Tolman, 1948).

It is important to emphasize that although Tolman's inclination toward a cognitive view of learning was partially motivated by a skepticism surrounding strict Watsonian behaviorism, his cognitive views were corroborated through extensive behavioral research, much of which was conducted in his own laboratory at Berkeley. Tolman used a variety of mazes to show that animals can acquire cognitive maps of a learning environment, and they could use this map to guide running behavior toward a palatable food reinforcer. For instance, contrary to the S-R view of learning, animals could make inferences about there being shortcuts in the maze and generate a novel series of navigational responses based on those inferences (Tolman, Ritchie, and Kalish, 1946b). The experiments coming from Tolman's laboratory promoted a shift in the field from a rather spartan S-R view toward a more purposeful, cognitive view of behavior. However, just as Tolman's cognitive expectancy theory was gaining traction in the field, another investigator took the stage and touted an impressive rejuvenation of the S-R view that could not be easily ignored.

4.2 A Hull in the Machine

When Clark L. Hull began studying psychology, he—like Tolman—developed a fascination with Watsonian behaviorism. However, also like Tolman, he had some reservations about the theory. Hull disagreed with some of Watson's "dogmatic claims." The result of his disagreement "was a belated conversion to a kind of neo-behaviorism—a behaviorism concerned with the determination of the quantitative laws of behavior and their deductive systemization" (Hull, 1952, pg. 154). Drawing from his love of mathematics and experience in chemistry and engineering, Hull developed an intricate series of "mathematico-deductive" formulas to explain and predict observable behavior (Hull, 1940). These formulas were leaps and bounds above the primitive S-R associations being proposed by classical behaviorists.

Although Hull and Tolman were similar in their urge to break away from the restrictions of Watsonian behaviorism, Hull, unlike Tolman, refrained from incorporating in his theory what he viewed as teleological concepts, such as purpose and expectation. He believed that such teleological concepts were the unfortunate products of anthropomorphic subjectivisim. To safeguard oneself against these pitfalls, Hull suggested that we view organisms as automatons:

> A device much employed by the author has proved itself to be… [an] effective prophylaxis. This is to regard, from time to time, the behaving organism as a completely self-maintaining robot, constructed of materials as unlike ourselves as may be. In doing this it is not necessary to attempt

> the solution of the detailed engineering problems connected with the design of such a creature. It is a wholesome and revealing exercise, however, to consider the various problems in behavior dynamics which must be solved in the design of a truly self-maintaining robot. (Hull, 1943, pg. 27)

Hull's inclination to view organisms as automatons that operate without purpose and free will remains evident in his mathematico-deductive view of behavior.

According to Hull, the probability of a particular behavior being performed (i.e. reaction potential) was a function of drive and habit strength. Habit strength, he defined, as the degree to which a stimulus (S) has the capacity to activate a response (R), with the performance of R leading to drive reduction. Habit strength increases over the course of many iterations of S being paired with R. Over time, when habit strength has reached asymptote, the S can activate the R automatically, even under conditions of low drive. Thus, much of behavior according to Hull is a series of S-R habits.

Hull's theory, though impressive in its completeness and explanatory power, was harshly criticized by cognitive learning theorists, including Tolman and his colleagues. Soon after the publication of Hull's neobehaviorist manifesto titled *Principles of Behavior,* when S-R sympathizers began to flock to this novel, Hullian view of behavior, Tolman's laboratory developed a new paradigm that placed the Hullian "habit" and Tolmanian "cognitive" theories in direct competition with each other. This Tolmanian paradigm was adopted by other laboratories and served as the battleground for the debate

between S-R and cognitive views of learning for many subsequent and contentious years.

4.3 Tolman versus Hull in the Plus-Maze

Early on, it became clear to investigators that if an animal is placed in a maze with food consistently placed at another "goal end" of the maze, the animal will eventually learn to retrieve the food (Small, 1901). However, exactly *how* animals learned to retrieve the food or *what* animals acquired that enabled them to guide behavior to the rewarded location remained debatable. According to Tolman and colleagues, there were 3 competing learning theories that had been applied to explain the learning that occurs during maze training:

> 1. Such training may have produced a disposition in the rats to run on a path which has certain specific characteristics (e.g. knotholes of such and such a pattern, or the like) and to avoid running on all paths which have certain other specific characteristics.
>
> 2. Such training may have produced a disposition to turn right whenever they come to the choice point.

> 3. Finally, such training may have produced a disposition to orient towards the place where the food is located (e.g., under the window, to the left of the radiator, etc.). (Tolman et al., 1946a, pg. 221)

Tolman and his colleagues quickly ruled out the first explanation based on earlier findings from Hoznik (1936), suggesting that it was difficult for rats to use intramaze cues to guide behavior. However, they suggested that no studies as yet had directly compared the last two explanations. Is it the case that—consistent with the Hullian view of learning—animals acquire a response, or that in contrast—consistent with Tolman's view of learning—animals learn to go to a place? Tolman and his colleagues (1946a) developed two plus-maze tasks to examine these hypotheses.

4.3.1 The Single-Solution Place Learning and Response Learning Tasks

Tolman's laboratory used a plus-maze that consisted of four arms arranged in a cross (+) formation. Two opposite arms (e.g. North and South) were designated as start arms from which the animals were released during maze training, and the other two arms (e.g. East and West) were designated as goal arms which may contain food reward during training. The two tasks that Tolman et al. (1946a) had run in the plus-maze were called the "place learning" and "response learning" tasks. In the place learning task (see Figure 4B), animals were released from the opposite starting positions, and a palatable food reward was located in a consistent goal arm. Thus, animals presumably needed to learn the spatial location of the food reward in order to accurately guide behavior from

different starting positions to the rewarded spatial location. In the response learning task (Figure 4A), animals were also released from opposite starting positions, but the food reward in this case was rotated to opposite goal arms in such a way that in order for animals to quickly retrieve the food, they needed to make a consistent body-turn response. For instance, if rats were released from the North arm, the food reward was in the West arm. If the animal were released from the South arm, the food reward was in the East arm. Thus regardless of where a rat was released from, the rat needed to learn a consistent right body-turn to quickly retrieve the food over the course of training.

Tolman et al. (1946a) wanted to determine which task animals learned faster. It was assumed at this time, perhaps erroneously, that if animals learned one task faster than the other, then the type of learning required by the former task is more dominant or "natural" to the animals than the type of learning required by the other, more slowly acquired task. The findings of their study indicated that animals learned the place learning task much faster than the response learning task, and therefore the investigators concluded that "both kinds of dispositions may be acquired by the rat, but that the disposition to orient towards the goal is simpler and more primitive than the disposition to make right turns" (Tolman et al., 1946a, pg. 228). However, many subsequent plus-maze experiments had challenged this notion.

4.3.2 The Dual-Solution Plus-Maze Task

Another team of investigators who were critical of Tolman's findings provided an alternative version of the plus-maze task (commonly referred to as a dual-solution

plus-maze) to examine the relative use of place and response learning. Hugh C. Blodgett, who obtained his PhD in psychology while in Tolman's laboratory, designed this dual-solution plus-maze task in his laboratory at the University of Texas with one of his students (Blodgett and McCutchan, 1948). This task was previously introduced in Chapter II, as it provided an elegant dissociation between hippocampus and DLS-dependent memory systems (Packard and Mcgaugh, 1996). However, for convenience, this task will be described again presently and in greater detail.

Over the course of maze training in the dual-solution plus-maze task (Figure 4C), rats were released from a consistent starting position in the plus-maze (e.g. North), and food was placed in a consistent goal arm (e.g. West). During this initial acquisition phase, animals could find the food by acquiring a place learning strategy (e.g. go to the West arm) or a response learning strategy (e.g. make a consistent right body-turn response). In either case, behavior looked the same during initial training. To determine which strategy the animals employed, a probe trial was conducted in which animals were released from the opposite start arm. If animals made the same body turn as they did during initial training (i.e. turning away from the original goal arm), the animals were believed to have acquired a response learning strategy. If animals instead made the opposite body-turn response in order to run to the original goal location, the animals were believed to have acquired a place learning strategy.

A surprising result from Blodgett and McCutchan's (1948) original dual-solution plus-maze study was that during the probe trial, when animals could either turn in the direction of the original goal location or make the original turning response (i.e. going

away from the original spatial location), animals predominantly made the same turning response. These findings suggest that animals preferentially acquired and/or expressed a response learning strategy over a place learning strategy. The authors concluded that under their experimental conditions and in contrast to what Tolman et al. (1946a) previously suggested, "a response disposition is… stronger than a place disposition" (Blodgett and McCutchan, 1948, pg. 23).

It should be noted that although this dual-solution task was originally designed by Blodgett and McCutchan (1948), it was markedly similar to a plus-maze task previously employed by Tolman, Ritchie, and Kalish (1947). During an initial acquisition phase of this previous experiment, Tolman et al. (1947) released rats from a consistent starting position and had the food located in a consistent goal location. Later, animals were trained from the opposite start arm, while food remained in the same spatial location. Tolman et al. (1947) predicted that, if rats had initially acquired the task using a spatial strategy, then the animals should display greater memory performance when being trained from the opposite start arm, relative to control animals who had not received prior maze training. The results and the accompanying conclusions from Tolman's experiment were mixed (Tolman et al., 1947).

Although this Tolmanian maze was similar and may have served as an ideological predecessor to the dual-solution task employed in Blodgett's laboratory, the latter maze task remained unique in its application of *single* probe trials to gauge the relative use of place and response learning. At the same time, given the similarities

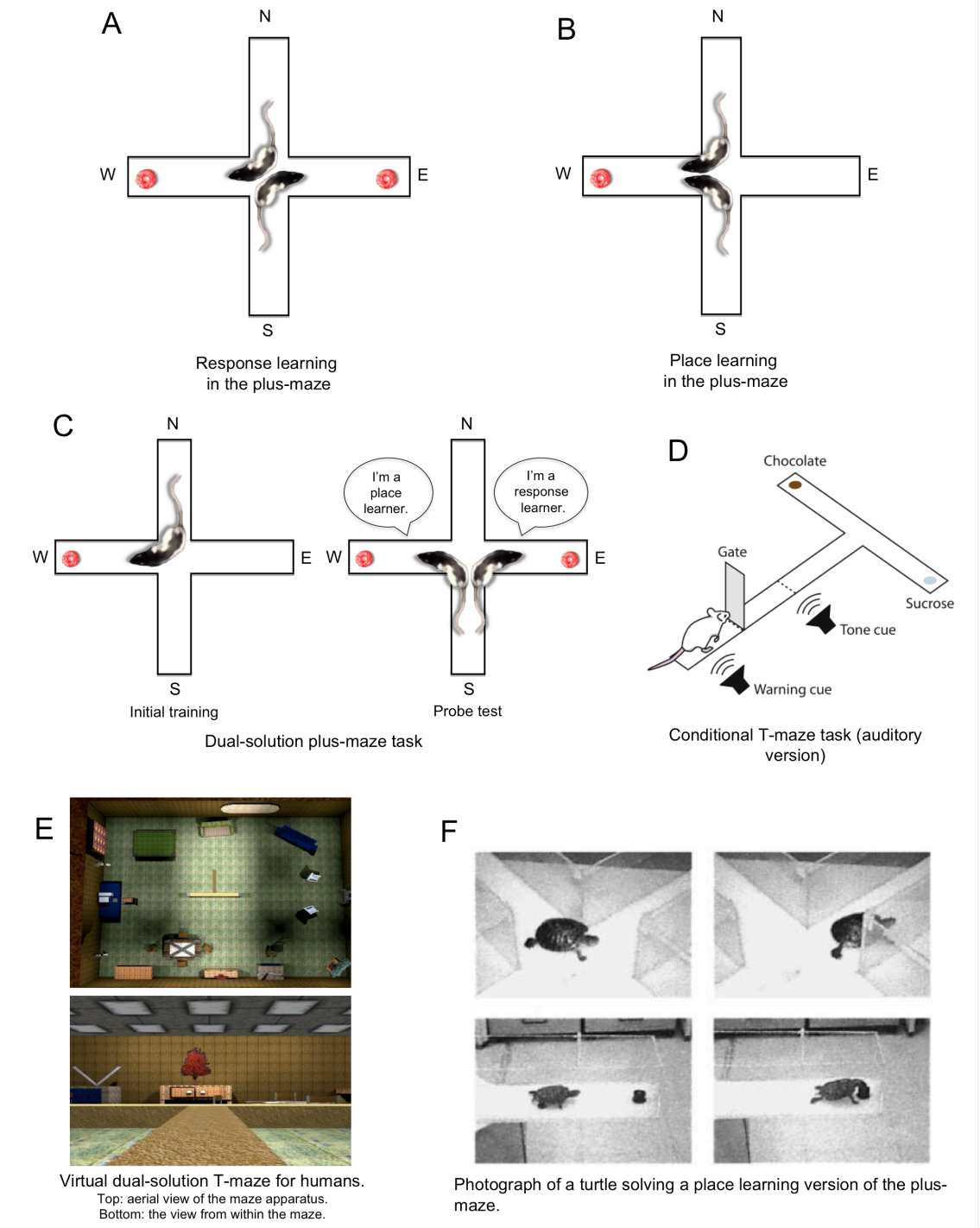

Figure 4. Place and response learning versions of the plus-maze/T-maze. See text for details

between the two tasks and the consideration that Blodgett was motivated to design the dual-solution plus-maze after reading about the place and response learning tasks employed in Tolman's laboratory, it may be fair that Blodgett's laboratory share the credit for designing this task with Tolman and his colleagues.

4.3.3 Other Versions of the Place and Response Learning Tasks

The plus-maze tasks coming from the laboratories of Tolman and Blodgett—that is, the single-solution place learning task, the single-solution response learning task, and the dual-solution place-response task—attracted the attention of numerous other investigators who later employed the tasks to examine Hullian S-R and Tolmanian cognitive views of learning. However, investigators had not only sought to replicate the original plus-maze tasks but were also wont to recast the designs with small changes. These novel place and response learning protocols allowed for investigators to examine other aspects of learning in these tasks, while also providing certain advantages—or in some cases, disadvantages—compared to the original designs. For instance, a virtual version of the dual-solution plus-maze task has been recently designed for use in humans (Astur et al., 2016; Figure 4E), precluding the cumbersome construction of a maze large enough for participants to walk through (but see also Overman et al., 1996). An exhaustive list of these nuanced versions of the place and response learning tasks would be tedious and perhaps unnecessary. However, a brief list of the more popular and potentially relevant incarnations will be discussed presently.

Water plus-maze tasks: Place and response learning tasks, including the dual-solution task, may be readily conducted in a water plus-maze (e.g. Schroeder, Wingard, and Packard, 2002; Packard and Wingard, 2004; Wingard and Packard, 2008). These tasks are conducted in a manner identical to the appetitive, food-reinforced versions designed by Tolman and Blodgett (Figure 4A–C). However, instead of animals running to retrieve food reward, animals are placed in a plus-maze filled with water and must swim to an invisible escape platform located in one of the goal arms. Thus, in contrast to the appetitive versions of the place and response learning tasks, which provide positive reinforcement (i.e. food reward), the water plus-maze versions of these tasks provide negative reinforcement (i.e. mounting an invisible platform to escape the water). These water-maze versions provide some advantages over the original appetitively reinforced tasks. Whereas animals trained in the appetitive versions need to be food restricted for several days to motivate food foraging behavior, animals trained in the water maze do not need any prior food deprivation. Not having to deprive laboratory animals of food not only saves time (i.e. experiments can start shortly after animals arrive in the laboratory), but this also rules out some potential confounding variables, such as the influence of hunger and food deprivation alone on learning and memory. In addition, animals tend to learn the water maze versions of the task much quicker than the appetitive versions, allowing investigators to complete experiments faster and increase experimental throughput in the laboratory. Finally, the water maze versions depend on the same neurobiological systems as the appetitive versions (Schroeder et al., 2002;

Compton, 2004; Asem and Holland, 2015), suggesting that the learning mechanisms underlying the appetitive and aversive versions of these tasks may be similar.

Conditional T-maze with an auditory or tactile cue: In addition to the standard response learning plus-maze task, response learning may also be conducted in an auditory- or tactile-cued version of the T-maze (Figure 4D). In this task, rats running down a long starting arm are exposed to a discrete stimulus (e.g. a tone in the auditory version; a strip of sandpaper underfoot in the tactile version), which indicates whether a right or left turn will be correct for that trial. For instance, a tone may signal that a left turn at the choice point will lead to the correct, food-reinforced arm, whereas a stint of white noise indicates that a right turn will lead to the correct arm. It is worth noting that, in contrast to the plus-maze tasks, this cued T-maze protocol may only be used to examine S-R response learning and may not be readily employed to examine place learning or the relative use of place and response learning strategies. However, investigators have examined the relative use of goal-directed and habitual responding in this task using reinforcer devaluation procedures. Investigators have demonstrated that, similar to instrumental lever pressing, animals initially express goal-directed running in the T-maze, but shift to habitual running behavior following extensive overtraining (Lin and Liao, 2003; Smith and Graybiel, 2013; Smith et al., 2012).

Place and response learning in other mazes: The present chapter focuses on the plus-maze (or T-maze) versions of the place and response learning tasks; however, it is only fair to mention that place and response learning may also be readily examined in other types of mazes, including the radial arm maze, Morris water maze, and Barnes

maze. In the S-R response learning or "win stay" version of the radial maze (Figure 5A; Packard et al., 1989), four of the eight arms in a radial maze are reinforced and signaled with a light stimulus, and rats may go to each of the illuminated arms twice within a daily training session to retrieve food. In this task, animals presumably acquire an S-R association between the light stimulus and the approach response, whereas entries into the unlit arms are scored as errors. On the other hand, in the place learning or "win-shift" version of the radial maze (Figure 5B), rats may visit each of the eight arms once within a daily training session to retrieve food reward, whereas re-entries into previously visited arms are scored as errors. Importantly, arms containing food are not marked with any proximal cues, and therefore the animal must presumably rely on allocentric spatial cues to determine which arms were already visited and which arms still contain food.

Interestingly, these radial maze tasks, although primarily conducted with rodents, have also been adapted to examine place and response learning in humans. These tasks typically involve computer-generated maze environments that the subject can navigate using a keyboard or joystick (e.g. Bohbot, Iaria, and Petrides, 2004; Bohbot et al., 2007; Banner et al., 2011; Konishi et al., 2013; Horga et al., 2015; Hussain et al., 2016b). However, some investigators have examined place and response learning using a built-to-scale radial arm maze that human participants can walk through (Overman et al., 1996; Figure 5D).

A dual-solution version of the radial arm maze may also be used to gauge the relative use of place and response learning strategies. In a dual-solution version of the radial arm maze designed for humans (Iaria et al., 2003; Figure 5C), participants

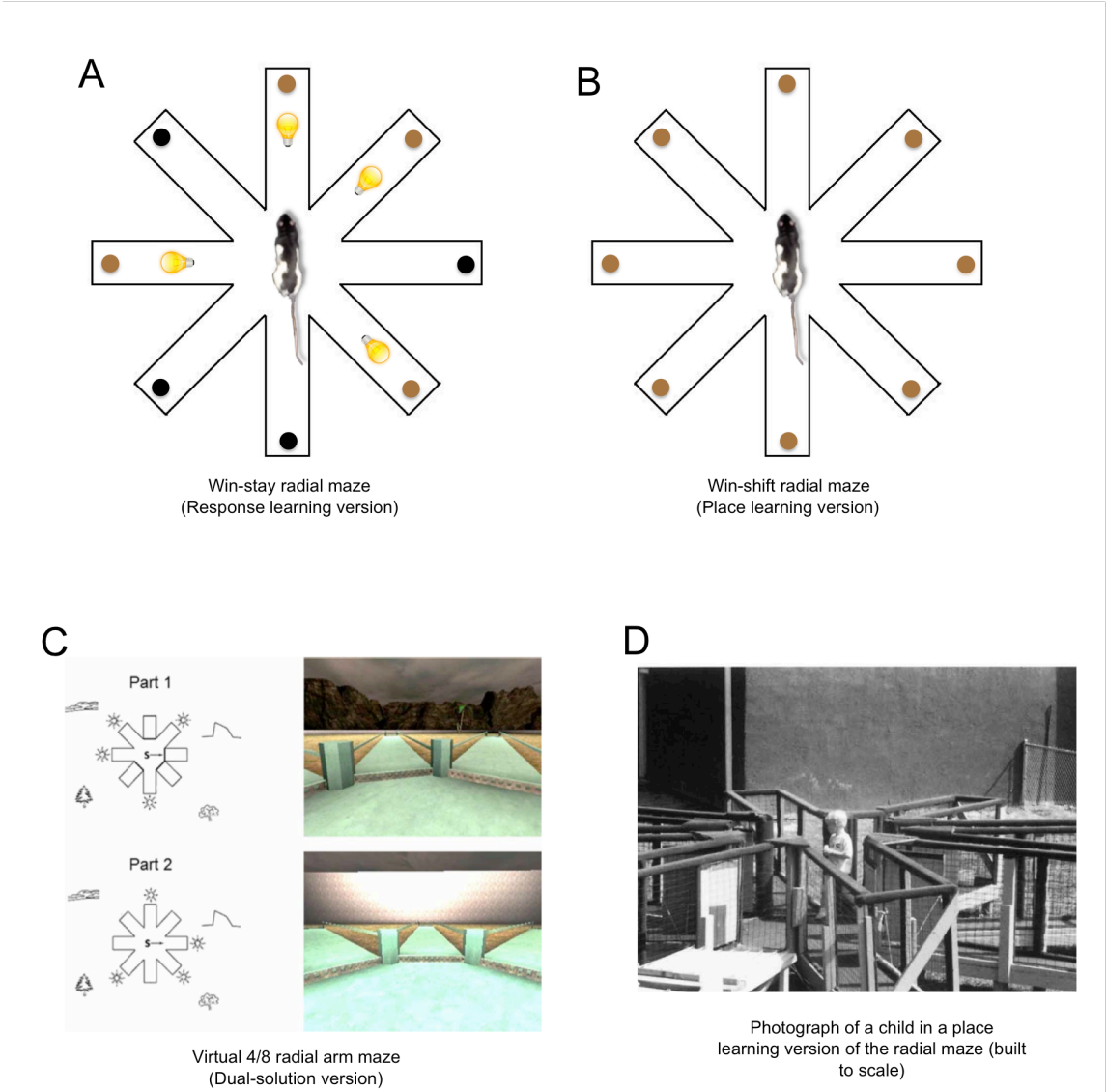

Figure 5. Place and response learning versions of the radial arm maze. See text for details.

navigate a virtual radial arm maze and retrieve reward objects by traveling to the ends of some arms. Given that there are numerous spatial cues in the distal virtual environment,

including mountains and trees, the participants may use the spatial cues to determine which arms contain reward objects. However, the participant may also use an egocentric strategy by learning the sequence of turns leading to the correct arms. To determine which strategy the participants employed, a probe test can be conducted in which walls surround the maze and prevent the subject from using the distal spatial cues. Thus, more errors during the probe test would suggest that the subjects had been using a place learning strategy, whereas few errors would suggest subjects had been using a response learning strategy. Also, and this is one of the advantages of conducting this task with humans, participants may also be debriefed and asked to report how they solved the maze. From these responses, investigators can determine whether the participants had used a place or response learning strategy.

Aside from the radial arm maze, place and response learning may also be investigated using the Morris water maze (Devan and White, 1999; Devan, McDonald, and White, 1999; Lee et al., 2008, 2013). In the standard place learning version of the Morris water maze (Morris, 1984; Figure 6B), rats are released into a circular pool of water from different starting positions and must rely on the allocentric spatial cues in the maze environment to learn the spatial location of an invisible escape platform. In the cued or response learning version of the Morris water maze (Figure 6A), the escape platform is visibly cued so that the animal may acquire an S-R association allowing the cued platform (S) to evoke approach behavior (R). The cued platform also moves to different spatial locations throughout training, making a spatial learning strategy unreliable in this response learning version of the task. In a dual-solution version, a cued

platform remains in the same spatial location across training, allowing animals to acquire either a spatial learning strategy (i.e. go to the same spatial location) or a response learning strategy (i.e. go to the cued platform). Learning strategy may be assessed using a probe trial in which the cued platform is moved to a new spatial location. If the rat

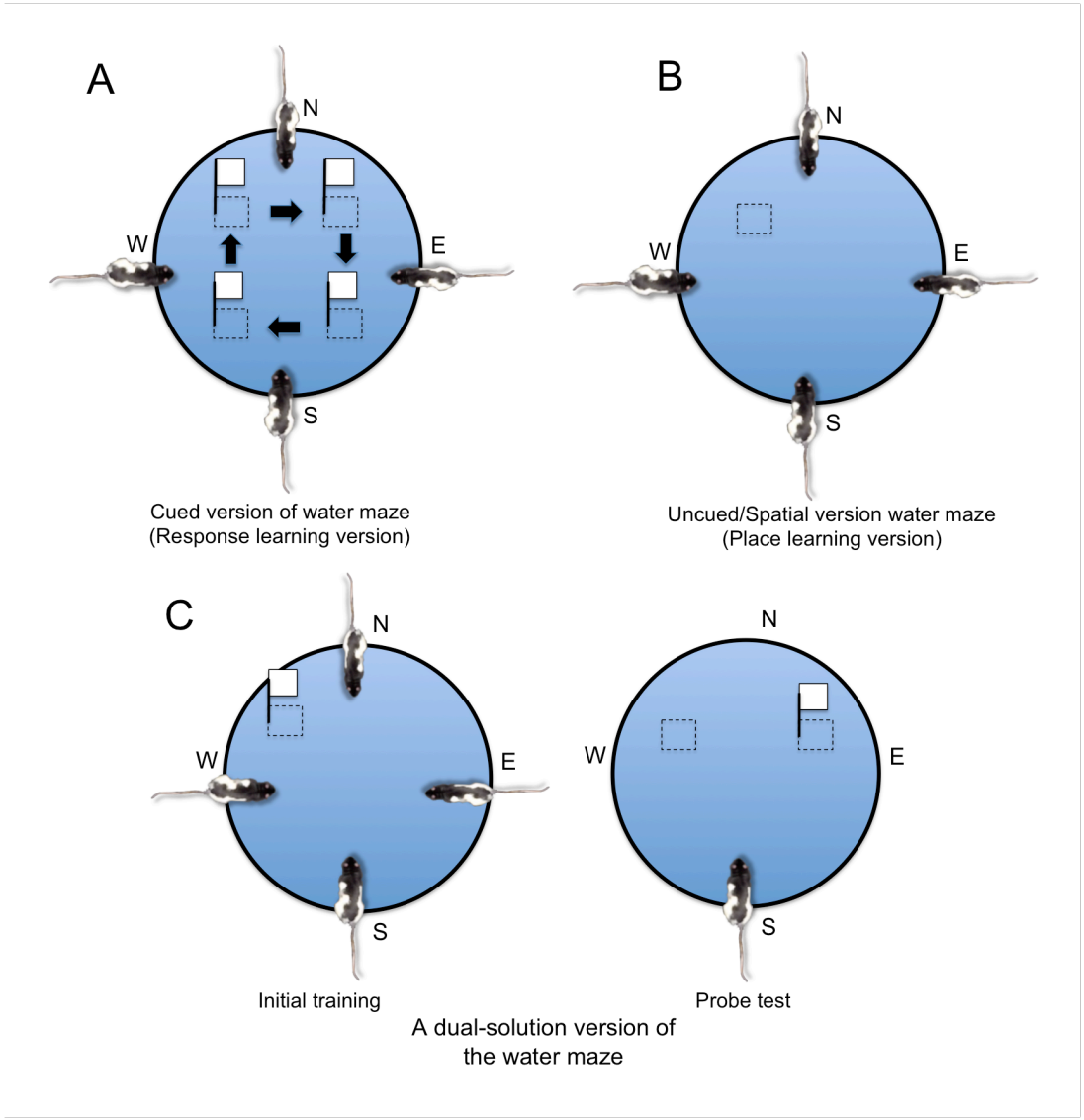

Figure 6. Place and response learning in the Morris water maze. See text for details.

continues to swim to the original spatial location, the rat is believed to have been using a place learning strategy, whereas if the rat follows the cued platform to the new spatial location, the rat is believed to have been using a response learning strategy.

Finally, place and response learning has also been investigated using the Barnes maze (Rueda-Orozco et al., 2008a; Harrison et al., 2006). The Barnes maze is a large circular platform with a series of holes lining the perimeter where one of these holes leads to a small escape compartment underneath the maze. In the standard place learning version, the animal may use distal cues to acquire the spatial location of the escape hole (Barnes, 1979). In the cued or response learning version, the escape hole is located in different spatial locations across training, but is reliably marked by a proximal visual cue (Reiserer et al., 2007). The relative use of place and response learning may also be examined using a dual-solution version of the Barnes maze (not shown), in which the escape hole is located in a consistent spatial location and is also reliably cued using a proximal visual stimulus (Harrison et al., 2006). During a subsequent probe trial, all the holes are blocked, and the proximal stimulus is relocated to a different hole. If animals spend more time near the original spatial location, they are considered to have been using a place learning strategy, whereas if animals spend more time near the hole near the proximal cue, they are assumed to have been using a response learning strategy. The relative use of place and response learning may also be observed by analyzing strategy use during the standard place learning version of the Barnes maze (Harrison et al., 2006; Rueda-Orozco et al., 2008a). If animals run directly to the escape hole, they are considered to be using a place learning strategy. However, if animals run to an

arbitrary hole and explore each adjacent hole in a serial fashion until finding the correct hole leading to the escape compartment, they are considered to be using a response learning strategy.

The place and response learning plus-maze in other species: Although the majority of studies employing the place and response learning tasks have used rats—and to a lesser extent mice and humans—these tasks have been adapted for use across a wide variety of species. The purpose of most of these experiments was to gauge the cognitive mapping abilities or spontaneous use of different navigational strategies across a range of species representing different branches on the phylogenetic tree. These findings may, over time, allow for inferences to be made regarding the evolution of spatial navigation (see Jacobs, 2003; Salas et al., 2003).

Plus-maze versions of the place and response learning tasks—including the dual-solution task—have been employed to examine learning and memory in chickens (Brookshire et al., 1961), terrestrial toads (Daneri et al., 2011), horses (Parker et al., 2009), salamanders (Kundey et al., 2016), and turtles (López et al., 2000; Rodríguez et al., 2002). In addition to studies in terrestrial and amphibious animals, water plus-maze versions of the place and response learning tasks have been readily employed to study memory in a variety of aquatic animals, including sharks (Fuss et al., 2014a,b), freshwater stingrays (Schluessel and Bleckmann, 2005), cuttlefish (Alves et al., 2007), crayfish (Tierney and Andres, 2013), and goldfish (Rodríguez et al., 1994; Salas et al., 1996a,b; Rodríguez et al., 2002; Romaguera and Matioli, 2008; McAroe et al., 2016). Aside from examining normal learning and memory abilities, some studies have used

lesion techniques to examine the neural substrates of place learning in these animals. Lesions delivered to certain areas of the telencephalon, believed to be homologous to the mammalian hippocampus, produce deficits in place learning, but not response learning, in sharks (Fuss et al., 2014a,b), goldfish (Salas et al., 1996a,b; Rodríguez et al., 2002; Romaguera and Mattioli, 2008), and turtles (Rodríguez et al., 2002). These findings demonstrate a similar role for the hippocampal formation in place learning functions across different species, suggesting that the ontogeny of hippocampal spatial memory processing may have an early evolutionary origin.

4.4 Factors that Influence Place and Response Learning in the Plus-Maze

Inspired by the original experiments conducted by Tolman and Blodgett, other experimenters began using these plus-maze tasks to examine cognitive versus S-R views of learning. Proponents of Tolman's cognitive view of learning believed that place learning was more dominant or natural to the animal than response learning, while proponents of Hull's S-R view believed the opposite. This conflict set the stage for the place vs. response learning controversy (Restle, 1957)—that is, the debate over whether animals in the plus-maze are naturally place learners or response learners—and directly motivated the immediate widespread use of the place and response learning plus-maze tasks in the 1940s–1950s. However, similar to the original studies conducted in the laboratories of Tolman and Blodgett, these experiments yielded mixed findings. In some

cases, investigators found place learning to be dominant, whereas in other cases response learning was dominant. The eventual resolution to this conundrum was that either place or response learning could be dominant in these plus-maze tasks, and that whether a particular kind of learning was dominant depended on a host of parametric factors (for reviews, see Restle, 1957; Packard and Goodman, 2013).

In his famous treatise on cognitive maps, Tolman (1948) suggested that rats and humans alike acquire cognitive maps to guide our thoughts and behavior and that cognitive maps vary in size and detail. Tolman believed that large and detailed cognitive maps allow for animals to flexibly generate new routes from novel starting positions and to take shortcuts when shorter paths are suddenly made available. On the other hand, a relatively slim "strip-map" that is lacking in detail may allow the animal to undertake a simple navigational response from point A to point B, but would not allow for animals to take shortcuts or to quickly reach the goal location from a novel starting position. Thus, broad and comprehensive maps may allow for place learning, whereas narrow-strip maps may allow for egocentric response learning in the plus-maze.

Tolman suggested that the relative smallness or bigness of a particular cognitive map may be influenced by a variety of factors. Tolman writes

> …what are the conditions which favor narrow strip-maps and what are those which tend to favor broad comprehensive maps? There is considerable evidence scattered throughout the literature bearing on this question both for rats and for men. Some of this evidence was obtained in

> Berkeley and some of it elsewhere… I can merely summarize it by saying that narrow strip-maps rather than broad comprehensive maps seem to be induced: (1) by a damaged brain, (2) by an inadequate array of environmentally presented cues, (3) by an overdose of repetitions on the original trained-on path and (4) by the presence of too strongly motivational or of too strongly frustrating conditions. (Tolman, 1948, pgs. 206-207)

Although this excerpt pertains directly to the factors influencing the relative narrowness or comprehensiveness of a cognitive map, it also serves as an impressive list of the major factors influencing the relative dominance of place learning or response learning in the plus-maze. The present section provides an overview of the behavioral factors, such as the amount of training (including consideration of reinforcement parameters and passage of time), the visual aspects of the learning environment, and the emotional state of the organism. The extent to which "a damaged brain" also influences learning and memory in the plus-maze will be discussed in a later section pertaining to the neurobiological mechanisms of place and response learning.

4.4.1 Amount of Training

According to the Hullian view of learning, the strength of an S-R habit is partially a function of the number of times that the S has been paired with the R. Therefore, it is reasonable to predict that after limited training an S-R association may be

weak, allowing for other learning mechanisms to guide behavior. This prediction is consistent with the findings from studies using single-solution versions of the place and response learning tasks. That is, as previously mentioned, more trials are required for animals to learn the S-R response learning version of the plus-maze relative to the cognitive place learning version of the task (Tolman, Ritchie, and Kalish, 1947). Moreover, in the dual-solution task, when animals are given a probe trial after limited training, most animals demonstrate a place learning strategy, whereas after extensive training, animals predominantly display response learning on the probe trial (Ritchie, Aeschliman, and Peirce, 1950; Hicks, 1964; Packard and McGaugh, 1996; Packard, 1999). Interestingly, a recent study demonstrated that the shift to response learning may be blocked if the animal is prompted to perform a concurrent working memory problem throughout task acquisition (Gardner et al., 2013). The shift to response learning originally demonstrated in the plus-maze has also been observed in later studies using instrumental lever pressing tasks whereby early in training, lever pressing is guided by cognitive goal-directed memory, and after extensive training, behavior is guided by S-R habit memory (Adams, 1982).

4.4.2 Massed versus Distributed Training

Evidence indicates that place and response learning may also differentially benefit from massed and distributed practice. During massed training, trials are separated by short inter-trial intervals, whereas during distributed (or spaced) training, trials are separated by considerably longer inter-trial intervals. Place learning is acquired

quickly when using a massed training protocol, in which trials are separated by 30 seconds, and slowly when using a distributed protocol, in which trials are separated by 15-30 minutes (Thompson and Thompson, 1949; Wingard, Goodman, Leong, and Packard, 2015). In contrast, response learning is acquired slowly when using the massed training protocol and quickly when using the distributed protocol (Thompson and Thompson, 1949; Wingard et al., 2015). Similar observations have been made in instrumental learning tasks, in which massed training favors cognitive goal-directed responding, and distributed training favors habitual responding (Adams, 1982).

4.4.3 Is It All a Matter of Time?

The above evidence indicates that either an extensive amount of training or a distributed training protocol favors response learning. It is possible, however, that the benefit of both of these factors may be related to passage of time. That is, the greater passage of time that transpires during an extensive number of trials or a distributed training protocol may be the critical factor favoring response learning over place learning. This was examined in our laboratory using a water maze version of the dual-solution plus-maze task (Figure 7). After initial training in the plus-maze (one day of training; 4 trials; 30s inter-trial intervals), one group of animals was given a probe trial 30s after the last training trial, and the other group was given a probe trial 48h after the last training trial. Animals given the 30s-probe trial predominantly displayed place learning, whereas animals given the 48h-probe trial predominantly displayed response learning. That is, the mere passage of time was sufficient to produce response learning.

In another experiment, we examined the possibility that the passage of time favors response learning by allowing for the place learning memory to decay. Animals were trained in a place learning task for one day (4 trials). This amount of training was

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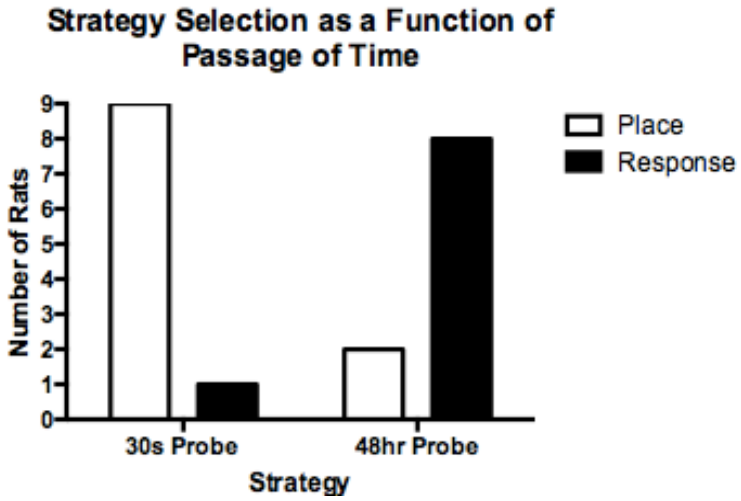

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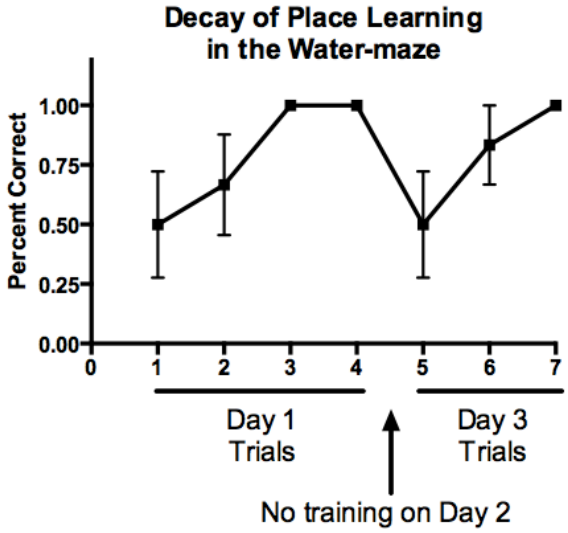

Figure 7. The effect of passage of time on the relative use of place and response learning. See text for details.

sufficient for all animals to achieve 100% accuracy during the last couple trials. After 48h, animals were given 3 more training trials. During the first couple trials on this second day (Day 3) of training, animals performed worse than their terminal accuracies on Day 1. These findings suggest that the spatial memory underlying place learning may decay over 48h, and this may be one mechanism allowing response learning to be the dominant strategy following the passage of time. Whether response learning is less sensitive to memory decay has yet to be examined. It should also be emphasized that the rats in these unpublished experiments were not naïve, and were previously trained in an appetitive response learning task and given extinction training. It is possible that this prior maze experience may have influenced these "passage of time" findings. In order to rule out this possibility these experiments will need to be replicated using naïve animals.

4.4.4 The Visual Aspects of the Learning Environment

Place and response learning in the plus-maze may also be influenced by the visual aspects of the learning environment. In learning environments containing abundant extra-maze visual cues (termed heterogeneous visual surrounds), place learning is achieved faster than response learning, and a place learning strategy is preferred over a response learning strategy in dual-solution versions of the task (Tolman et al., 1946a, 1947; Blodgett and McCutchan, 1948; Blodgett et al., 1949; Tolman and Gleitman, 1949; Galanter and Shaw, 1954; Waddell et al., 1955). Visually heterogeneous learning environments may favor place learning by allowing animals to acquire a cognitive spatial map (Tolman, 1948). In contrast, learning environments

containing few or no extra-maze visual cues (homogenous visual surrounds) allow for response learning to be acquired faster than place learning and lead to the use of response learning strategies over place learning strategies in the dual-solution plus-maze (Blodgett and McCutchan, 1948; Ritchie et al., 1950; McCutchan et al., 1951; Hill and Thune, 1952; Scharlock, 1955). Interestingly, adding extramaze visual cues to make the learning environment more heterogeneous impairs acquisition in a response learning task (Chang and Gold, 2004). It is possible that extra-maze cues may engage the acquisition of a cognitive map and make animals more likely to go to the same place where they found food on a previous trial. This would lead to errors in a response learning task, but would lead to correct responses in a place learning task. On the other hand, a relatively homogenous visual surround would presumably prevent acquisition of a spatial cognitive map, which could (1) eliminate spatial interference and lead to faster acquisition of response learning and (2) greatly impair the subject's ability to encode the spatial location of the reinforcer and therefore impair acquisition in a place learning task.

4.4.5 Emotion

Another factor that profoundly influences place and response learning is stress and anxiety. Behavioral stressors, such as restraint stress or exposure to predator odor, enhance acquisition in the response learning version of the plus-maze and lead to greater use of response learning over place learning in the dual-solution version of the task (Sadowski et al., 2009; Leong and Packard, 2014; Taylor et al., 2014). Chronic restraint and unpredictable shock also lead to greater response learning in other kinds of maze

tasks (Kim et al., 2001; Schwabe et al., 2008). Aside from behavioral stressors, high levels of trait anxiety or hypertension favor response learning in the plus-maze and in a dual-solution version of the Morris water maze (Robertson, Clements, and Wainwright, 2008; Wells et al., 2010; Hawley Grissom, and Dohanich, 2011). Finally, conditioned emotional stimuli (e.g. a tone previously paired with a shock) may also enhance response learning and lead to greater use of response learning strategies over place learning strategies in the plus-maze (Leong, Goodman, and Packard, 2015; Goode, Leong, Goodman, Maren, and Packard, 2016). Presentation of a conditioned emotional stimulus may similarly promote the use of a response learning strategy in a dual-solution Morris water maze task (Hawley et al., 2013).

Systemic infusion of stress hormones (e.g. corticosterone or epinephrine) or anxiogenic drugs (e.g. α-2 adrenoreceptor antagonists yohimbine or RS 79948-197) appears to mimic the effects of trait anxiety and behavioral stressors by enhancing response learning (Packard and Wingard, 2004; Elliot and Packard, 2008; Wingard and Packard, 2008; Packard and Gabriele, 2009; Leong, Goodman, and Packard, 2012). The enhancing effect of corticosterone or RS 79948-197 on response learning may be blocked by concurrent infusion of anxiogenic drugs (Leong et al., 2012; Goodman et al., 2015). Interestingly, the enhancement of response learning following stress/anxiety has not only been demonstrated in rats using plus-maze tasks (Packard and Wingard, 2004), but has also been observed in human participants using response learning or "habit" memory tasks designed for humans (Schwabe et al., 2007, 2008; Schwabe & Wolf, 2009, 2010; Schwabe et al., 2010, 2013; Guenzel, Wolf, and Schwabe, 2014).

The enhancement of response learning may be attributed to the impairing effect of stress/anxiety on spatial memory processing. Infusions of anxiogenic drugs impair acquisition in a place learning version of the plus-maze, and similar doses enhance acquisition of response learning (Wingard and Packard, 2008; Packard and Gabriele, 2009; Sandowski et al., 2009). Consistent with a competitive interaction between memory systems mediating place and response learning (Poldrack and Packard, 2003), stress/anxiety may enhance response learning and lead to greater use of response learning strategies indirectly by impairing acquisition of place learning.

4.4.6 Biological Sex

The potential influence of biological sex on place and response learning has also received some investigation. As reviewed previously, rats typically prefer a place learning strategy in the early stages of training in the dual-solution plus-maze and gradually shift toward a response learning strategy following extensive additional training; however, recent evidence indicates that only male rats display this shift in preference, whereas the strategy preference of female rats depends on estrogen levels (for review see Korol, 2004). Female rats at proestrus (i.e. when estrogen levels are high) predominantly display place learning, whereas female rats at estrus (i.e. when estrogen levels are relatively low) predominantly display response learning (Korol et al., 2004; see also Korol and Kolo, 2002; Zurkovsky, Serio, and Korol, 2011; Zurkovsky, Brown, Boyd, Fell, and Korol, 2007; Zurkovsky, Brown, and Korol, 2006). In contrast, other research has found no preference between spatial and stimulus-response strategies

in female rats even when estrous cycle is taken into account, relative to male rats which continue to show either a place or response learning preference under various conditions (Hawley, Grissom, Barratt, Conrad, and Dohanich, 2012; Grissom, Hawley, Bromley-Dulfano, Marino, Stathopoulos, and Dohanich, 2012; Grissom et al., 2013). In addition, men and women do not differ significantly from each other in terms of strategy preference across a variety of dual-solution tasks (e.g. Iaria et al., 2003; Schwabe et al., 2007; Schwabe et al., 2008; Andersen, Dahmani, Konishi, and Bohbot, 2012; Schwabe and Wolf, 2012).

4.4.7 Resolving the Place vs. Response Controversy

Although early investigators examining place and response learning in the plus-maze were originally concerned with whether a Tolmanian place learning disposition was more dominant or "natural" to the animal than a Hullian response learning disposition, and vice versa, numerous studies have indicated that either disposition may be dominant depending on certain experimental factors. Many of these factors were quickly identified by the first wave of investigators who, inspired by the original Tolman and Blodgett experiments, sought to tackle the heated place vs. response controversy. The ensuing observation that the relative dominance of place and response learning depended on a myriad of experimental variables provided a potential resolution to the debate. In a highly cited review article summing up the findings from this first wave of experiments, Frank Restle, a prominent cognitive psychologist at the time, had this to say:

> There is nothing in the nature of a rat which makes it a "place" learner, or a "response" learner. A rat in a maze will use all relevant cues, and the importance of any class of cues depends on the amount of relevant stimulation provided as well as the sensory capacities of the animal… The writer's general conclusion is that further "definitive" studies of the place-vs.-response controversy, to prove that rats are by nature either place or response learners, would be fruitless… (Restle, 1957, pgs. 226-227)

It is reasonable to infer that Restle's general conclusion that further analysis of the place vs. response question would be "fruitless" was probably espoused by some of his contemporaries. A comment on Restle's review, which was published about a decade later, noted that "since the appearance of Restle's article, few further studies have been published dealing with the place vs. response issue" and that "it is unlikely that the issue will ever be reopened in its earlier form" (Goldstein, Krantz, and Rains, 1965, pg. 229). In hindsight, we can determine that this prediction was correct—investigators were no longer concerned over which kind of learning was more "natural"—however these commentators had failed to foresee just how valuable the place and response learning tasks would become when later investigators began to examine the neural substrates of learning and memory.

4.5 Neural Mechanisms of Place and Response Learning

Consistent with the multiple memory systems hypothesis discussed in Chapter II, place learning and response learning may be achieved through distinct learning mechanisms mediated by different parts of the brain. Thus, these tasks, although once used to test Hullian vs. Tolmanian views of learning, were later employed to examine the neurobiology of different kinds of memory. These tasks are ideal for not only determining what brain regions are involved in cognitive or habit forms of memory, but may also be used to demonstrate double dissociations between the mnemonic functions of different brain regions. For instance a double dissociation may be demonstrated when damage to brain region A impairs place learning, but not response learning, whereas damage to brain region B impairs response learning, but not place learning. A major benefit to using the place and response learning tasks to demonstrate double dissociations is that these tasks involve similar motivational, sensory, and motoric processes, and only differ in terms of their mnemonic requirements. Therefore, if damage to brain region A disrupts acquisition in the place learning task, but not the response learning task, the effect may be attributed to an impairment in the type of memory underlying place learning rather than to some impairment in the non-mnemonic processes shared between the place and response learning tasks.

4.5.1 Brain Regions

Hippocampus and DLS: The two principal brain regions that have been implicated in place and response learning in the plus-maze are the hippocampus and DLS. As mentioned earlier, the differential mnemonic functions of the hippocampus and DLS in mediating cognitive spatial and S-R habit memory, respectively, were originally demonstrated using win-shift and win-stay versions of the eight-arm radial maze, as well as place and response learning versions of the Morris water maze (Packard, Hirsh, and White, 1989; Packard and McGaugh, 1992a,b; Devan and White, 1999; Devan, McDonald, and White, 1999). These original studies provided excellent precedent for examining potential differences in the mnemonic contributions of the hippocampus and DLS to place and response learning in the plus-maze. In the dual-solution plus-maze task, temporary inactivation of the hippocampus leads to the predominant use of a response learning strategy, whereas inactivation of the DLS leads to use of a place learning strategy (Thompson et al., 1980; Packard and McGaugh, 1996; Ramos and Vaquero, 2000; Ramos, 2002; Yin and Knowlton, 2004; Espina-Marchant et al., 2009; Schumacher et al., 2011; Jacobsen et al., 2012; Middei et al., 2004a). Also, intra-ventricular infusion of beta-amyloid protein, which is associated with Alzheimer's disease and hippocampal memory deficits, leads to greater use of response learning over place learning in the dual-solution version of the maze (Ammassari-Teule et al., 2002; see also, Middei et al., 2004b).

In addition, reversible or irreversible lesion of the hippocampus impairs acquisition in the single-solution place learning version of the plus-maze, but not in the

response learning version of the plus-maze (Oliveira et al., 1997; Ramos, 2002; Schroeder et al., 2002; Chang and Gold, 2003a; Compton, 2004; Boucard et al., 2009; Jacobsen et al., 2012). In fact, consistent with a competitive interaction between memory systems, sometimes inactivation of the hippocampus is associated with enhanced acquisition in the response learning plus-maze (Schroeder et al., 2002; Chang and Gold, 2003a; Compton, 2004). In contrast, reversible or irreversible lesion of the DLS impairs acquisition in the single-solution response learning plus-maze, but not in the place learning version of the task (Chang and Gold, 2004; Compton, 2004; Asem and Holland, 2015; Gornicka-Pawlak et al., 2015).

Consistent with the findings from hippocampal and striatal lesion studies, extensive electrophysiological evidence also indicates involvement of the hippocampus and DLS in place and response learning, respectively (Jog et al., 1999; Mizumori et al., 2004; Mulder et al., 2004; Schmitzer-Torbert and Redish, 2004; Barnes et al., 2005; Eschenko and Mizumori, 2007; Schmitzer-Torbert and Redish, 2008; Thorn et al., 2010; Barnes et al., 2011; Thorn and Graybiel, 2014; Hawes et al., 2015; Smith and Graybiel, 2016a). Likewise, studies examining transcription factors and molecular markers of activity also provide evidence that the hippocampus and DLS have critical roles in place and response learning (Colombo et al., 2003; Martel et al., 2006; Daberkkow et al., 2007; Pittenger et al., 2006; Gill et al., 2007; Sung et al., 2008; Dagnas et al., 2013; Kathirvelu and Colombo, 2013; Kathirvelu et al., 2013; Gardner et al., 2016).

Medial prefrontal cortex: Later studies indicated that the prelimbic-infralimbic regions of the medial prefrontal cortex are critically involved in switching from a

hippocampus-dependent place learning strategy to a DLS-dependent response learning strategy, and vice versa, in the plus-maze (Ragozzino et al., 1999a,b; Rich and Shapiro, 2009). In addition, dissociable roles for the infralimbic and prelimbic subregions in strategy selection have been demonstrated using instrumental lever-pressing tasks. Whereas the prelimbic region mediates expression of goal-directed lever pressing, the infralimbic region is selectively involved in habitual lever pressing (Coutureau and Killcross, 2003; Killcross and Coutureau, 2003; Haddon and Killcross, 2011; Schmitzer-Torbert et al., 2015). The infralimbic region has also been recently associated with expression of habit learning in an auditory cued-version of the T-maze (Smith et al., 2012; Smith and Graybiel, 2013). Interestingly, optogenetic inhibition of the infralimbic region disrupts expression of a habitual turning response, but after a new turning response is formed re-inhibition of the infralimbic region disrupts the new habit and compels animals to revert back to the old habit (Smith et al., 2012).

Dorsomedial striatum: As described in Chapter III, the dorsal striatum is functionally heterogeneous. Whereas the DLS mediates S-R habit memory, the DMS mediates cognitive memory mechanisms akin to the hippocampus (for review, see Devan et al., 2011). Consistent with a potential role in spatial learning, DMS lesions impair acquisition in a variety of hippocampus-dependent spatial memory tasks, including place learning versions of the radial maze (Devan, 1997) and Morris water maze (Devan, McDonald, & White, 1999; Devan & White, 1999; Lee, Andre, Pittenger, 2014). In the dual-solution plus-maze, pre-training DMS lesions impair the use of a place learning strategy and lead to greater use of a response learning strategy (Yin &

Knowlton, 2004). The role of the DMS in acquiring/expressing a place learning strategy may be partially attributed to dopaminergic mechanisms (Lex et al., 2011) and synaptic plasticity in the DMS (Hawes et al., 2015).

The DMS has also been critically implicated in reversal learning tasks. In a "response" reversal learning task, rats are initially trained in a response learning version of the plus-maze to make a consistent body-turn response (e.g. turn left) and are subsequently given reversal training in which the opposite body turn (e.g. a right turn) is now reinforced. In the "place" reversal task, rats are initially given place learning in the plus-maze and then receive reversal training in which the opposite goal arm now contains food. Pre-training DMS lesions impair both place and response reversal learning (Pisa & Cyr, 1990; Ragozzino, Jih, & Tzavos, 2002; Ragozzino & Choi, 2004). Likewise DMS lesions spare acquisition of a response learning strategy, but impair the shift from a response learning strategy to a cue-guided strategy and vice versa (Ragozzino, Ragozzino, Mizumori, & Kesner, 2002). Extensive evidence indicates that cholinergic and glutamatergic transmission in the DMS may be required for behavioral flexibility in these tasks (Ragozzino, Jih, & Tzavos, 2002; Ragozzino, 2003; Palencia & Ragozzino, 2004; Ragozzino & Choi, 2004; Tzavos, Jih, & Ragozzino, 2004; Palencia & Ragozzino, 2006; McCool, Patel, Talati, & Ragozzino, 2008; Ragozzino et al., 2009; Watson & Stanton, 2009; Baker & Ragozzino, 2014).

Finally, it is important to mention that the DMS is critically implicated in the relative use of goal-directed A-O learning versus habitual S-R learning. DMS lesions impair goal-directed A-O learning and promote S-R habitual responding in instrumental

lever-pressing tasks (Yin et al., 2005a,b). In addition, the DMS has also been implicated in goal-directed responding in the dual-solution plus-maze task. In this experiment, mice were trained for three weeks so that they predominantly expressed a response learning strategy during the probe trial. Subsequently mice were given devaluation, in which the food reward was devalued with lithium chloride injections, causing illness (De Leonibus et al., 2011). Despite devaluation, mice continued running for the food reward during training trials, indicating S-R/habitual responding. However, during the probe trial, mice that had received reinforcer devaluation decreased the expression of a response learning strategy, suggesting that response learning may only be sensitive to devaluation when the animal is released from a novel start position. In contrast, animals given DMS lesions displayed continued use of a response learning strategy during the probe test, despite devaluation (De Leonibus et al., 2011). This suggests that the DMS may be needed for goal-directed learning in not only instrumental learning tasks, but also in maze learning tasks.

Amygdala: Another brain region implicated in place and response learning is the basolateral complex of the amygdala (BLA). Although the BLA is not critically needed for the acquisition of place or response learning, this brain region may still be involved to the extent that it mediates the emotional modulation of memory in these tasks (Packard, 2009b; Packard and Goodman, 2012; Schwabe, 2013). As described above, stress/anxiety enhances acquisition of response learning and impairs acquisition of place learning. In addition, stress/anxiety leads to the greater relative use of a response learning strategy in the dual-solution plus-maze. The BLA has been critically implicated

in each of these effects. Intra-BLA administration of anxiogenic drugs is sufficient to enhance response learning, impair place learning, and lead to greater use of a response learning strategy in the dual-solution plus-maze (Packard and Wingard, 2004; Elliott and Packard, 2008; Wingard and Packard, 2008). In addition, the enhancement of response learning produced by exposure to predator odor or systemic administration of anxiogenic drugs is blocked by neural inactivation of the BLA (Elliot and Packard, 2008; Packard and Gabriele, 2009; Leong and Packard, 2014). Likewise, enhancement of response learning produced by exposure to a fear-conditioned stimulus (i.e. tone previously paired with shock) is blocked following intra-BLA administration of the β-adrenergic receptor antagonist propranolol (Goode et al., 2016).

Other brain regions: The dorsal striatum, hippocampus, medial prefrontal cortex, and amygdala comprise the major brain regions popularly associated with place and response learning. However, a handful of other brain regions have been implicated in these tasks, but investigations of these alternative neural substrates have not received extensive investigation. Thus, they will only be mentioned briefly. Lesion of the vestibular system promotes response learning in the dual-solution plus-maze, suggesting this system may be involved in place learning (Machado et al., 2014). Lesion of the posterior parietal cortex impairs acquisition in the response learning task, but spares acquisition in the place learning task (McDaniel et al., 1995). On the other hand, posterior cingulate cortex lesions impair the use of a place learning strategy, whereas anterior cingulate cortex lesions impair the use of a response learning strategy in the dual-solution plus-maze (Noblejas and Poremba, 2003). Dopaminergic neurons in the

ventral tegmental area (VTA) and substantia nigra are also needed for acquisition of a response learning strategy in the dual-solution task (Wang et al., 2011). Finally ibotenic acid lesion of the medial septum/vertical limb of the diagonal band completely disrupts strategy preference, leading to a comparable number of place and response learners on probe trials (Cahill and Baxter, 2001). It is likely that a variety of additional brain regions may eventually be implicated in place and response learning. These brain regions may cooperate within neural circuits to mediate the cognitive or S-R habit memory mechanisms underlying successful memory performance and strategy use in these tasks.

4.5.2 Neurotransmitter Systems

Glutamate: Glutamate serves as the major excitatory neurotransmitter in the brain and plays profound roles in synaptic plasticity and memory function, including the mnemonic processes underlying place and response learning. In a dual-solution plus-maze task, pre-training systemic administration of MK-801, an antagonist of the glutamate-sensitive NMDA receptor, does not impair initial acquisition, but decreases the use of a place learning strategy and increases the use of a response learning strategy during a subsequent probe trial (Mackes and Willner, 2006). In another study, direct post-training infusions of glutamate into the hippocampus or DLS also influenced the relative use of place and response learning in the dual-solution plus-maze. As mentioned previously, control animals typically express a place learning strategy after limited training, but then shift to the use of a response learning strategy following

extensive training. However, post-training infusion of glutamate directly into the hippocampus during initial acquisition of the dual-solution task is associated with use of a place learning strategy even after extensive training, suggesting that intra-hippocampal glutamate blocks the shift to response learning (Packard, 1999). In contrast, post-training infusion of glutamate into the DLS during initial acquisition in this task leads to greater use of a response learning strategy during the probe test even after only limited training, suggesting that intra-DLS glutamate accelerates the shift to response learning (Packard, 1999). Also, pre-training or post-training intra-DLS administration of the NMDA receptor antagonist AP5 impairs acquisition/consolidation of memory in the response learning version of the plus-maze (Palencia and Ragozzino, 2005; Leong and Packard, 2013). Finally, deletion of NMDA receptors exclusively from dopaminergic neurons in the ventral tegmental area and substantia nigra impairs the use of a response learning strategy in the dual-solution task (Wang et al., 2011). It is also worth highlighting that investigators have demonstrated similar roles for the hippocampus and DLS glutamatergic systems in place and response learning versions of the Morris water maze (Packard and Teather, 1997; Packard and Teather, 1999).

Dopamine: Extensive evidence has indicated a selective role for dopamine in the hippocampus and DLS in place and response learning, respectively (e.g. Packard and White, 1991; Packard and McGaugh, 1994; Packard et al., 1994; Packard and Teather, 1998; Legault, Smith, and Beninger, 2006). However these studies have been primarily conducted in cognitive and habit versions of the radial maze and Morris water maze, whereas few studies have been conducted in the place and response learning plus-maze

tasks. In one experiment using the response learning version of the plus-maze, systemic administration of either the dopamine D1 receptor antagonist SCH23390 or the dopamine D2 receptor antagonist eticlopride impaired acquisition of response learning (Daniel et al., 2006). Electrophysiological evidence also indicates a role for DLS dopamine in response learning during memory performance in the conditional T-maze (see Figure 4D). In one study (Lemaire et al., 2012), animals received unilateral dopamine depletion in the DLS before training in the conditional T-maze. Although dopamine depletions did not impair acquisition in this task, dopamine depletion increased oscillations in local field potentials in the DLS during maze performance, but only following extensive training in the task (Lemaire et al., 2012). In another study (Eddy et al., 2014), investigators found that wheel-running exercise enhanced acquisition in a tactile/visual version of the conditional T-maze task, and this partially depended on dopaminergic mechanisms. Intra-DLS infusion of the D1 receptor antagonist SCH23390 enhanced acquisition in the conditional T-maze task for the non-exercising rats, but had no effect in the exercising rats. On the other hand, intra-DLS infusion of the D2 receptor antagonist eticlopride impaired T-maze acquisition for the exercising rats, but had no effect on the non-exercising rats. Thus, the mnemonic benefit of exercise in this task may depend on downregulation of D1 receptor activity and upregluation of D2 activity in the DLS (Eddy et al., 2014).

In contrast to the DLS, the DMS as discussed above mediates acquisition of a place learning strategy in the dual-solution plus-maze. Some evidence indicates that the role of the DMS in this kind of learning may partially depend on the dopaminergic

system. That is, dopamine depletion in the DMS leads to the preferential use a response learning strategy over a place learning strategy in the dual-solution plus-maze (Lex et al., 2011). In contrast, and this was also noted in a previous section, dopaminergic neurons in the ventral tegmental area and substantia nigra also play a role in the relative use of place and response learning, in that deleting NMDA receptors from these dopaminergic neurons impairs the use of a response learning strategy in the dual-solution plus-maze (Wang et al., 2011). However, another study indicated that daily exposure to atrazine for one year, which damages the striatonigral dopamine system, did not influence the relative use of place and response learning in the dual-solution task (Bardullas et al., 2013).

Acetylcholine: Several microdialysis studies have indicated that cholinergic mechanisms may be critically involved in place and response learning in the plus-maze. In a dual-solution plus-maze task, acetylcholine release in the hippocampus surges early in training (i.e. when animals typically use a place learning strategy during the probe trial) and remains elevated throughout extended training (Chang and Gold, 2003b). On the other hand, acetylcholine release in the DLS rises steadily throughout training and only asymptotes following extensive training when animals begin to express response learning during the probe trials (Chang and Gold, 2003b). In addition, measures of acetylcholine release both prior to and during dual-solution training indicate that rats using a response learning strategy on a subsequent probe trial had a higher ratio of intra-DLS acetylcholine release relative to intra-hippocampal acetylcholine (McIntyre et al., 2003). This study also observed that acetylcholine release in the hippocampus was

much higher for animals displaying a place learning strategy relative to animals displaying a response learning strategy (McIntyre et al., 2003). Similar findings suggesting a role for hippocampal and DLS acetylcholine release in place and response learning, respectively, have also been obtained in a dual-solution version of a Y-maze task (Pych et al., 2005a). Finally, acetylcholine release in the striatum is higher when animals are trained in the response learning version of the plus-maze task relative to the place learning version of the task, whereas hippocampal acetylcholine release increases similarly when animals are trained in the place or response learning task (Pych et al., 2005b). However, hippocampal acetylcholine release will begin to decrease when the response learning task is conducted in relatively homogenous learning environment with few extra maze spatial cues (Pych et al., 2005b).

Similarly, rats given pyrithiamine-induced thiamine deficiency, which presumably mimics the mnemonic impairments observed in Wernicke-Korsakoff syndrome, display greater response learning in a dual-solution plus-maze task, relative to control animals (Vetreno et al., 2008). Moreover, acetylcholine release in the striatum was greater in the thiamine-deficient rats relative to the control rats (Vetreno et al., 2008). Finally, high levels of choline acetyltransferase—an enzyme involved in acetylcholine synthesis—has been associated with the preferential use of a spatial strategy over a response learning strategy in a dual-solution version of the Morris water maze (Hawley et al., 2015).

A role for acetylcholine in place and response learning has also been observed through ablation of cholinergic neurons in different brain areas. Selective ablation of

cholinergic neurons in the striatum impairs acquisition in a conditional T-maze task, but does not impair place learning in the Morris water maze (Kitabatake et. al., 2003), suggesting that striatal acetylcholine may selectively benefit response learning. As mentioned above, one brain region implicated in place and response learning is the medial septum/vertical limb of the diagonal band (MS/VDB), a brain region that releases acetylcholine into the hippocampus. Although lesion of the MS/VDB disrupts strategy preference in the dual-solution plus-maze task, selective ablation of the cholinergic neurons in this brain region fail to influence place and response learning (Cahill and Baxter, 2001). This suggests that the role of the MS/VDB in strategy preference may be achieved through other neurotransmitter systems not involving acetylcholine. However, in contrast to this study, another experiment indicated that selective ablation of MS/VDB cholinergic neurons enhanced the use of a place learning strategy in the dual-solution Morris water maze (Jonasson et al., 2014). To complicate matters even further, in direct contrast with this study, another experiment indicated that toxic ablation of MS/VDB cholinergic neurons impaired acquisition in a place learning version of the Morris water maze and led to greater use of a response learning strategy over a place learning strategy in a dual-solution version of the task (Janis et al., 1998). The reason for these discrepancies remains undetermined.

Regarding what receptor subtypes may be implicated in the effects of acetylcholine on place and response learning, evidence points to the involvement of muscarinic acetylcholine receptors. Intra-hippocampal infusion of the muscarinic receptor antagonist scopolamine impairs acquisition of the place learning version of the

plus-maze, while sparing acquisition in the response learning version of the plus-maze (Soares et al., 2013). In contrast, intra-DLS infusion of scopolamine impairs acquisition in the response learning plus-maze, while preserving acquisition in the place learning plus-maze task (Soares et al., 2013). In another study using the dual-solution version of the Morris water maze, a higher ratio of muscarinic receptor binding in the hippocampus relative to the DLS was associated with preference for a place learning strategy over a response learning strategy (Grissom et al., 2013). In the same study, investigators found that a higher ratio of muscarinic receptor binding in the amygdala relative to the hippocampus was associated with a response learning strategy in the dual-solution Morris water maze (Grissom et al., 2013).

Cannabinoids: Recent evidence indicates a prominent role for the endocannabinoid system in mnemonic functions of the dorsal striatum and hippocampus (for review, see Riedel and Davies, 2005; Goodman and Packard, 2015a). Studies suggest that either disrupting or enhancing function of the endocannabinoid system may impair response learning. Systemic or intra-DLS infusions of CB1 receptor agonists and antagonists have been associated with impaired acquisition in the response learning plus-maze task (Gerdeman et al., 2006, 2007; Goodman and Packard, 2014; Goodman and Packard, unpublished findings). In a simple T-maze task (in which animals could acquire place learning or response learning), acquisition is impaired following systemic administration of the cannabinoid agonist Δ^9THC (the major psychoactive constituent of marijuana) or intra-DLS administration of cannabinoid receptor antagonist AM251 (Henriksson and Järbe, 1972; Järbe and Henriksson, 1973; Marichal-Cancino et al.,

2015). In addition, intra-DLS AM251 impairs, whereas intra-hippocampal AM251 enhances, reversal learning in the simple T-maze (Rueda-Orozco et al., 2008b). It should be emphasized, however, that since these studies used the simple T-maze task without a subsequent probe trial, it remains unclear whether the kind of learning being targeted was place learning or response learning.

Cannabinoids also participate in place and response learning in other maze tasks. In a response learning version of the Morris water maze, systemic or intra-DLS infusions of the cannabinoid agonist WIN 55,212-2 impairs consolidation of memory (Goodman and Packard, 2014). In the standard Barnes maze task, which can be solved using different learning strategies, post-training intra-DLS infusion of either CB1 receptor agonists or antagonists decreases the use of a response learning strategy and increases the use of a place learning strategy or random strategy (Rueda-Orozco et al., 2008a). Finally, in contrast to acute administrations of cannabinoid drugs, repeated cannabis use may be associated with *enhanced* response learning. A history of cannabis use in humans leads to the preferential use of a response learning strategy in the virtual radial arm maze (Bohbot et al., 2013). The mnemonic effects of cannabinoid drugs or CB1 receptor deletion on response learning have also been demonstrated in instrumental learning tasks (Hilário et al., 2007; Crombag et al., 2010; Nazzaro et al., 2012; Gremel et al., 2016).

Estrogen: The mnemonic effects of estrogen in the place and response learning tasks have attracted a great deal of attention. As mentioned above, whether female rats display place learning or response learning in the dual-solution plus-maze partially

depends on the estrous cycle (Korol et al., 2004). During proestrus (i.e. when ovarian hormone levels are high), female rats preferentially employ a place learning strategy, whereas during estrous (i.e. when ovarian hormones are low) female rats display a response learning strategy (Korol et al., 2004). However, it has been suggested that the influence of estrogen on learning strategy in the dual-solution plus-maze may only occur during the early stages of acquisition. Once the task is well learned, cycling estrogen does not influence the ability to use a place or response learning strategy in the dual-solution plus-maze (Schmidt et al., 2009).

Aside from simply examining the effects of endogenous cycling estrogen, the influence of estrogen on place and response learning may also be demonstrated through estrogen replacement in ovariectomized female rats. Estrogen replacement through systemic administration of estrogen or selective ERα or ERβ agonists enhances acquisition in the place learning version of the plus-maze and impairs acquisition in the response learning version of the plus-maze (Korol and Kolo, 2002; Hussain et al., 2013; Pisani et al., 2015), and similar effects of estrogen have been observed in the place and response learning versions of the eight-arm radial maze (Davis et al., 2005) and open-field tower maze (Lipatova et al., 2014). In addition, estrogen replacement through administration of botanical compounds containing estrogenic properties may also enhance acquisition in the place learning plus-maze and impair acquisition in the response learning plus-maze (Pisani et al., 2012; Neese et al., 2014). Likewise, in a conditional T-maze task, estrogen replacement in ovariectomized rats impairs initial acquisition, yet enhances extra-dimensional set shifting (Lipatova et al., 2016).

Interestingly, the enhancing effect of very low estrogen levels on response learning in both the dual-solution and single-solution plus-maze tasks (e.g. Korol and Kolo, 2002) is blocked in female rats with prior reproductive experience (Hussain et al., 2013).

The effects of estrogen in these tasks may be attributed to estrogen activity in regions associated with place and response learning, i.e. the hippocampus, DLS, and medial prefrontal cortex. Direct infusion of estradiol into the hippocampus or DLS of female ovariectomized rats selectively enhances acquisition of place learning or response learning, respectively, in the Y-maze (Zurkovsky et al., 2007). Also, the increased use of a place learning strategy in the plus-maze during proestrus may be blocked by intra-hippocampal inactivation with muscimol (McElroy and Korol, 2005). Similar roles for the hippocampus and DLS have also been demonstrated using c-Fos immunohistochemistry labeling. Systemic estradiol administration is associated with increased c-Fos expression in the dentate gyrus, DMS, and DLS following acquisition in a place learning version of the plus-maze (Pleil et al., 2011). In contrast, systemic estradiol administration is associated with *decreased* c-Fos expression in the dentate gyrus, DMS, and DLS following acquisition in the response learning version of the plus-maze (Pleil et al., 2011). Importantly, in control animals, acquisition in the response learning task was associated with greater c-Fos expression in the DLS, whereas this increase was blocked by estradiol administration (Pleil et al., 2011). Estradiol-induced decrease of c-Fos activity in the DLS may be one factor contributing to the impairment in response learning following estradiol administration. Aside from hippocampal and striatal regions, evidence also indicates a role for the medial prefrontal cortex in the

effects of estrogen on place and response learning. Infusion of estradiol into the medial prefrontal cortex, but not the anterior cingulate cortex, biases female rats toward the use of a place learning strategy over a response learning strategy in the dual-solution plus-maze (Almey et al., 2014).

Some evidence has suggested that estrogen might interact with the dopamine system to influence place and response learning. Estrogen replacement in ovariectomized rats augments the impairing effect of systemic administration of D2 receptor antagonist eticlopride, but not the impairing effect of D1 receptor antagonist SCH 23390, on acquisition of response learning in the plus-maze (Daniel et al., 2006). The preference for response learning in the dual-solution task during low levels of estrogen may be reversed into a place learning preference following administration of either D1 receptor antagonist SKF 83566 or D2 receptor antagonist raclopride (Quinlan et al., 2008). Moreover, the preference for place learning produced by high levels of estrogen may be eliminated following SKF 83566 or raclopride administration, such that these animals subsequently show no preference for either place or response learning (Quinlan et al., 2008). In another study conducted in the dual-solution plus-maze, the response learning bias in low estrogen animals was reversed into a place learning bias following intra-DLS administration of the D1 receptor antagonist SCH 23390, but not the D2 receptor antagonist raclopride (Quinlan et al., 2013). Conversely, the place learning bias in high estrogen animals was reversed into a response learning bias following intra-DLS SCH 23390, but not intra-DLS raclopride administration (Quinlan et al., 2013). Although intra-DLS raclopride did not reverse strategy preference, a

moderate dose of the drug was sufficient to eliminate strategy preference altogether in the high- and low-estrogen animals, producing a comparable number of place and response learners in these groups. Also observed in this study was that administration of SCH 23390 or raclopride into the nucleus accumbens had no notable effects on strategy preference in high- or low-estrogen animals. Thus, the influence of high or low estrogen levels on strategy preference in the dual-solution task may depend on dopamine receptor activation selectively in the DLS (Quinlan et al., 2013). In a similar study, systemic administration of apomorphine or amphetamine at doses that increase D2 autoreceptor activity reverses the place learning bias into a response learning bias in high-estrogen animals, whereas no effects of these drugs were observed in the low estrogen rats (Hussain et al., 2016a). In addition, amphetamine administration was associated with higher intra-DLS dopamine release in high estrogen rats relative to low estrogen rats, but DLS dopamine release itself was not reliably associated with strategy preference (Hussain et al., 2016a).

Finally, in contrast to studies using rodents in the plus-maze, a recent study in humans using a virtual radial arm maze indicated that high levels of estrogen might be associated with the use of spatial strategy over a response strategy (Hussain et al., 2016b). The reason for this discrepancy remains uncertain.

Others: A variety of other neurotransmitter systems have been implicated in place and response learning, albeit not as extensively as the neurotransmitters described above. For instance, low doses of testosterone lead to greater use of a response learning strategy in the dual-solution plus-maze and Morris water maze tasks, whereas a higher

dose of testosterone leads to the predominant use of a place learning strategy in the dual-solution Morris water maze (Spritzer et al., 2013). In addition, mice lacking delta-opioid receptors display a delay in the acquisition of a place learning strategy in the dual-solution plus-maze and also an enhancement in the acquisition of the response learning version of the plus-maze (Le Merrer et al., 2013). In another study, mice lacking GPR88 receptors were quicker to acquire a dual-solution plus-maze task and also began using a response learning strategy sooner, relative to wild-type mice (Meirsman et al., 2015). Later, when the same group of mice was given reversal training in the dual-solution task, the GPR88 knockout mice were quicker to acquire the reversal and displayed a place learning strategy in a subsequent probe test, whereas the wild-type mice displayed a response learning strategy (Meirsman et al., 2015).

Aside from neurotransmitters, place and response learning may also depend on metabolic substrates, such as glucose and lactate. Increasing striatal function through injections of glucose into the DLS impairs acquisition in a place learning version of the Y-maze (Pych et al., 2006), which is consistent with a competitive interaction between hippocampus and DLS memory systems (Poldrack and Packard, 2003). However, the intra-DLS infusions of glucose were not sufficient to facilitate acquisition in the response learning Y-maze (Pych et al., 2006). In another study, extracellular levels of glucose in the hippocampus were significantly higher when trained in a place learning version of the plus-maze, relative to animals trained in the response learning plus-maze or relative to control animals that received no training (see Gold et al., 2013). A similar pattern was observed for extracellular levels of lactate in the hippocampus, whereby

animals trained in the place learning task had higher hippocampal lactate levels relative to animals trained in the response learning task or animals that received no training (Gold et al., 2013). These substrates may be activated when a task requires hippocampus-dependent spatial processing, and may provide the necessary energy for neurons to meet the demands of the task and encode the memory.

The hippocampus and DLS contain multiple neurotransmitter systems and signaling substrates that have yet to be investigated within the context of place and response learning, including serotonin, GABA, adenosine, and neuropeptide Y, to name a few. In addition, few studies have explored how neurotransmitter systems might interact to influence place and response learning. It is likely that additional neurotransmitter systems, as well as interactions between these systems, may participate in these tasks. Past and current research examining the neurobiology of place and response learning, despite decades of research, has yet to scratch the surface.

4.6 Extinction of Habit Memory: The Present Dissertation Project

The present dissertation project examines the behavioral and neurobiological mechanisms of habit memory, and uses the response learning task as a model system. In some experiments, the place learning task will also be used for dissociation purposes, i.e. to control for other types of learning as well as non-mnemonic factors. In other words, the place learning task is used to verify that the mechanisms which appear to underlie response learning may be unique to habit memory. In addition, the dissociation

methodology was employed to verify that the influence of parametric and neurobiological manipulations on response learning may be attributed to their effects on habit memory rather than to some non-mnemonic factor shared between the place and response learning tasks.

Response learning in the plus-maze may be viewed as an exemplar of habit memory given that memory performance is guided by an autonomous turning response. Response learning is not sensitive to reinforcer devaluation (Sage and Knowlton, 2000; Lin and Liao, 2003; Leonibus et al., 2011; Smith et al., 2012; Smith and Graybiel, 2013) or to the allocentric spatial environment (e.g. Packard and McGaugh, 1996). In addition, acquisition in the response learning task is critically dependent on a region of the brain known to be critical for habit formation in a multitude of different tasks, i.e. the DLS. Thus, the response learning plus-maze task may be a suitable paradigm to study extinction of DLS-dependent habit memory.

The experiments described in the present dissertation examined the mechanisms underlying extinction of DLS-dependent response learning on three levels, constituting the three aims of the project. Aim 1 examined the behavioral mechanism; Aim 2, the neurobiological substrate; and Aim 3, the neurotransmitter mechanism.

In Aim 1, Experiment 1 examined whether an animal needs to make the unrewarded response for extinction of response learning to occur, or if knowledge that the goal locations no longer contain food contributes to extinction of response learning. This will be investigated by using a latent extinction procedure. Experiment 2 verified that the parameters used for latent extinction in Experiment 1 may be effective at

producing extinction in a place learning task. In Aim 2, Experiment 3 examined whether extinction of response learning depends on DLS function. Experiment 4 examined whether the role of the DLS in extinction is selective to response learning or whether it might also be implicated in extinction of place learning.

In Aim 3, Experiment 5 examined whether extinction of response learning is subserved by NMDA receptor activity in the DLS using the NMDA receptor antagonist AP5. In contrast, Experiment 6 examined whether increasing NMDA receptor activity with the NMDA receptor agonist D-cycloserine *enhances* extinction of response learning.

CHAPTER V.[1]

AIM 1: BEHAVIORAL MECHANISMS OF HABIT MEMORY EXTINCTION

5.1 Introduction

After training a rat in a maze to retrieve food reward from a particular goal arm, memory performance may be readily extinguished by removing the food reinforcer, and extinction learning becomes evident when the animal suppresses the original running approach response. However, the nature of *how* the new extinction memory is achieved or *what* information constitutes this extinction memory is not always clear. It is possible that consistent with Tolman's cognitive view of learning (Tolman, 1932), the rat acquires a change in expectation, i.e. that the food reinforcer is no longer available in the goal arm. Thus, when the animal is returned to the original starting position, the animal recalls that the food is no longer available, and therefore does not run to the goal arm as readily. The S-R learning theory of extinction, on the other hand, suggested that mechanisms relating to response-produced inhibition may be involved. For instance, according to Hull's S-R theory of extinction, an inhibitory association may materialize between the stimuli in the learning situation (S) and the running approach response (R). Thus, following extinction training when an animal is returned to the original starting position, the animal does not run to the goal arm as readily, because cues in the learning

[1] Reprinted from "The memory system engaged during acquisition determines the effectiveness of different extinction protocols" (Goodman and Packard, 2015a) under the terms of the Creative Commons Attribution License (CC BY). Originally published in *Frontiers in Behavioral Neuroscience,* 9, 314, Copyright [2015] by Goodman and Packard.

environment suppress the approach response. Importantly, according to the S-R view of extinction, an animal must have the opportunity to perform the original to-be-extinguished behavior in order for the inhibitory S-R association to be formed. Performance of the original response, however, is not necessary in Tolman's cognitive view of learning. There is evidence in favor of both the cognitive and S-R theories of extinction (Seward and Levy, 1949; Rescorla, 1993); however, it possible that each theory might only account for extinction in some learning situations.

Learned behavior in the straight alley maze, a maze in which rats learn to traverse a runway for food reward located at the opposite end of the maze, may be extinguished using two distinct protocols. In a typical "response extinction" protocol, rats are placed in the same starting position as during training, but with the food reward at the opposite end of the maze removed. Thus, during response extinction trials, animals can execute the running approach response, only now this response leads to an empty food well. Importantly, this kind of extinction training may be conducive to the proposed S-R mechanisms of extinction, given that the animal has the opportunity to perform the original approach response. In contrast, during "latent extinction," rats are confined to the original goal location with the empty food well. Thus, animals cannot execute the running approach response. Nevertheless, this kind of extinction training may be conducive to Tolmanian mechanims of extinction, given that animals have the opportunity to form a new learned association between the goal location and absence of reinforcement. As emphasized in Chapter II, the effectiveness of latent extinction figured prominently in learning theory, because it demonstrated that—in contrast to the

Hullian S-R view of extinction (Hull, 1943; Hull, 1952)—a subject does not need to make the previously acquired response for extinction to occur (Seward and Levy, 1949; Deese, 1951; Moltz, 1955; Denny and Ratner, 1959; Dyal, 1962; Clifford, 1964).

The effectiveness of typical response extinction is easily explained through classical S-R models of extinction learning, whereas latent extinction has summoned heated debates between proponents of expectancy theory and proponents of a neo-Hullian view involving the fractional anticipatory approach response (Moltz, 1957; Deese, 1967). Although the precise mechanisms underlying latent extinction have yet to be completely elucidated, evidence from our laboratory indicates that latent extinction indeed depends on a dissociable neural system. In the straight-alley maze inactivation of the hippocampus, but not the DLS, impairs latent extinction (Gabriele and Packard, 2006; Gabriele, 2008). In contrast, inactivation of the DLS, but not the hippocampus, impairs response extinction (Gabriele and Packard, 2006; Gabriele, 2008). A corollary to the contention that these extinction protocols depend on operatively and anatomically distinct learning systems is that response and latent extinction may not be equally effective across all learning situations. For instance, if a critical feature needed for latent extinction mechanisms to occur is absent from the learning situation, then it is reasonable to hypothesize that latent extinction would not be effective, whereas response extinction could still work.

Latent and response extinction protocols may be useful in determining what mechanisms are involved in extinction of specific kinds of memory. For instance, if latent extinction effectively produces a response decrement in one task, then the

particular kind of memory being extinguished in that task may be susceptible to changes in expectation. On the other hand, if in another task latent extinction proves ineffective, while response extinction remains effective, then it is possible that the animal needs to make the previously acquired response in order for that particular kind of memory to be extinguished.

5.1.1 Experiment 1

The present experiments were designed to examine whether an animal needs to perform the original behavior for extinction of response learning to occur. In experiment 1, rats were trained in a response learning task dependent on DLS-dependent habit memory (Chang and Gold, 2004; Palencia and Ragozzino, 2005; Asem and Holland, 2015), in which rats were reinforced to make a consistent egocentric body-turn at the maze choice point. Subsequently, animals were given latent extinction, response extinction, or no extinction training. Initial memory performance in the response learning task is presumably guided by automatic execution of the egocentric turning response and not by knowledge—or *expectation*—that a particular spatial location contains reinforcement. In fact, considerable evidence suggests that spatial information interferes with acquisition in the response learning task (Poldrack and Packard, 2003).

As noted, latent extinction produces a response decrement in some tasks by generating a new expectation that the goal location no longer contains reinforcement. Thus, it is reasonable to hypothesize that latent extinction may not be effective at producing extinction in the response learning task, because memory performance in this

task is not guided by expectation that the goal arms contain food reinforcement, but rather it is guided by an egocentric turning response. Thus, the changes in expectation acquired through the latent extinction protocol would not be relevant to the original response learning memory. In contrast, it is reasonable to predict that response extinction, on the other hand, will be effective at producing extinction in the response learning task. Consistent with the S-R view of extinction learning, the response extinction protocol should allow for direct inhibition of the original response and therefore have the capacity to target the egocentric behavior that represents response learning.

5.1.2 Experiment 2

To determine whether the parameters used for latent extinction in Experiment 1 would be effective for targeting another kind of memory, we conducted a positive control experiment in which animals received training in a place learning task. In this place learning version of the plus-maze, which is dependent on hippocampal function (Schroeder, Wingard, and Packard, 2002; Compton, 2004), rats were reinforced to approach a consistent spatial location. Following initial acquisition, animals were given latent extinction, response extinction, or no extinction training. Importantly, the place and response learning tasks employed in experiments 1 and 2 share similar motoric, sensory, and motivational requirements, and may only differ in terms of their mnemonic requirement. Therefore, if we see a difference in the effectiveness of latent and response

extinction protocols across these tasks, the differences may be readily attributed to the distinct kinds of memory that are being extinguished rather than non-mnemonic differences shared between the two tasks.

In contrast to the response learning task, memory performance in the place learning task is presumably guided by knowledge that a spatial location contains reinforcement. Thus, it is reasonable to predict that confining the animal to the goal location without reinforcement (i.e. latent extinction) should produce a new expectation that effectively competes with the original place learning memory, thereby producing a response decrement. Response extinction could also be effective at targeting the place learning memory directly inhibiting the turning response(s) leading to the original goal location. Response extinction could also be effective by allowing the animals running to the original goal location to form an association between the goal location and the absence of reinforcement, similar to latent extinction. However, this alternative spatial mechanism for response extinction is not likely given that response extinction is effective in the absence of extramaze visual cues and is not impaired following lesions of the hippocampus-dependent spatial memory system (Gabriele and Packard, 2006).

5.2 Method

5.2.1 Subjects

The subjects were 46 male Long-Evans rats weighing 375-425 g upon arrival. Animals were subsequently food-restricted and maintained at 85% of the their ad lib

weight throughout all behavioral procedures. Water was provided ad libitum. Animals were housed individually in a temperature-controlled vivarium with a 12 h light-dark cycle (lights on at 7AM), and all behavioral procedures were conducted during the light phase of this cycle. Age, weight, and housing conditions did not differ between animals in experiments 1 and 2. Animal use in this study was carried out in accordance with the ethical guidelines of the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University. The protocol was approved by IACUC.

5.2.2. Apparatus

An eight arm radial maze was modified by removing four of the original arms to create a plus-maze configuration consisting of north, south, east, and west arms. The arms of the cross maze measured 60 X 9 cm, and the center platform of the maze connecting the four arms measured 40 cm in diameter. At the end of each arm was a recessed food well. A clear Plexiglas cross-shaped structure was placed in the center of the cross maze, serving as the intersection of the four arms. A separate Plexiglas divider was used to block off the arm opposite to the start arm for each trial, creating a T-maze configuration that could be adjusted between trials. The maze was situated in a room with multiple extra maze cues, including posters, a door, a cabinet, and a table.

5.2.3 Behavioral Procedures

Maze habituation: Before maze training, animals in experiments 1 and 2 were given two days of habituation to the maze. For each day of habituation, a rat was placed

on the maze apparatus (from the north arm on day 1 and from the south arm on day 2) and was given 5 minutes to explore the maze. No food was located on the maze at this time. Immediately after the 5 minutes, each rat was removed from the maze and placed in a holding container with 3 Froot Loops cereal pieces (Kellog's). Rats were monitored to confirm consumption of the Froot Loops.

Maze training: Maze training began 24 h following the last day of habituation and lasted 8 days. For the first 2 days of training, animals were given 6 trials per day, and for the remainder of training animals were given 15 trials per day. The maze was rotated 90º after every two trials to discourage the use of intramaze cues. A wide-angle digital camera was fixed over the maze and attached to a computer monitor (only visible to the experimenter) allowing for a clear aerial view of arm entries, and a stopwatch was used to record latencies during task performance.

In experiment 1, animals (N = 25) received training in a response learning version of the plus-maze task whereby animals were reinforced to make a consistent egocentric body-turn response at the maze choice point (Leong, Goodman, and Packard, 2012; Goodman and Packard, 2014; Leong, Goodman, and Packard, 2015; Wingard, Goodman, Leong, and Packard, 2015). Animals were released from north and south starting positions (counterbalanced) throughout training. When animals began in the north arm, the food reward (1/2 Froot Loop) was located in the recessed food well of the east arm. When animals began in the south arm, the food reward was located in the west arm. Thus, regardless of the starting position, animals were reinforced to make a left body-turn response at the choice point to receive food reward. Learning in this task

constitutes an exemplar of egocentric/S-R learning mediated by the DLS (Packard and McGaugh, 1996; Chang and Gold, 2004; Palencia and Ragozzino, 2005; Asem and Holland, 2015; for reviews, see Packard, 2009; Goodman and Packard, in press).

In experiment 2, animals (N = 21) received training for 8 days in a place learning version of the plus-maze task whereby animals were reinforced to approach a consistent spatial location. At the start of each training trial, the animal was placed on the north or south arm facing the outside of the maze (the start arm sequence was counterbalanced across training), and the food reward (1/2 Froot Loop) was always located in the recessed food well of the east arm. This place learning protocol presumably compelled rats to acquire a cognitive map of the learning environment that enabled them to guide behavior from different starting positions to the correct spatial location. Extensive evidence indicates that spatial learning in the plus-maze critically involves hippocampal function (Packard and McGaugh, 1996; Packard, 1999; Schroeder, Wingard, and Packard, 2002; Colombo, Brightwell, and Countryman, 2003; Compton, 2004; Jacobson, Gruenbaum, and Markus, 2012).

For each training trial in experiments 1 and 2, if the animal made an initial full-body entry into the correct arm (i.e. the arm containing the food), the trial was scored as correct. If the animal made an initial full body entry into the incorrect arm, the trial was scored as incorrect. A trial ended once the animal found the food or after 120 seconds had elapsed. When finding the food, the animal was allowed to finish eating before being removed from the maze and placed in an opaque holding container for a 30 second

intertrial interval (ITI). The percentage of correct trials and the latency to reach the correct food well were used as measures of acquisition.

Extinction. Extinction was conducted 24 h after the last day of maze training and lasted 3 days. No food was located in the maze throughout extinction training. The maze was rotated 90º after every 2 trials to prevent the use of intramaze cues.

In experiment 1, animals that previously received response learning were subsequently assigned to response extinction (n = 6), limited latent extinction (n = 6), extended latent extinction (n = 6), or "no extinction" control (n = 7) groups. Groups were matched on average latency and percent correct responses during the last 3 days of acquisition. Response extinction was conducted over 3 days (10 trials per day). For each trial of response extinction, animals were started from the north or south arm and were given the opportunity to run to the previously correct food well. An animal was removed from the maze after reaching the previously correct food well or after 120 seconds had elapsed. For each trial, if the animal made an initial full-body entry into the previously correct arm and ran directly to the food well, the trial was identified as "perseverative." A trial was not considered perseverative if the animal at any point made an entry into the incorrect arm or failed to enter either the correct or incorrect arm within 120s. After each trial the animal was removed from the maze and placed in an opaque holding container for a 30s ITI. For limited and extended latent extinction (conducted over 3 days), animals were confined to the east or west goal arm for 60s for each trial with the sequence of goal arm confinements mimicking the counterbalanced sequence of food locations throughout initial response learning. Animals were confined

to the goal locations using a Plexiglas shield secured 20 cm from the end of the maze arm. For each day of limited latent extinction, animals received 10 trials (5 trials on each arm). The parameters for limited latent extinction were chosen based on previous evidence indicating that 10 latent extinction trials per day produced extinction in the straight alley (Gabriele and Packard, 2006). However, given that latent extinction trials had to be divided between east and west goal arms, this only permitted 5 trials on each arm per day. In order to allow for 10 trials on each arm, an additional group was given extended latent extinction, in which animals received 20 trials (10 trials on each arm) per day. For the "no extinction" control group, animals were not placed in the maze for the 3 extinction days, but rather remained in their holding containers for the duration of an extinction session, i.e. while animals in the latent and response extinction groups were receiving extinction training.

In experiment 2, rats that were previously given place learning were subsequently assigned to response extinction (n = 7), latent extinction (n = 7), or "no extinction" control (n = 7) groups. Groups were matched on average latency and percent correct responses during the last 3 days of acquisition. The behavioral procedures for response extinction and no extinction control groups were identical to that described for Experiment 1. The behavioral procedure for latent extinction was adapted from previous work from our laboratory indicating the effectiveness of latent extinction in the straight alley maze (Gabriele and Packard, 2006, 2007; Gabriele, Setlow, and Packard, 2009). For each trial of latent extinction, an animal was confined to the previously correct goal arm (i.e. the east arm for the place learning task) for 60s using a Plexiglas shield secured

20 cm from the end of the maze arm. After each trial, the animal was placed in an opaque holding container for a 30s ITI.

Extinction probes: 24 h following the last day of extinction, all animals in experiments 1 and 2 were given 4 probe trials. No food was located in the maze for the extinction probe trials. For each probe trial, an animal was released from the north or south arm (start arm sequence: SNNS), and after reaching the previously correct food well or after 120s had elapsed, animals were removed from the maze and placed in an opaque holding container for a 30s ITI. The maze was rotated 90º after every 2 trials. Latency to reach the previously correct food well and the number of perseverative trials (see above) were recorded and used as measures of extinction. The experimenter conducting the probe trials and scoring the animals was blind to the experimental conditions.

5.3 Results

5.3.1 Experiment 1

Initial acquisition: Initial acquisition of the response learning task is depicted in Figure 8. A two-way repeated measures 4 X 8 ANOVA (Group X Day) computed on percentage of correct turning responses over the course of training (Figure 8A) indicated a significant main effect of Day ($F(7, 147) = 23.74$, $p < .001$), but no effect of Group ($F(3, 21) = .224$, $p = .878$) and no Group X Day interaction ($F(21, 147) = .753$, $p = .771$). Similarly, a two-way repeated measures 4 X 8 ANOVA (Group X Day) computed on

latency (Figure 8B) also indicated a significant effect of Day ($F(7, 147) = 95.52$, $p < .001$), no effect of Group ($F(3, 21) = .330$, $p = .800$), and no Group X Day interaction ($F(21, 147) = .88$, $p = .620$). These results indicate that all groups acquired the task about equally. Therefore, any subsequent differences between groups during extinction may not be readily attributed to differing rates of initial task acquisition.

Response extinction: Figure 9 depicts learning over the course of extinction training for animals in the "response extinction" group. Tests of within-subjects contrasts computed on number of perseverative trials (Figure 9A) for extinction days 1-3 revealed a significant linear effect of Day ($F(1, 5) = 24.98$, $p = .004$), indicating that the number of perseverative trials decreased over the course of response extinction training. In addition, within-subjects contrasts computed on latency (Figure 9B) also revealed a significant effect of Day ($F(1, 5) = 23.90$, $p = .005$), indicating that latency increased over the course of response extinction training.

Extinction probes: The results from the extinction probe trials are depicted in Figure 10. To assess the effectiveness of the different types of extinction training for each group, comparisons were made between the probe day and the last day of initial acquisition. The first 4 trials (versus the last 4 trials) of the last acquisition day were selected for this comparison based on the observation that during initial acquisition, animals were typically slower and more likely to make errors for the first few trials of each training day versus the final training trials of the previous day (see Figure 8C–D). Therefore, it was reasonable to expect that the extinction probe trials would also have higher latencies and more errors than the terminal trials of the last acquisition day,

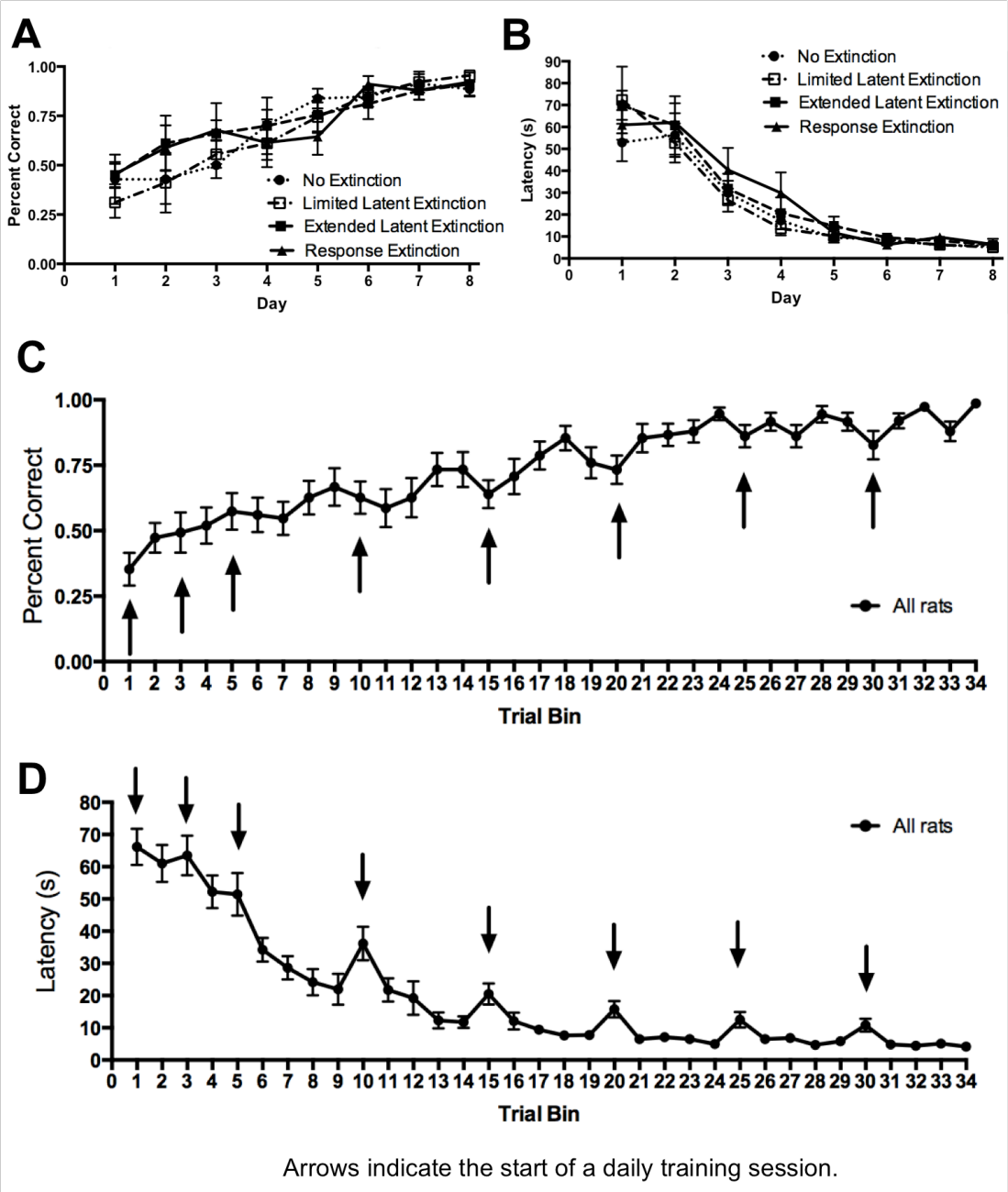

Figure 8. Acquisition of DLS-dependent response learning in the plus-maze. A-B. The percentage of correct turns increased (**A**) and the latency to reach the correct food well decreased (**B**) over the course of training in the response learning task. There were no differences between groups, suggesting all groups acquired the task about equally. **C-D.** All groups were combined, and the trials of each day were averaged into trial bins (1 trial bin = 3 trials). Animals were more likely to make incorrect turns (**C**) and were slower (**D**) on the first few trials of a given training day versus the last few trials of the previous day.

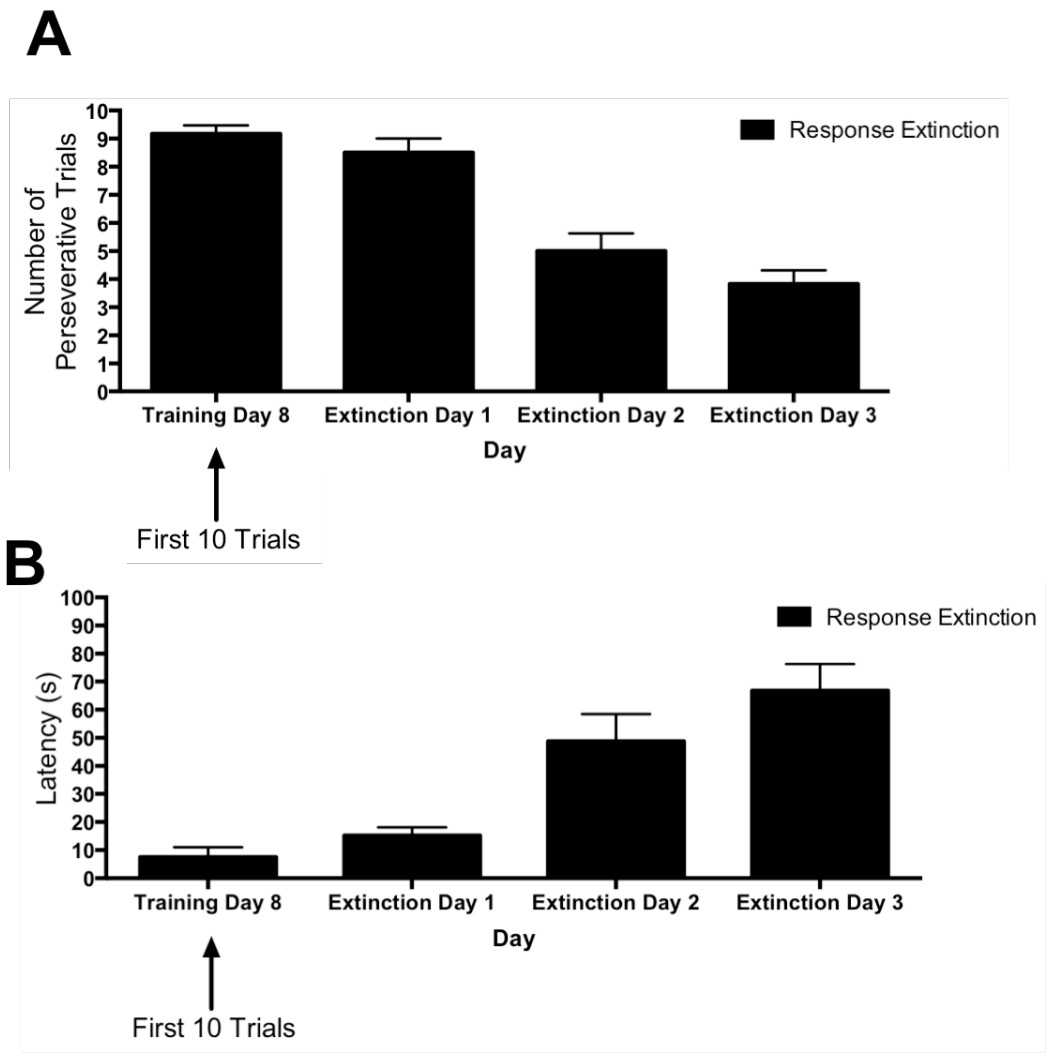

Figure 9. Response extinction of DLS-dependent response learning. A-B. For animals in the response extinction group, the number of perseverative trials decreased (**A**) and latency increased (**B**), indicating the effectiveness of response extinction training.

regardless of whether an extinction protocol was effective. Thus, for a more accurate measurement of the effectiveness of each extinction protocol, we compared the extinction probe trials with the *first* 4 trials of the final acquisition day.

A two-way repeated measures 4 X 2 ANOVA (Group X Day) was computed for number of perseverative trials on the last acquisition day (i.e. training day 8; first 4 trials) and the extinction probe day (Figure 10A). Results indicated a significant main effect of Group ($F(3, 21) = 3.73$, $p = .027$), a significant effect of Day ($F(1, 21) = 7.66$, $p = .012$), and a significant Group X Day interaction ($F(3, 21) = 4.48$, $p = .014$). Multiple pairwise comparisons using Fisher's LSD test indicated that there were no significant differences in number of perseverative trials between groups on the last acquisition day. This is consistent with data presented above indicating that the groups did not differ during initial task acquisition. For animals in the "response extinction" group, Fisher's LSD test indicated that there was a significant decrease in the number of perseverative trials from the last acquisition day ($M = 3.50$) to the probe day ($M = 1.33$), $p < .001$. No other groups showed a significant change in number of perseverative trials between the last acquisition day and the probe day. On the extinction probe day, Fisher's LSD test indicated that the response extinction group ($M = 1.33$) displayed a significantly lower number of perseverative trials than animals in the no extinction control group ($M = 3.23$), $p < .001$. Number of perseverative trials for the limited latent extinction group ($M = 3.00$) did not differ from the no extinction group, $p = .642$. In addition, perseverative trials for the extended latent extinction group ($M = 3.17$) did not differ from the no extinction group, $p = .790$. There was a significantly lower number of perseverative

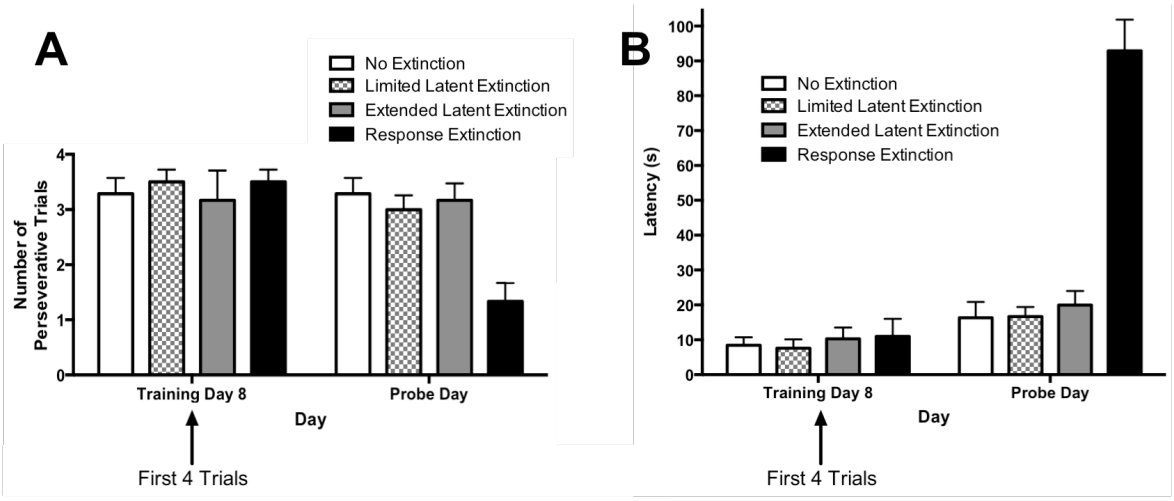

Figure 10. Extinction probe trials in the DLS-dependent response learning task. A. There were no differences between groups in perseveration during the first few trials of the last acquisition day (i.e. training day 8). Only the response extinction group displayed a decrease in number of perseverative trials from the last acquisition day to the probe day. On the probe day, the response extinction group displayed lower perseveration than all other groups. The latent extinction groups (limited and extended) did not differ in perseveration from the no extinction control group on the probe day. **B.** There were no between-group differences in latency on the last training day. All groups increased latency from the last acquisition day to the probe day. On the probe day, the response extinction group had higher latency than all other groups. Latency was not higher in the latent extinction groups (limited and extended), relative to the no extinction group. Results indicate that response extinction was effective and latent extinction was ineffective at extinguishing memory of DLS-dependent response learning.

trials in the response extinction group versus the limited latent extinction group, $p < .001$, and the extended latent extinction group, $p < .001$.

A two-way repeated measures 4 X 2 ANOVA (Group X Day) was computed for latency on the last acquisition day (i.e. training day 8; first 4 trials) and the extinction probe day (Figure 10B). Results indicated a significant main effect of Group ($F(3, 21) = 22.00$, $p < .001$), a significant effect of Day ($F(1, 21) = 183.9$, $p < .001$), and a

significant Group X Day interaction ($F(3, 21) = 81.57$, $p < .001$). Multiple pairwise comparisons using Fisher's LSD test indicated that there were no significant differences in latency between groups on the last acquisition day. Comparing the mean latencies between the last acquisition day and the probe day for each group indicated a significant increase in latency between the 2 days for all groups: no extinction (last acquisition day $M = 8.46$, probe day $M = 16.32$, $p = .049$), limited latent extinction (last acquisition day $M = 7.58$, probe day $M = 16.67$, $p = .037$), extended latent extinction (last acquisition day $M = 10.29$, probe day $M = 19.96$, $p = .027$), and response extinction (last acquisition day $M = 11.00$, probe day $M = 92.92$, $p < .001$). On the probe day, Fisher's LSD test indicated that latency for the response extinction group ($M = 92.92$) was significantly higher than latency in the no extinction control group ($M = 16.32$), $p < .001$. Latency did not differ significantly between limited latent extinction ($M = 16.67$) and the no extinction control group, $p = .957$, and latency also did not differ between extended latent extinction ($M = 19.96$) and the no extinction control group, $p = .567$. Response extinction latency was significantly higher than latency in limited latent extinction, $p < .001$, and extended latent extinction groups, $p < .001$.

Taken together, the results of experiment 1 indicate that following acquisition in the response learning task, animals given response extinction displayed higher latency and lower perseveration during the extinction probe trials, relative to animals given no extinction. In contrast, animals given limited or extended latent extinction protocols did not differ significantly in latency or perseveration from animals given no extinction. The results suggest that in contrast to typical response extinction, latent extinction

protocols may not be effective at extinguishing memory in a DLS-dependent response learning task.

5.3.2 Experiment 2

Initial acquisition: Initial acquisition of the place learning task is depicted in Figure 11. A two-way repeated measures 3 X 8 ANOVA (Group X Day) computed on percentage of correct turning responses over the course of training (Figure 11A) indicated a significant main effect of Day ($F(7, 126) = 22.22$, $p < .001$), but no effect of Group ($F(2, 18) = .15$, $p = .860$) and no Group X Day interaction ($F(14, 126) = 1.51$, $p = .118$). Likewise, a 3 X 8 ANOVA (Group X Day) computed on latency (Figure 11B) indicated a significant effect of Day ($F(7, 126) = 52.41$, $p < .001$), but no effect of Group ($F(2, 18) = .00$, $p = 1.00$) and no Group X Day interaction ($F(14, 126) = 1.47$, $p = .131$). Together, these results indicate that all groups acquired the task about equally over the course of training, and any subsequent differences between groups during extinction may not be readily attributed to differing rates of initial task acquisition.

Response extinction: Figure 2 depicts learning rates over the course of extinction training for animals in the "response extinction" group. Tests of within-subjects contrasts computed on number of perseverative trials (Figure 12A) revealed a significant linear effect of Day ($F(1, 6) = 39.06$, $p = .001$), indicating a decrease in number of perseverative trials during response extinction training. In addition, within-subjects

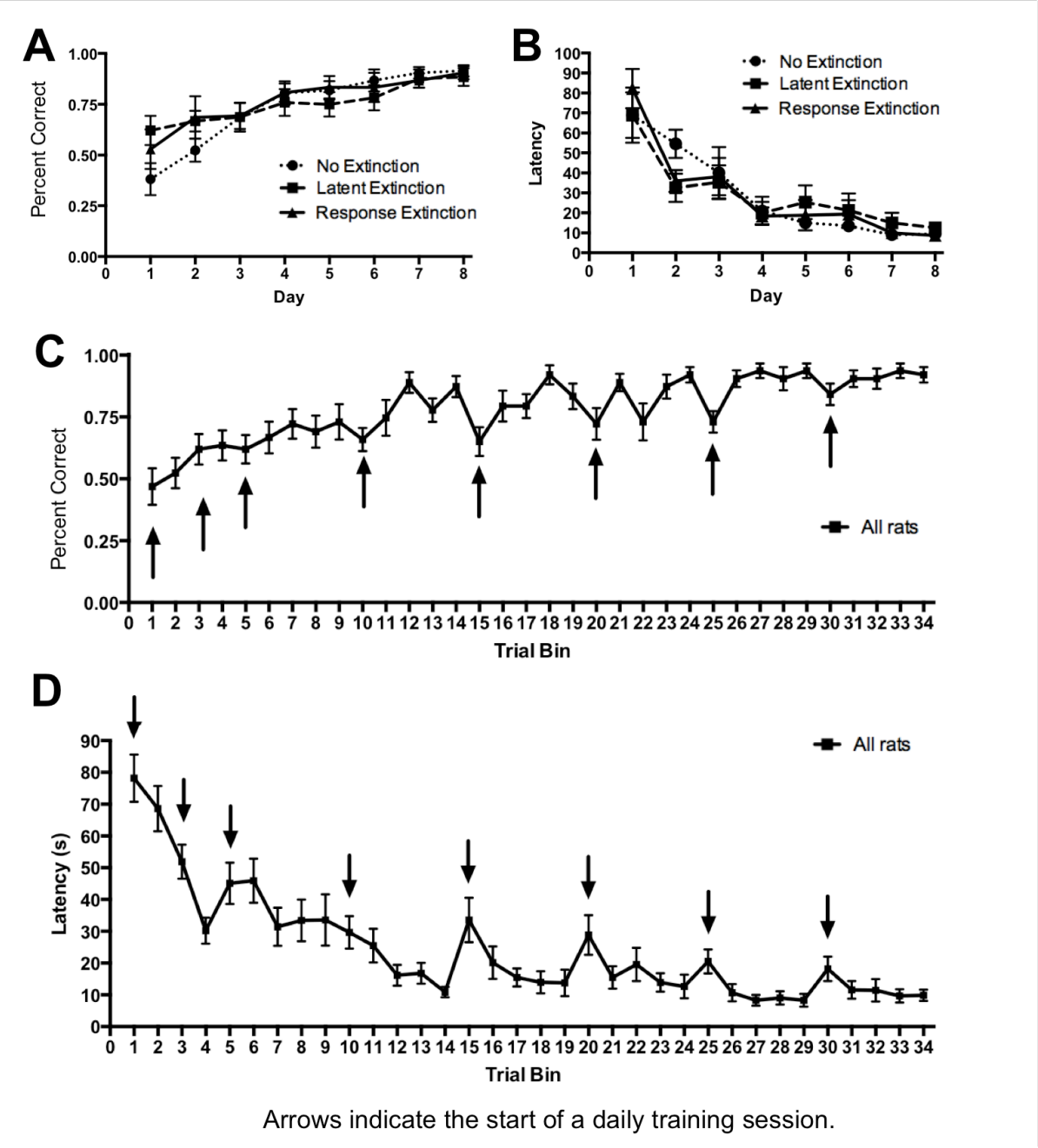

Figure 11. Acquisition of hippocampus-dependent place learning in the plus-maze. **A-B.** The percentage of correct turns increased (A) and the latency to reach the correct food well decreased (B) over the course of training, with no differences between groups. **C-D.** Subsequently, all groups were combined, and the trials of each day were averaged into trial bins (1 trial bin = 3 trials). Animals were more likely to make incorrect turns (C) and were slower (D) on the first few trials of a given training day versus the last few trials of the previous day.

contrasts computed on latency for extinction training days 1-3 (Figure 12B) also revealed a linear effect of Day ($F(1, 6) = 113.56$, $p < .001$), indicating that latency increased over the course of response extinction training.

Extinction probes: The results from the extinction probe trials are depicted in Figure 13. The rationale for comparing extinction probe performance with the first 4 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 3 X 2 ANOVA (Group X Day) was computed for number of perseverative trials on the last acquisition day (i.e. training day 8; first 4 trials) and the extinction probe day (Figure 13A). Results indicated no significant main effect of Group ($F(2, 18) = 1.79$, $p = .195$), but there was a significant effect of Day ($F(1, 18) = 10.89$, $p = .004$) and a significant Group X Day interaction ($F(2, 18) = 5.37$, $p = .015$). Multiple pairwise comparisons using Fisher's LSD test indicated that there were no significant differences in number of perseverative trials between groups on the last acquisition day. This is consistent with data presented above indicating that the groups did not differ during initial task acquisition. For animals in the latent extinction group, Fisher's LSD test indicated that there was a significant decrease in the number of perseverative trials from the last acquisition day ($M = 3.57$) to the probe day ($M = 2.43$), $p = .007$. In addition, the response extinction group showed a significant decrease in number of perseverative trials between the last acquisition day ($M = 3.29$) and the probe day ($M = 2.00$), $p = .003$. Animals given no extinction did not show a significant change

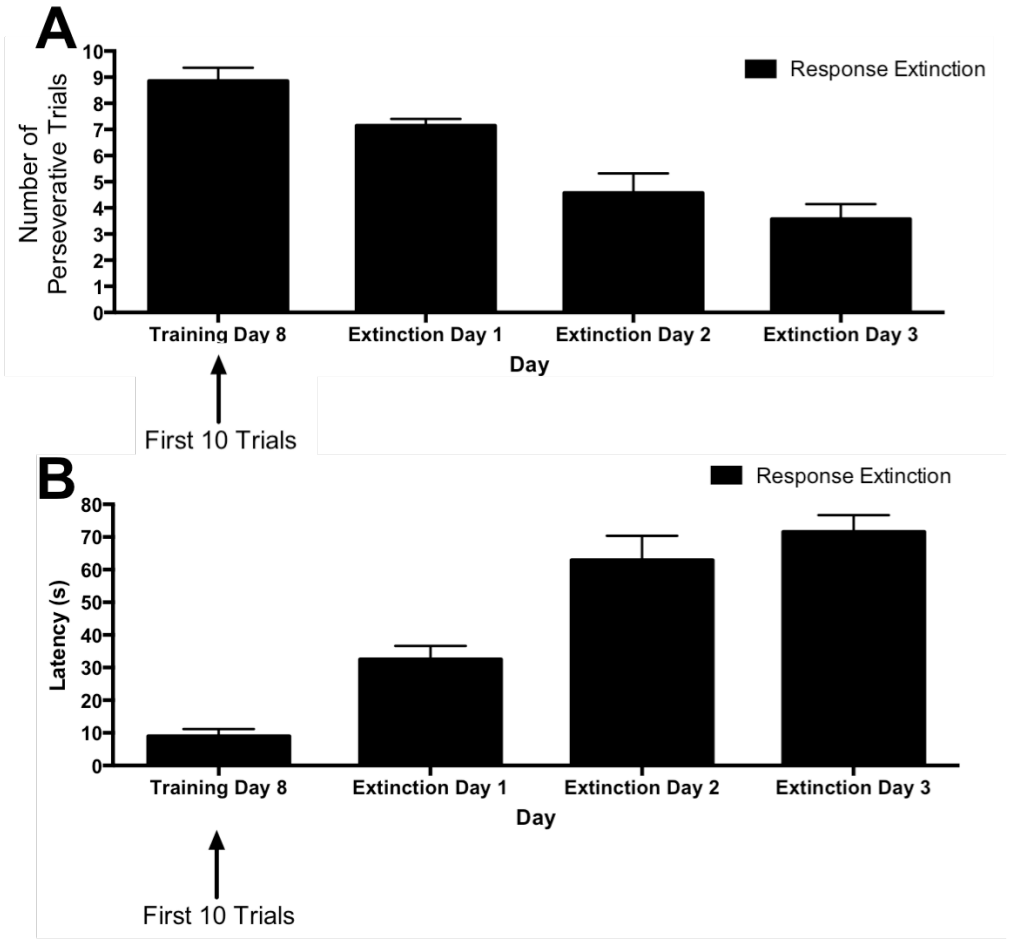

Figure 12. Response extinction of hippocampus-dependent place learning. A-B. For animals in the response extinction group, the number of perseverative trials decreased (A) and latency increased (B) over the course of extinction training, indicating the effectiveness of response extinction.

in number of perseverative trials from the last acquisition day (M = 3.14) to the probe day (M = 3.43), p = .456.

On the extinction probe day, Fisher's LSD test indicated that the latent extinction group (M = 2.42) displayed a significantly lower number of perseverative trials than animals in the no extinction control group (M = 3.42), p = .026. Similarly, number of

perseverative trials during probe day for the response extinction group (M = 2.00) was also significantly lower than perseverative trials for the no extinction group, p = .002. In contrast, perseverative trials for the latent extinction group and response extinction group did not differ on the probe day, p = .327.

A two-way repeated measures 3 X 2 ANOVA (Group X Day) was computed for latency on the last acquisition day (i.e. training day 8; first 4 trials) and the extinction probe day (Figure 13B). Results indicated a significant main effect of Group ($F(2, 18) = 5.48$, $p = .014$), a significant effect of Day ($F(1, 18) = 36.84$, $p < .001$), and a significant Group X Day interaction ($F(2, 18) = 17.92$, $p < .001$). Multiple pairwise comparisons using Fisher's LSD test indicated that there were no significant differences in latency between groups on the last acquisition day. For animals given latent extinction, there was a significant increase in latency from the last acquisition day (M = 14.77) to the probe day (M = 40.71), p = .002. There was also a significant increase in latency between the last acquisition day (M = 11.61) and the probe day (M = 65.00) for animals given response extinction, p < .001. Animals given no extinction did not show a significant change in latency from the last acquisition day (M = 17.39) to the probe day (M = 11.61), p = .419. On the probe day, Fisher's LSD test indicated that latency for the latent extinction group (M = 40.71) was significantly higher than latency in the no

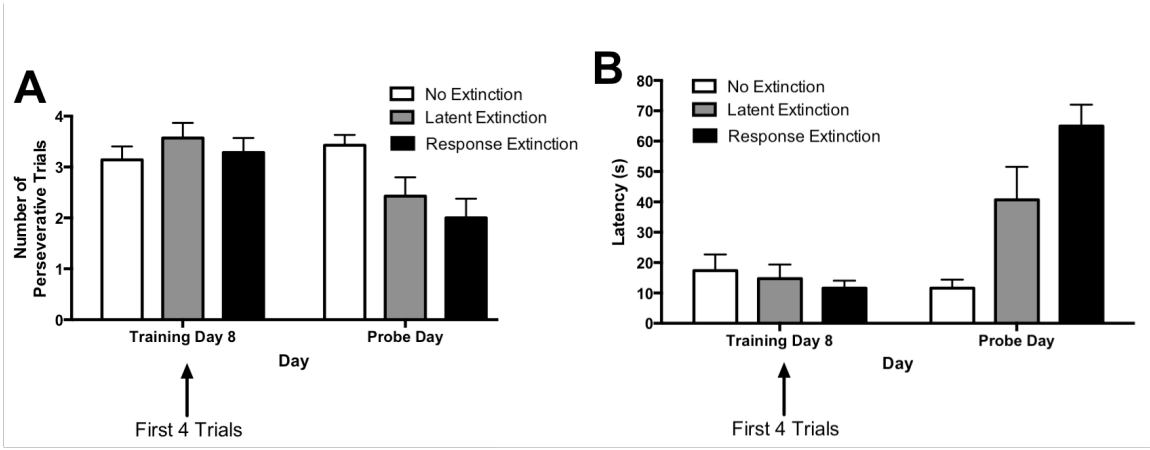

Figure 13. Extinction probe trials in the hippocampus-dependent place learning task. A. There were no between-group differences in perseveration during the first few trials of the last training day (i.e. training day 8). Response and latent extinction groups, but not the "no extinction" group, displayed a decrease in number of perseverative trials from the last acquisition day to the probe day. On the probe day, the latent and response extinction groups displayed lower perseveration than the no extinction group, but the latent and response extinction groups did not differ from each other in perseveration. **B.** There were no differences in latency between groups on the last training day. Response and latent extinction groups, but not the "no extinction" group, increased latency from the last acquisition day to the probe day. On the probe day, the latent and response extinction groups had higher latency than the no extinction group. Latency was also higher in the latent extinction group versus the response extinction group on the probe day. Results indicate the effectiveness of latent and response extinction protocols in extinction of hippocampus-dependent place learning.

Taken together, the results of experiment 1 indicate that following acquisition in a place learning task animals given latent or response extinction displayed higher latency and lower perseveration during the extinction probe trials, relative to animals given no extinction. These results suggest that either a latent or response extinction protocol may be effective at extinguishing hippocampus-dependent place learning in the plus-maze.

5.4 Discussion

The present findings indicate a dissociation regarding the effectiveness of latent extinction across two learning and memory tasks. Latent extinction was effective at extinguishing memory in a hippocampus-dependent place learning task, but not in a DLS-dependent response learning task. In contrast, typical "response extinction" was effective in both place and response learning tasks. These findings suggest that in order for DLS-dependent response learning to be extinguished, the animal must have the opportunity to perform the original response during extinction training. Creating conditions to allow the animal to form a new expectation about the original goal location no longer containing food (i.e. latent extinction) is not effective at targeting the response learning memory.

In experiment 1, following acquisition of a response learning task, animals given response extinction displayed higher latencies and fewer perseverative trials than animals given no extinction, indicating the effectiveness of response extinction in this task. In contrast, animals given limited or extended latent extinction did not differ in latency or perseveration from animals given no extinction, suggesting that these latent extinction protocols were not effective at producing extinction in the response learning task. Even though latencies in the limited and extended latent extinction groups showed a slight increase from the last acquisition day to the probe day, a comparable increase was also observed for animals in the "no extinction" control group. Therefore, this increase in latency from the last acquisition day to the probe day may not be readily attributed to the latent extinction protocols. The reason for this increase in latency

remains uncertain, however it is possible that some extinction learning had occurred during the four extinction probe trials for all groups, regardless of prior extinction training. However, if some extinction learning had occurred during the four probe trials, it was only reflected in extinction latencies; the latent extinction group and no extinction control group did not show a decrease in number of perseverative trials across the 2 days.

In experiment 2, following acquisition of the place learning task, animals given latent or response extinction displayed greater latency and fewer perseverative trials than animals given no extinction. Interestingly, animals given response extinction displayed higher latencies than animals given latent extinction, suggesting response extinction may have had greater efficacy than latent extinction in the place learning task. However, there was no difference in number of perseverative trials between latent and response extinction groups. It is possible that, relative to latent extinction, response extinction was more efficient at slowing the running approach response, but not necessarily more effective at extinguishing the location of food reward.

A finding secondary to the differential effects of the extinction protocols, but of considerable relevance to classical learning theories, pertains to the initial acquisition curves in the place and response learning tasks. During most days of initial acquisition, the first few trials were accompanied with greater latencies and more errors than the last few trials of the previous training day (see Figure 8C–D and Figure 11C–D). However, this rise in latency and inaccuracy on the first few training trials of a given day became progressively less pronounced on subsequent training days. The present finding is

consistent with early principles in learning theory pertaining to decay theory (e.g. Ebbinghaus, 1913; Thorndike, 1913). Thorndike (1913) proposed that following acquisition, a memory begins to fade as a function of its disuse over time (i.e. decay). However, some traces of the memory survive this decay, and thus relearning not only proves faster than initial learning, but also results in a stronger memory that is less sensitive to memory decay. Although the precise mechanisms of memory decay have been disputed (McGeoch, 1932), the general predictions of Thorndike's model resemble the acquisition curves obtained in the present study. It is possible that some decay (or, more generally, forgetting) occurred in between daily training sessions, but that with each subsequent session of relearning the memory became more firmly engrained and less sensitive to decay.

The principal finding that latent extinction was effective in the place learning task but not the response learning task may be related to differences between the memories acquired in each task. That is, latent extinction might only be effective when the to-be-extinguished memory contains certain critical features. The tasks selected for the present experiments depended on distinct neural systems, and solving each task hinged on different learning requirements. The hippocampus-dependent place learning task presumably required animals to encode the spatial location of the food reward to guide behavior to the correct arm, whereas the DLS-dependent response learning task only required that animals encode a left body-turn response at the maze choice point. Although animals being trained in the response learning task could also encode the spatial locations of the food reward, this information was not necessary for acquisition

and ongoing performance in this task. In fact, extensive evidence indicates that spatial information might interfere with acquisition in the response learning task (for reviews, see Poldrack and Packard, 2003; Packard and Goodman, 2013).

Latent extinction in maze learning tasks might only be effective when the spatial location of the reinforcer is a critical part of the to-be-extinguished memory. Previous studies examining latent extinction have typically employed maze tasks, such as the straight alley maze, that could be solved adequately using either spatial or non-spatial learning strategies. In "dual-solution" tasks such as these, animals typically employ spatial learning strategies when the learning environment constitutes a heterogeneous visual surround, whereas animals employ response learning strategies when the task is conducted in a homogeneous visual surround (for reviews, see Restle, 1957; Packard and Goodman, 2013). Interestingly previous studies have indicated that latent extinction was only effective in heterogeneous visual surrounds conducive to allocentric spatial learning (e.g. Seward and Levy, 1949; Denny and Ratner, 1959; Dyal, 1962). Latent extinction was not effective in homogenous visual surrounds that prevented the use of allocentric spatial learning (e.g. Bugelski, Coyer, and Rogers, 1952; Scharlock, 1954; Denny and Ratner, 1959). These previous findings are consistent with the suggestion that in maze learning tasks, latent extinction might be selectively effective at extinguishing allocentric spatial memory.

The finding that latent extinction might only be successful at extinguishing certain types of memory could be attributed to the distinct learning mechanisms through which latent extinction operates. Unlike response extinction, latent extinction does not

conform to classical models of extinction that suggest the animal must make the previously acquired response for extinction to occur (e.g. Hull, 1943, 1952). Proponents of the Hullian S-R view of learning have suggested that latent extinction, although it may not be readily explained by Hull's traditional response-inhibition theory of extinction, could still be accounted for through a Hullian fractional anticipatory response mechanism (Hull, 1931; Spence, 1951). According to this view (Moltz, 1957), an unobservable component of the consumatory goal response is elicited by cues throughout the maze during initial acquisition of the task, and this partially guides behavior to the correct goal location. When an animal is confined to the goal box during latent extinction, this fractional goal response is elicited and, over time, becomes extinguished to the goal box cues. To the extent that the goal box cues might resemble earlier sections of the maze, extinction of the fractional goal response will generalize to other parts of the maze, resulting in increased latency and incorrect turns during extinction probe trials. Several cogent arguments have been raised indicating the inadequacy of this potential S-R mechanism in explaining latent extinction (Gleitman, Nachmias, and Neisser, 1954; Treisman, 1960). In addition, this putative mechanism is not supported by the present findings. If latent extinction were to operate by extinguishing a fractional response in the goal box that generalizes to other parts of the maze, then it would be reasonable to predict that latent extinction would be effective across both place and response learning tasks, which presently was not observed.

Previous evidence from our laboratory suggests that latent extinction may involve spatial memory mechanisms (Gabriele and Packard, 2006). Temporary

inactivation of the dorsal hippocampus with bupivacaine blocks the effectiveness of latent extinction in the straight alley maze (Gabriele and Packard, 2006). Considering that a principal function of the hippocampus involves spatial memory formation (O'Keefe and Nadel, 1978; Morris, Garrud, Rawlins, & O'Keefe, 1982), it is possible that hippocampal inactivation blocked latent extinction by disrupting hippocampus-dependent spatial memory processing. That latent extinction might depend in part on spatial memory processing is largely consistent with previous behavioral evidence. As mentioned previously, latent extinction is selectively effective in heterogeneous visual environments conducive to spatial memory formation, but not homogenous visual environments that prevent spatial memory formation (Seward and Levy, 1949; Bugelski, Coyer, and Rogers, 1952; Scharlock, 1954; Denny and Ratner, 1959; Dyal, 1962).

Latent extinction may involve spatial memory processing insofar as confining an animal to a previously rewarded spatial location without food (i.e. latent extinction) might allow the animal to acquire a new memory in which the spatial location becomes associated with absence of food. Thus, for latent extinction to be successful, a rat must be confined to the previously rewarded spatial location. Confining a rat to an empty goal box located in a different room (Iwahara, Asami, Okano, and Shibuya, 1953) or a different spatial location in the same room (Clifford, 1964) does not produce extinction. This proposed mechanism for latent extinction is consistent with its dependence on hippocampal function, i.e. in addition to acquiring information about food rewarded locations, the hippocampus is similarly involved in linking spatial locations with the *absence* of food reward (Gaskin and White, 2006).

This putative spatial mechanism could also explain why latent extinction was effective in the place learning task, but not the response learning task. In the place learning task, memory performance was presumably guided by a learned association in which a spatial location had been associated with the food reward. Thus, if the same spatial location were subsequently associated with the absence of food reward, which putatively occurs during latent extinction, we should expect memory performance in the place learning task to decline. In contrast, memory performance in the response learning task was presumably not guided by the spatial locations of the food reward, and therefore associating spatial locations with the absence of food reward should not affect later retrieval of the previously acquired response.

Given the effectiveness of typical response extinction across both place and response learning tasks, it is tempting to speculate that response extinction might depend on a distinct learning mechanism. Previous evidence from our laboratory indicates that in contrast to latent extinction, the effectiveness of response extinction in the straight alley maze is not impaired following hippocampal inactivation (Gabriele and Packard, 2006). Rather, response extinction in the straight alley maze is attenuated following lesion or temporary inactivation of the DLS (Dunnett and Iversen, 1981; Thullier, LaLonde, Mahler, Joyal, and Lestienne, 1996; Gabriele, 2008). Considering that the DLS is a chief neural substrate implicated in S-R learning and memory processes (Packard and Knowlton, 2002; Goodman and Packard, in press), one possibility is that during response extinction the DLS forms S-R associations between visual cues in the learning situation (i.e. the stimuli) and the inhibition of a behavior (i.e. the response).

Several investigators have proposed similar S-R mechanisms to account for extinction across maze learning, operant lever pressing, and Pavlovian conditioning paradigms (Guthrie, 1935; Hull, 1943; Rescorla, 1993; Delameter, 2004). Importantly, the learned inhibition of behavior during response extinction could potentially explain the effectiveness of this protocol in both place learning and response learning tasks.

Aside from the direct involvement of multiple memory systems, another potential mechanism underlying the selective effectiveness of latent extinction pertains to the immediate differences between the two tasks. Although the place and response learning tasks were identical in terms of their motivational, sensory, and motoric requirements, it was necessary that the tasks differed slightly in some respects so that each task invoked a different memory system. We cannot rule out the possibility that slight differences between the two tasks (e.g. in the place learning task, animals received food in one location; in the response learning task, animals received food in two locations) may have partially influenced the effectiveness of latent extinction.

The present findings may have important implications for understanding the mechanisms behind extinction of response learning. During typical response extinction, the animal has the opportunity to form a new inhibitory response that may directly produce a response decrement. However, considering that the animal is running to the previously rewarded goal locations, the animal could also be forming a new association between the original goal arms and the absence of reinforcement, which could indirectly produce a response decrement. However, the present observation that latent extinction proved ineffective in the response learning task, potentially rules out this second

possibility. It appears that changes in expectation acquired through latent extinction may not be a critical part of the behavioral mechanisms underlying extinction of response learning. Rather, it appears the animal needs to make the previously acquired response in order for extinction to occur, suggesting that response-produced inhibition may be a potential mechanism underlying extinction of response learning. Whether this response-produced inhibition is achieved through inhibitory S-R associations, as predicted in Hull's theory of extinction, has yet to be definitely examined.

The present finding that response learning is only sensitive to certain kinds of extinction training may not only be important for its theoretical implications, but might also lead to important clinical applications. Multiple researchers have suggested that the formation and expression of habit-like behavioral features in human psychopathologies might reflect heightened engagement of dorsal striatum-dependent memory processes (White, 1996; Everitt and Robbins, 2005; Schwabe, Dickinson, and Wolf, 2011; Goodman, Leong, and Packard, 2012; Berner and Marsh, 2014; Gillan and Robbins, 2014; Goodman, Marsh, Peterson, and Packard, 2014). Moreover, several investigators have proposed that animal models of extinction may be adapted into treatments that combat maladaptive memory formation in some human neuropsychiatric disorders (e.g. Quirk and Mueller, 2008; Maren, Phan, and Liberzon, 2013; Goode and Maren, 2014). One potential limitation to this idea drawn from the present experiments is that not all extinction strategies may be effective at suppressing DLS-dependent habit memory. It is possible that in order to adequately extinguish DLS-dependent habit memory, behavioral interventions that mimic response extinction protocols might be more beneficial than

treatments that depend on cognitive memory mechanisms. The progression from recreational drug taking to habitual drug addiction may reflect a shift from cognitive control of behavior toward habitual control of behavior mediated by the DLS (White, 1996; Everitt and Robbins, 2005; Schwabe, Dickinson, and Wolf, 2011; Everitt and Robbins, 2013; Hogarth, Balleine, Corbit, and Killcross, 2013). Thus, one prediction is that response extinction might be more effective than latent extinction in suppressing habitual drug seeking. Previous evidence indicates that latent and response extinction prove equally effective following acquisition of a running approach response for sucrose reinforcement. However, if the reinforcer during initial acquisition is cocaine, response extinction proves more effective than latent extinction (Gabriele, Setlow, and Packard, 2009).

In sum, the present findings indicate that whereas response extinction successfully extinguished memory in hippocampus-dependent place learning and DLS-dependent response learning tasks, latent extinction was selectively effective in the place learning task and not the response learning task. The suggestion that the principal learning mechanisms underlying latent extinction involve an acquired association between the spatial location and the absence of food reward may provide an explanation for the selective effectiveness of latent extinction across these learning tasks. Importantly, these findings suggest that behavioral mechanisms underlying extinction of response learning may involve a kind of response-produced inhibition consistent with the Hullian S-R view of extinction.

CHAPTER VI

AIM 2: A NEUROBIOLOGICAL SUBSTRATE OF HABIT MEMORY EXTINCTION

6.1 Introduction

Memory is organized into multiple neural systems that mediate different kinds of memory (Squire, 2004; White, Packard, and McDonald, 2013). However, the role of anatomically dissociable neural systems in different kinds of memory has been demonstrated primarily during initial acquisition, consolidation, and retrieval of memory, whereas few studies have adopted a multiple memory systems approach when examining *extinction* of memory.

As reviewed in Chapter II, research in our laboratory indicates that multiple memory systems are indeed differentially implicated in extinction (Gabriele and Packard, 2006). In the straight alley maze, inactivation of the DLS impairs the effectiveness of response extinction, but not latent extinction. Inactivation of the hippocampus, on the other hand, completely blocks latent extinction, but has no effect on response extinction. These findings suggest a double dissociation regarding the role of multiple memory systems in extinction. The hippocampus may be involved in acquiring changes in expectation that indirectly produce a response decrement, whereas the DLS may be involved in response-produced inhibition. In addition, the multiple memory systems approach might also be relevant to extinction insofar as the effectiveness of an extinction protocol might partially depend on the kind of memory

that is being extinguished (e.g. Goodman et al., 2016; see also previous Chapter). However, different roles for memory systems in extinction have only been demonstrated when using different extinction protocols (i.e. latent and response extinction), not when extinguishing different kinds of memory. Thus, whether a brain region can be implicated in extinction of one type of memory and not another has yet to be definitively demonstrated. The present aim examines whether a particular neural substrate, the DLS, selectively mediates extinction of habit memory in a response learning task.

6.1.1 Experiment 3

In experiment 3, we examined whether extinction of response learning is mediated by the DLS. There are three good reasons to consider the DLS as a candidate neural structure mediating extinction of response learning. For one, as observed in extinction of some kinds of memory, the neural substrates implicated in the initial acquisition of a memory may also be implicated in its extinction. Examples of this may be observed in fear conditioning experiments, whereby for instance the BLA and ventral hippocampus are implicated in both the initial acquisition and extinction of conditioned fear (e.g. Sierra-Mercado, Padilla-Coreano, and Quirk, 2011). In addition, the dorsal hippocampus has been implicated in both initial acquisition and extinction of place learning in the plus-maze (e.g. Schroeder et al., 2002; Gabriele and Packard, 2006). As noted extensively in chapter IV, the DLS mediates initial acquisition of response learning (Chang and Gold, 2004; Compton, 2004; Palencia and Ragozzino, 2005; Asem and Holland, 2015), and therefore this brain region might also be involved in extinction

of response learning. One possible reason that brain regions implicated in initial acquisition of a particular memory may also be implicated in extinction of the same memory is that, during extinction training, plastic changes might transpire in the original locus of acquisition/storage that ultimately reverse or suppress the original memory.

Another reason the DLS should be considered as a candidate neural structure underlying extinction of response learning is that, according to some learning theories, extinction may involve the acquisition of an inhibitory S-R association, and the DLS is a principal neural substrate of S-R learning. According to the classic Hullian S-R view of extinction discussed at length in earlier sections, stimuli in the extinction learning environment may acquire the capacity to inhibit the original response. Strong evidence for an S-R view of extinction was observed in a series of experiments conducted in instrumental learning tasks (for review, see Rescorla, 2001). In these experiments, investigators demonstrated that extinction training influences specific S-R combinations. That is, if during extinction training, the animal makes a specific unreinforced response (e.g. lever pressing) in the presence of a distinct stimulus (e.g. a light), later performance of the response will be suppressed when the same extinction stimulus, but not another stimulus, is presented during the extinction test. Nevertheless, the stimulus that was presented during extinction could still be associated with other responses (e.g. chain pulling) that were not made available during extinction training. Thus, it appears that extinction leads to a decrement in the original S-R (e.g. light-lever press) association, creating a novel inhibitory association whereby the specific stimulus presented during extinction training suppresses the specific response that was unreinforced. On the other

hand, extinction training does not disrupt the original response-outcome or stimulus-outcome contingency, as animals that received extinction training remain sensitive to outcome devaluation and display normal Pavlovian-to-instrumental transfer. Thus, the only association that appears to be disrupted following extinction training in the instrumental learning task is the S-R association. DLS activity is needed for learning in tasks that may be acquired using S-R inhibitory learning (e.g. passive avoidance; Salado-Castillo et al., 1996), but whether the DLS may be involved in acquisition of an inhibitory S-R association during *extinction* remains unexamined.

A third reason to consider the DLS as a candidate neural structure mediating extinction of response learning is that the DLS has been associated with extinction across multiple learning and memory tasks. Evidence indicates that the DLS is involved in extinction in the straight alley maze (Thullier et al., 1996), T-maze (Campus et al., 2014), instrumental learning (Schmaltz and Isaacson, 1972), and cocaine self-administration tasks (Ghasemzadeh et al., 2009a,b; Knackstedt et al., 2014), as well as others (Herz and Peeke, 1971; Makarova, 2001). Each of these tasks can be acquired using a response learning strategy. In the straight alley maze and T-maze, stimuli in the learning environment (S) may activate the approach response (R), leading to the correct food well. In addition, acquisition in the straight alley and T-maze tasks sometimes involves DLS-dependent memory function (e.g. Dunnett and Iversen, 1981; Kirkby et al., 1981; Packard and McGaugh, 1996), further implicating a response learning system in initial acquisition of these tasks. Likewise, instrumental learning tasks may also be achieved using DLS-dependent S-R mechanisms (Yin, Knowlton, and Balleine, 2004).

Thus, the involvement of the DLS during *extinction* in each of these tasks may be related to its presumed role in the suppression of DLS-dependent response learning. However, even though each of these tasks can be acquired using S-R learning strategies, each of these tasks can also be acquired just as easily using learning strategies that do not depend on S-R learning mechanisms. Straight alley and T-maze tasks can be acquired using a hippocampus-dependent place learning strategy (Rawlins et al., 1985; Salinas and White, 1998), and instrumental learning tasks may be achieved using a DMS-dependent response-outcome strategy (Yin, Ostlund, Knowlton, and Balleine, 2005). Therefore, we cannot determine what type of memory was being extinguished in these tasks nor whether the DLS is selectively involved in extinction of only specific kinds of memory.

For experiment 3, animals were trained in the response learning plus-maze task, and were then given two days of extinction training using the response extinction protocol. A latent extinction protocol was not used for this experiment, because as indicated in the previous chapter this kind of extinction training does not effectively produce extinction in the response learning task. Immediately following the first day of extinction training, neural inactivation of the DLS was conducted to determine if this brain region is implicated in consolidation of the extinction memory. Consolidation denotes the phase of memory processing in which a short-term memory is consolidated into a long-term memory. Thus, if the drug disrupted consolidation of the extinction memory, a memory impairing effect should be evident on the second day of extinction training, relative to animals that did not receive drug. Post-training drug administrations are useful not only in examining the consolidation of memory that occurs following

initial learning, but also in ruling out the possibility that the drug influenced online non-mnemonic processes that occur during acquisition, such as motoric, sensory, or motivational processes. Moreover, post-training drug infusions are particularly useful in examining extinction. The problem with administering drug infusions immediately *before* extinction training is that drug infusions could be influencing not only extinction learning, but also retrieval of the original memory. Indeed, the DLS is involved in retrieval of response learning in the plus-maze (Packard and McGaugh, 1996). Thus, the administration of drugs immediately *after* extinction training should prevent the drug manipulations from influencing retrieval of the original memory during extinction training. It is hypothesized that DLS inactivation will impair extinction in the response learning task.

6.1.2 Experiment 4

As noted previously, DLS-dependent memory function may underlie extinction in a variety of learning and memory tasks (e.g. maze learning and instrumental learning tasks), each of which may be acquired using a response learning strategy. However, each of these tasks could also be acquired using hippocampus-dependent cognitive strategies. Thus, since it remains unclear what kind of memory was being initially acquired in these tasks, we also do not know what kind of memory was being extinguished. Moreover, since we do not know what kind of memory was being extinguished, we also do not know whether the DLS is needed for extinction of response

learning, place learning, both place learning and response learning, or some other kind of memory underlying performance in these tasks.

The purpose of experiment 4 was to determine whether the presumed role of the DLS in extinction proves selective to response learning, or whether the DLS may be implicated in extinction of another kind of memory mediated by a different neural system. In experiment 4, animals were trained in a place learning task and then given response extinction for two days. Similar to experiment 3, animals received post-training inactivation of the DLS immediately following the first day of extinction training, and potential effects of DLS inactivation of consolidation of the extinction memory were examined on the second day of extinction training. Unlike the response learning task, acquisition in the place learning task is critically dependent on hippocampal function and not DLS function (Oliveira et al., 1997; Ramos, 2002; Schroeder et al., 2002; Chang and Gold, 2003; Compton, 2004; Boucard et al., 2009; Jacobsen et al., 2012). Thus, the mnemonic processes underlying acquisition of place learning are operatively and anatomically dissociable from those underlying response learning. However, importantly, the place learning task shares similar non-mnemonic features with the response learning task (i.e. similar sensory, motivational, and motor processes). Thus, if we examine an effect of DLS inactivation on extinction of response learning, but not extinction of place learning, the difference may be attributed to the different kinds of memory that were being extinguished rather than to some non-mnemonic difference between the two tasks.

As opposed to the response learning task, it was hypothesized that the DLS would not be involved in extinction of place learning. This is because extinction of place learning may be achieved through changes in expectation regarding the original goal location and the absence of reinforcement, and this kind of extinction learning is presumably mediated by the hippocampus and not the DLS (Gabriele and Packard, 2006; Gabriele, 2008). Nevertheless, it is possible that response-produced inhibition mediated by the DLS may be partially involved in extinction of place learning, especially when using a response extinction protocol. However, this kind of extinction learning does not seem *necessary* to produce a response decrement in the place learning task. Thus, removal of this presumed response-produced inhibition mechanism through DLS inactivation would only leave the hippocampus-dependent cognitive system to achieve adequate extinction of the place learning memory by acquiring changes in expectation.

6.2 Method

6.2.1 Subjects

The subjects were 34 male Long-Evans rats weighing 275-350 g upon arrival. Animals were subsequently food-restricted and maintained at 85% of the their ad lib weight throughout all behavioral procedures. Water was provided ad libitum. Animals were housed individually in a temperature-controlled vivarium with a 12 h light-dark cycle (lights on at 7AM), and all behavioral procedures were conducted during the light phase of this cycle. Age, weight, and housing conditions did not differ between animals

in experiments 3 and 4. Animal use in this study was carried out in accordance with the ethical guidelines of the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University. The protocol was approved by IACUC.

6.2.2 Apparatus

The apparatus was the plus-maze used for experiments 1 and 2 (see previous chapter).

6.2.3 Surgical Procedures

Animals were anesthetized with gaseous isoflurane and implanted with bilateral guide cannulae (23 gauge, 15mm length) targeting the DLS. Guide cannulae were anchored to the skull with jewelers screws and dental cement. Stereotaxic coordinates for cannula placements were anterior-posterior (AP) = – 0.3 mm from bregma, medial-lateral (ML)= ± 4.2 mm, and dorsal-ventral (DV)= -4.0 mm. These coordinates were chosen based on previous research indicating that drug infusions into this region of the striatum were associated with modulation of response learning (e.g. Goodman and Packard, 2014). Rats were given 7 days of post-operative recovery before the behavioral protocol was implemented.

6.2.4 Histology

Cannulated rats were sacrificed with a 1ml injection of pentobarbital and perfused intracardially with 0.9% saline, followed by 10% formol-saline solution.

Brains were removed and stored in 10% formol-saline before being sliced to 40µm sections with a cryostat. Every fourth slice was collected and scanned into a computer wherein brain slices were closely examined for cannula placements and injection needle tip locations using a rat brain atlas (Paxinos and Watson, 2007). Histologies for animals in experiment 3 are depicted in Figure 14. Histologies for animals in experiment 4 are depicted in Figure 17.

6.2.5 Drug and Injection Procedures

Drugs were administered with the same injection procedures used in our previous studies (Packard and Wingard, 2004; Wingard and Packard, 2008; Goodman and Packard, 2014). To reversibly inactivate the DLS, animals received bilateral intra-DLS infusions of the sodium channel blocker bupivacaine (0.75% solution; Sigma-Aldrich). Bupivacaine produces a temporary disruption of neural activity through blockade of sodium channels, thereby preventing conductance of action potentials. The duration of bupivacaine activity has been estimated at 30–50 min (Catterall & Mackie, 1986). Control animals received intra-DLS infusions of physiological saline.

Intra-DLS infusions were administered bilaterally over a period of 52 s via a microsyringe pump using 10µl Hamilton syringes connected to polyethylene tubing. Following infusions, the injection needles (16mm) were left in the guide cannulae for an additional 60s to allow for diffusion of drug from the needle tip.

6.2.6 Behavioral Procedures

Maze habituation and training: In experiment 3, rats (N = 20) were trained in a response learning version of the plus-maze, and in experiment 4 rats (N = 14) were trained in a place learning version of the plus-maze. The behavioral procedures for maze habituation and initial acquisition training in the response and place learning tasks were identical to those described in experiments 1 and 2 (see previous chapter). The only difference was that the response and place learning tasks in the present experiments were conducted for only 7 days, instead of 8 days.

Extinction training: Twenty-four hours following the last day of initial acquisition of the response learning task (experiment 3) or place learning task (experiment 4), animals received response extinction training for 2 days (10 trials/day). The parameters for response extinction were identical to those described in experiments 1 and 2 (see previous chapter).

The purpose of experiment 3 was to examine whether the DLS is required for extinction of response learning. Thus, in experiment 3, immediately after the first day of extinction training, rats were given bilateral intra-DLS infusions of the sodium channel blocker bupivacaine (n = 7) or physiological saline (n = 7). A third group was given bupivacaine infusions two hours after extinction training (n = 6) to control for potential proactive effects of the drug. Whether post-training drug infusions influenced consolidation of the extinction memory would be determined by looking at average latency and number of perseverative trials on the *second* day of extinction training, i.e. 24 hours after drug administration.

In experiment 4, immediately following training on the first extinction day, rats received bilateral intra-DLS infusions of the sodium channel blocker bupivacaine (n = 7) or physiological saline (n = 7). It was hypothesized that DLS inactivation with bupivacaine would not influence extinction of place learning, and therefore we did not run a third group that received delayed injections of bupivacaine. Potential effects of post-training DLS inactivation were examined 24 hours following drug administration (i.e. on the second day of extinction training) by measuring average latency and number of perseverative trials.

6.3 Results

6.3.1 Experiment 3: Response Learning Task

Initial acquisition: Initial acquisition of the response learning task is depicted in Figure 15. A two-way repeated measures 3 X 7 ANOVA (Group X Day) computed on percentage of correct turning responses over the course of training (Figure 15A) indicated a significant main effect of Day ($F(6, 102) = 13.01$, $p < .001$), but no effect of Group ($F(2, 17) = .37$, $p = .697$) and no Group X Day interaction ($F(12, 102) = .49$, $p = .919$). Likewise, a 3 X 7 ANOVA (Group X Day) computed on latency (Figure 15B) indicated a significant effect of Day ($F(6, 102) = 39.39$, $p < .001$), but no effect of Group ($F(2, 17) = .243$, $p = .787$) and no Group X Day interaction ($F(12, 102) = .18$, $p = .999$). Together, these results indicate that all groups acquired the task about equally over the

course of training, and any subsequent differences between groups during extinction may not be readily attributed to differing rates of initial task acquisition.

Extinction: Number of perseverative trials across extinction training is depicted in Figure 16A. The rationale for comparing extinction performance with the first 10 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 3 X 3 ANOVA (Group X Day) was computed for number of perseverative trials across acquisition day 7 (first 10 trials), extinction day 1, and extinction day 2 (Figure 16A). Results indicated a trend toward a significant main effect of Group ($F(2, 17) = 3.175$, $p = .067$). There was also a significant main effect of Day ($F(2, 34) = 67.89$, $p < .001$) and a significant Group X Day interaction ($F(4, 34) = 53.56$, $p = .016$). These findings suggest that an effect of Group may only be observable on certain days.

A one-way ANOVA indicated that there was no main effect of Group on number of perseverative trials during the last acquisition day (i.e. Training Day 7; first 10 trials; $F(2, 17) = .412$, $p = .669$) or on Extinction Day 1 ($F(2, 17) = .46$, $p = .641$). These findings suggest that all groups had similar baseline measures of perseveration during Training Day 7 and Exinction Day 1, i.e. before drugs were administered. A two-way 3 X 2 (Group X Day) ANOVA computed on perseverative trials across Training Day 7 and Extinction Day 1 indicated an effect of Day ($F(1, 17) = 35.40$, $p < .001$), but no main effect of Group ($F(2, 17) = .18$, $p = .833$) and no Group X Day interaction ($F(2, 17) = .79$, $p = .469$). Thus, all groups displayed a comparable decrease in the number of

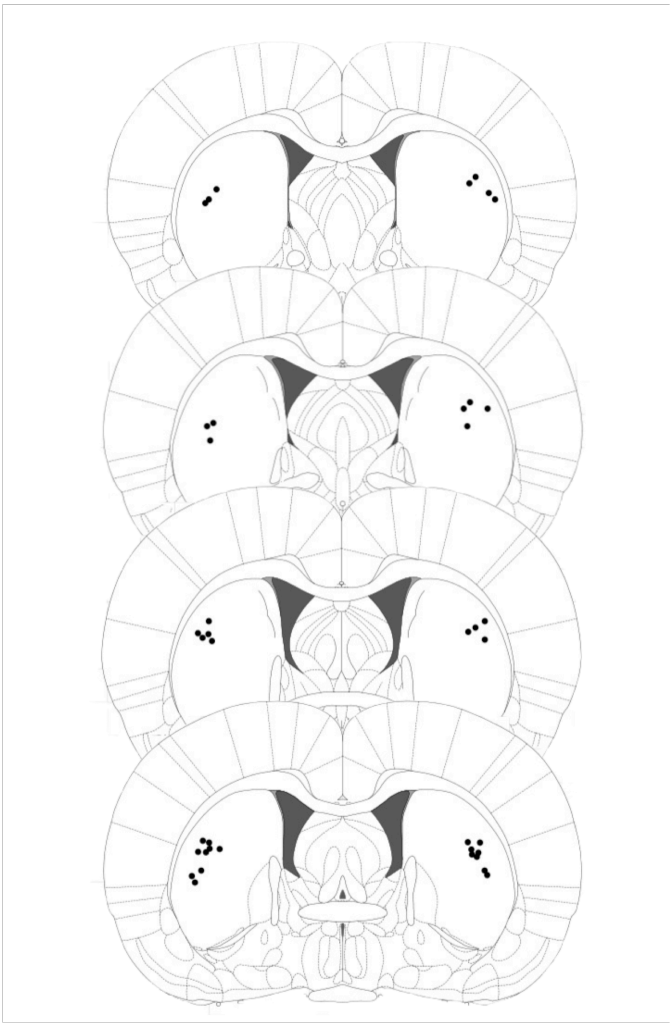

Figure 14. Injection needle placements in the DLS. Dorsolateral striatum cannula placements for experiment 3 showing the anterior/posterior extent of needle tip locations at 40-µm sections. Placements ranged from +0.24 to –0.36 mm from bregma. Images were adapted from Paxinos and Watson, 2007.

perseverative trials from Training Day 7 to Extinction Day 1. This indicates that all groups extinguished about equally before drugs were administered.

A one-way ANOVA computed on number of perseverative trials during Extinction Day 2 (i.e. 24h *after* post-training drug administration) indicated a main

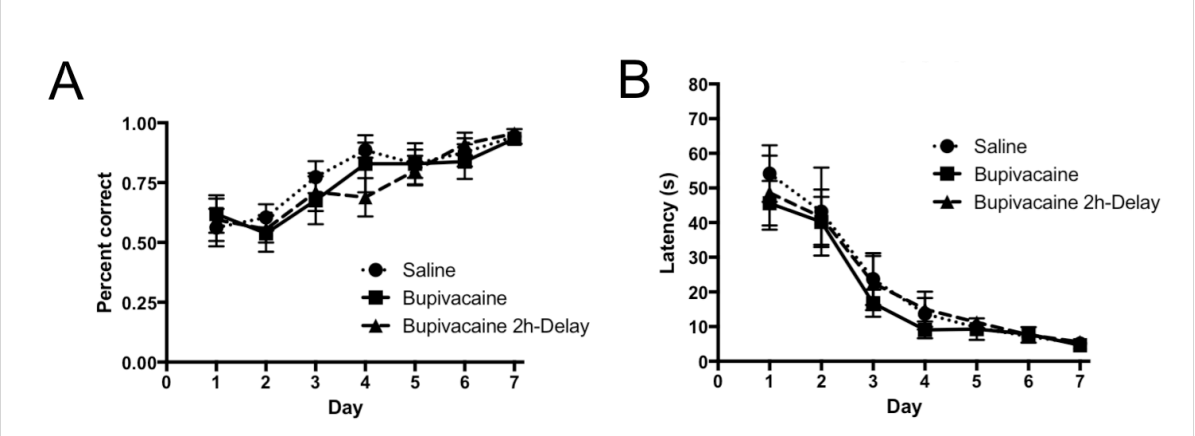

Figure 15. Acquisition of DLS-dependent response learning in the plus-maze. A-B. The percentage of correct turns increased (A) and the latency to reach the correct food well decreased (B) over the course of training, with no differences between groups.

effect of Group ($F(2, 17) = 7.55$, $p = .005$). Fisher's LSD test indicated that animals given immediate post-training bupivacaine displayed a higher number of perseverative trials ($M = 6.14$) than control animals given saline ($M = 3.00$), $p = .002$. In contrast, animals given bupivacaine after a 2h delay displayed a comparable number of perseverative trials ($M = 3.33$) relative to animals given saline, $p = .721$. These findings suggest that immediate post-training DLS inactivation with bupivacaine, but not bupivacaine administered after a 2h delay, impaired extinction of response learning, as measured by number of perseverative trials.

A two-way 3 X 2 (Group X Day) ANOVA computed on number of perseverative trials across Extinction Day 1 and Extinction Day 2 indicated a main effect of Day ($F(1, 17) = 37.05$, $p < .001$), a main effect of Group ($F(2, 17) = 4.36$, $p = .029$), and a trend toward a significant Group X Day interaction ($F(2, 17) = 3.15$, $p = .069$). Fisher's LSD

test indicated that the saline group displayed a significant decrease in number of perseverative trials from Extinction Day 1 (M = 6.43) to Extinction Day 2 (M = 3.00), $p < .001$. Animals given delayed bupivacaine also displayed a significant decrease in perseverative trials from Extinction Day 1 (M = 6.833) to Extinction Day 2 (M = 3.33), $p < .001$. However, animals given immediate post-training bupivacaine did not show a significant reduction in the number of perseverative trials from Extinction Day 1 (M =

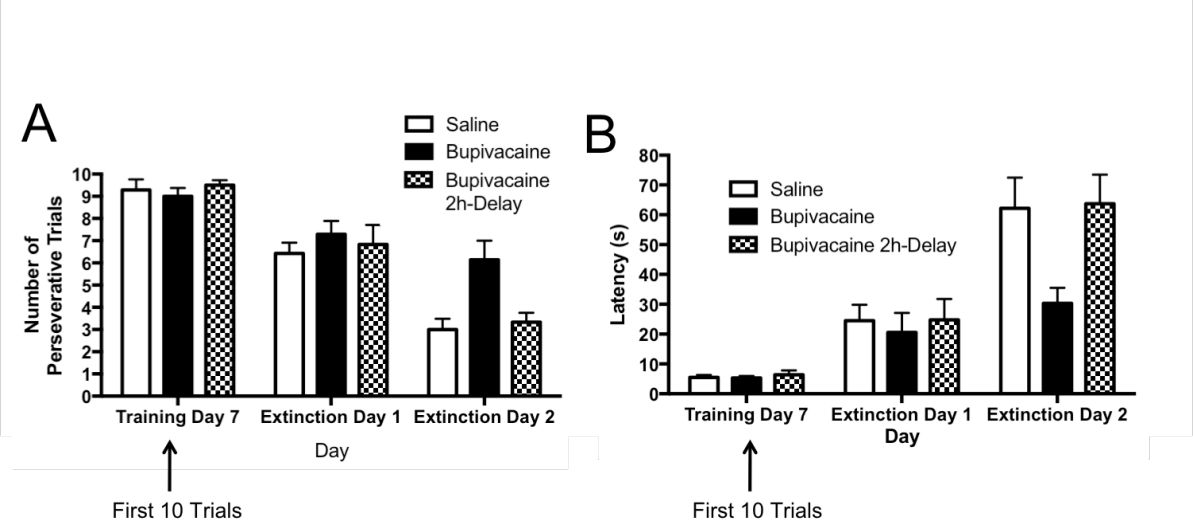

Figure 16. Influence of DLS inactivation on consolidation of extinction in the DLS-dependent response learning task. There were no differences between groups in perseveration (**A**) or latency (**B**) during the first few trials of the last acquisition day (i.e. training day 7) or during the first day of extinction training (i.e. extinction day 1). This indicates that there were no differences between groups before drugs were administered. On the second day of extinction training (Extinction Day 2, i.e. 24h after post-training drug administration), animals given immediate post-training DLS inactivation with bupivacaine displayed a significantly higher number of perseverative trials and lower latencies relative to animals given saline or delayed bupivacaine. Also, whereas the saline and bupivacaine-delay groups displayed a significant decrease in number of perseverative trials and an increase in latency across extinction days 1 and 2, animals given immediate DLS inactivation with bupivacaine did not show a significant change in extinction behavior across the two days of extinction, indicating a blockade of extinction. Results indicate that the DLS plays an essential role in the consolidation of extinction in the response learning task.

7.29) to Extinction Day 2 (M = 6.14), p = .144. These findings suggest that immediate post-training DLS inactivation with bupivacaine completely blocked consolidation of extinction in the response learning task, when measuring extinction with number of perseverative trials.

Average latency across extinction training is depicted in Figure 16B. The rationale for comparing extinction performance with the first 10 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 3 X 3 ANOVA (Group X Day) was computed for latency across acquisition day 7 (first 10 trials), extinction day 1, and extinction day 2 (Figure 16B). Results indicated that there was no main effect of Group ($F(2, 17) = 2.51$, $p = .111$), but there was a significant main effect of Day ($F(2, 34) = 64.26$, $p < .001$) and a significant Group X Day interaction ($F(4, 34) = 4.13$, $p = .008$). These findings suggest that an effect of Group on latency may only be observable on certain days.

A one-way ANOVA indicated that there was no main effect of Group on latency during the last acquisition day (i.e. Training Day 7; first 10 trials; $F(2, 17) = .34$, $p = .714$) or on Extinction Day 1 ($F(2, 17) = .14$, $p = .868$). These findings suggest that all groups had similar baseline measures of latency before drugs were administered. A two-way 3 X 2 (Group X Day) ANOVA computed on latency across Training Day 7 and Extinction Day 1 indicated an effect of Day ($F(1, 17) = 24.71$, $p < .001$), but no main effect of Group ($F(2, 17) = .18$, $p = .836$) and no Group X Day interaction ($F(2, 17) = .11$, $p = .899$). Thus, all groups displayed a comparable increase in latency from

Training Day 7 to Extinction Day 1. This indicates that all groups extinguished about equally before drugs were administered.

A one-way ANOVA computed on latency during Extinction Day 2 (i.e. 24h *after* post-training drug administration) indicated a main effect of Group ($F(2, 17) = 4.92$, $p = .021$). Fisher's LSD test indicated that animals given immediate post-training bupivacaine displayed lower running latencies ($M = 30.31$) than control animals given saline ($M = 62.19$), $p = .016$. In contrast, animals given bupivacaine after a 2h delay displayed comparable latency ($M = 63.73$) relative to animals given saline, $p = .902$. These findings suggest that immediate post-training DLS inactivation with bupivacaine, but not bupivacaine administered after a 2h delay, impaired extinction of response learning, as measured by latency to reach the previously correct food well.

A two-way 3 X 2 (Group X Day) ANOVA computed on latency across Extinction Day 1 and Extinction Day 2 did not reveal a significant main effect of Group ($F(2, 17) = 2.60$, $p = .103$), but there was a significant main effect of Day ($F(1, 17) = 49.51$, $p < .001$) and a significant Group X Day interaction ($F(2, 17) = 5.57$, $p = .014$). Fisher's LSD test indicated that the saline group displayed a significant increase in latency from Extinction Day 1 ($M = 24.47$) to Extinction Day 2 ($M = 62.19$), $p < .001$. Animals given delayed bupivacaine also displayed a significant increase in latency from Extinction Day 1 ($M = 24.78$) to Extinction Day 2 ($M = 63.73$), $p < .001$. In contrast, animals given immediate post-training bupivacaine did not show a significant increase in latency from Extinction Day 1 ($M = 20.57$) to Extinction Day 2 ($M = 30.31$), $p = .176$. These findings suggest that immediate post-training DLS inactivation with bupivacaine

completely blocked consolidation of extinction in the response learning task, when extinction is operationalized as an increase in latency.

6.3.2 Experiment 4: Place Learning Task

Initial acquisition: Initial acquisition of the place learning task is depicted in Figure 18. A two-way repeated measures 2 X 7 ANOVA (Group X Day) computed on percentage of correct turning responses over the course of training (Figure 18A) indicated a significant main effect of Day ($F(6, 72) = 21.91$, $p < .001$), but no effect of Group ($F(1, 12) = .58$, $p = .455$) and no Group X Day interaction ($F(6, 72) = .49$, $p = .992$). Likewise, a 2 X 7 ANOVA (Group X Day) computed on latency (Figure 18B) indicated a significant effect of Day ($F(6, 72) = 43.30$, $p < .001$), but no effect of Group ($F(1, 12) = .00$, $p = .994$) and no Group X Day interaction ($F(6, 72) = .30$, $p = .932$). Together, these results indicate that all groups acquired the task about equally over the course of training, and any subsequent differences between groups during extinction may not be readily attributed to differing rates of initial task acquisition.

Extinction: Number of perseverative trials across extinction training is depicted in Figure 19A. The rationale for comparing extinction performance with the first 10 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 2 X 3 ANOVA (Group X Day) was computed for number of perseverative trials across acquisition day 7 (first 10 trials), extinction day 1, and extinction day 2 (Figure 19A). Results indicated a main effect of Day ($F(2, 24) =$

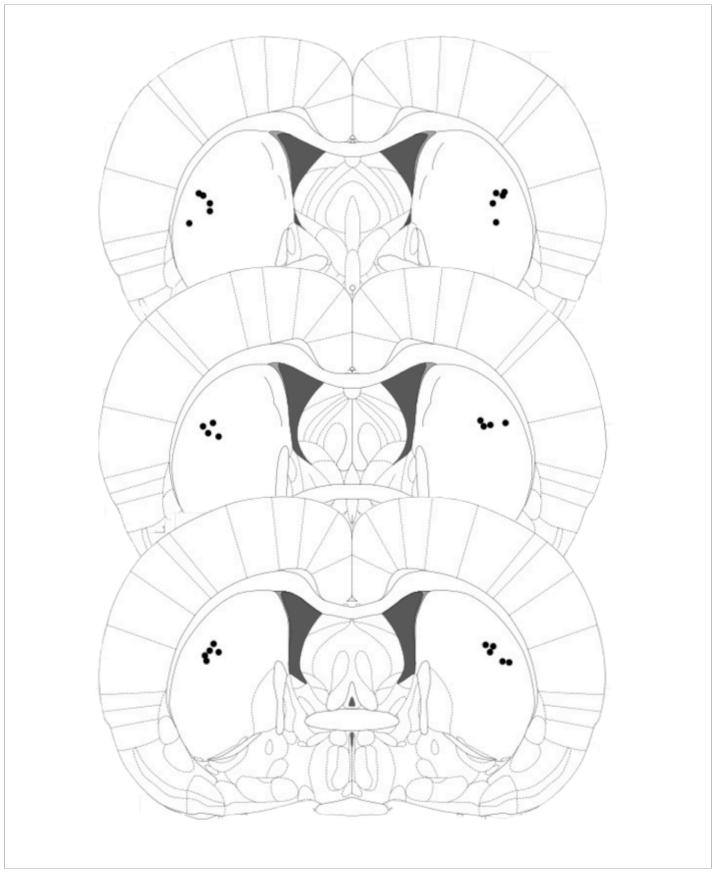

Figure 17. Injection needle placements in the DLS. Dorsolateral striatum cannula placements for experiment 4 showing the anterior/posterior extent of needle tip locations at 40-µm sections. Placements ranged from +0.00 to –0.36 mm from bregma. Images were adapted from Paxinos and Watson, 2007.

58.95, $p < .001$), but no main effect of Group ($F(1, 12) = 1.59$, $p = .231$) and no significant Group X Day interaction ($F(2, 24) = 2.27$, $p = .125$).

An independent samples t-test indicated no difference in number of perseverative trials between the bupivacaine group and the saline group during the last acquisition day (i.e. Training Day 7; first 10 trials; $t(12) = .245$, $p = .811$) or on Extinction Day 1 ($t(12)$

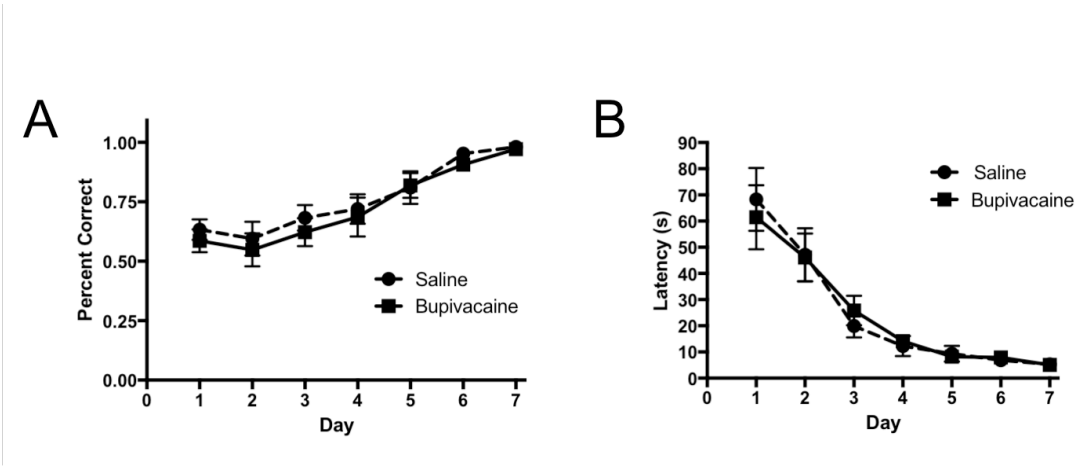

Figure 18. Acquisition of hippocampus-dependent place learning in the plus-maze. The percentage of correct turns increased (**A**) and the latency to reach the correct food well decreased (**B**) over the course of training, with no differences between groups.

= .47, p = .645). These findings suggest that both groups had similar baseline measures of perseveration during Training Day 7 and Exinction Day 1, i.e. before drugs were administered. A two-way 2 X 2 (Group X Day) ANOVA computed on perseverative trials across Training Day 7 and Extinction Day 1 indicated an effect of Day ($F(1, 12) = 16.76$, $p = .002$), but no main effect of Group ($F(1, 12) = .05$, $p = .820$) and no Group X Day interaction ($F(1, 12) = .40$, $p = .541$). Thus, both groups displayed a comparable decrease in the number of perseverative trials from Training Day 7 to Extinction Day 1. This indicates that both groups extinguished about equally before drugs were administered.

Interestingly, an independent samples t-test examining number of perseverative trials during Extinction Day 2 (i.e. 24h *after* post-training drug administration) indicated that animals given post-training bupivacaine displayed a significantly lower number of

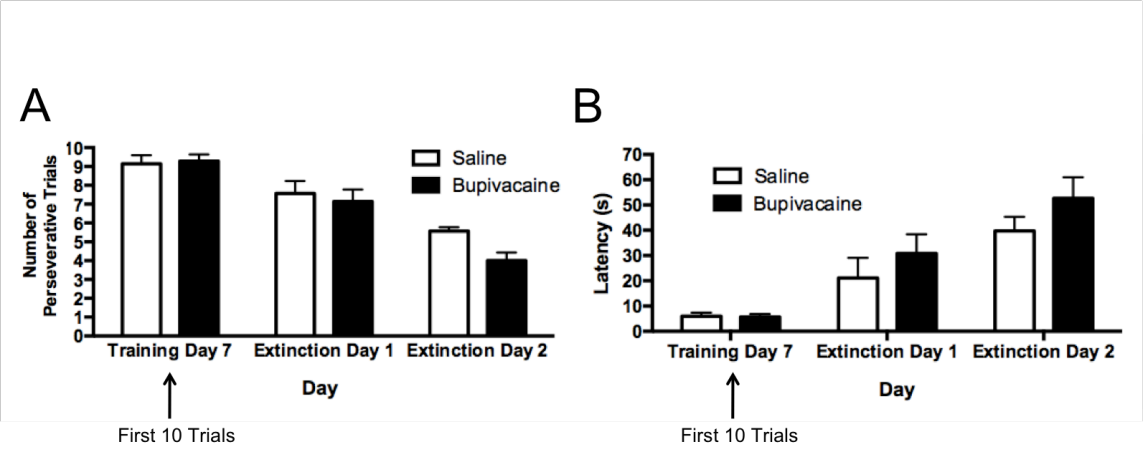

Figure 19. Influence of DLS inactivation on consolidation of extinction in the hippocampus-dependent place learning task. There was no difference between groups in perseveration (**A**) or latency (**B**) during the first ten trials of the last acquisition day (i.e. training day 7) or during the first day of extinction training (i.e. extinction day 1). This indicates that there were no differences between groups before drugs were administered. On the second day of extinction training (extinction day 2, i.e. 24h after post-training drug administration), animals given immediate post-training DLS inactivation with bupivacaine displayed a significantly lower number of perseverative trials relative to animals given saline. This suggests that DLS inactivation might have enhanced extinction of place learning. However, the bupivacaine and saline animals did not show a difference in latency on extinction day 2.

perseverative trials (M = 4.00) relative to animals given post-training saline (M = 5.57), $t(12) = 3.27$, $p = .007$. This suggests that DLS inactivation with bupivacaine *enhanced* extinction of place learning. However, a two-way 2 X 2 (Group X Day) ANOVA computed on number of perseverative trials across Extinction Day 1 and Extinction Day 2 indicated a main effect of Day ($F(1, 12) = 28.59$, $p < .001$), but no main effect of Group ($F(1, 12) = 3.38$, $p = .091$) and no significant Group X Day interaction ($F(1, 12) = 1.41$, $p = .258$). Thus, even though there was a difference between groups in the number of perseverative trials on Extinction Day 2, both groups displayed a comparable decrease

in number of perseverative trials across Extinction Days 1 and 2. Therefore, these findings do not provide conclusive evidence that DLS inactivation enhanced consolidation of extinction in a place learning task. However, these findings at the very least clearly indicate that DLS inactivation does not *impair* extinction of place learning, when examining extinction as a decrease in the number of perseverative trials.

Average latency across extinction training is depicted in Figure 19B. The rationale for comparing extinction performance with the first 10 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 2 X 3 ANOVA (Group X Day) was computed for latency across acquisition day 7 (first 10 trials), extinction day 1, and extinction day 2 (Figure 19B). Results indicated a main effect of Day ($F(2, 24) = 32.06$, $p < .001$), but no main effect of Group ($F(1, 12) = 1.36$, $p = .267$) and no significant Group X Day interaction ($F(2, 24) = .931$, $p = .408$).

An independent samples t-test indicated no difference in latency between the bupivacaine group and the saline group during the last acquisition day (i.e. Training Day 7; first 10 trials; $t(12) = .15$, $p = .885$) or on Extinction Day 1 ($t(12) = .88$, $p = .395$). These findings suggest that both groups had similar baseline measures of latency during Training Day 7 and Extinction Day 1, i.e. before drugs were administered. A two-way 2 X 2 (Group X Day) ANOVA computed on perseverative trials across Training Day 7 and Extinction Day 1 indicated an effect of Day ($F(1, 12) = 16.11$, $p = .002$), but no main effect of Group ($F(1, 12) = .60$, $p = .453$) and no Group X Day interaction ($F(1, 12) = .99$, $p = .339$). Thus, both groups displayed a comparable increase in latency from

Training Day 7 to Extinction Day 1. This indicates that both groups extinguished about equally before drugs were administered.

An independent samples t-test examining average latency during Extinction Day 2 (i.e. 24h *after* post-training drug administration) indicated no difference between animals in the bupivacaine group and animals in the saline group, $t(12) = 1.29$, $p = .221$. In addition, a two-way 2 X 2 (Group X Day) ANOVA computed on latency across Extinction Day 1 and Extinction Day 2 indicated a main effect of Day ($F(1, 12) = 13.30$, $p < .003$), but no main effect of Group ($F(1, 12) = 1.61$, $p = .229$) and no significant Group X Day interaction ($F(1, 12) = .08$, $p = .778$). Thus, in contrast to some evidence in the previous section on number of perseverative trials suggesting that DLS inactivation might enhance extinction, the present results examining latency do not provide evidence that DLS inactivation enhanced extinction of place learning task. However, these findings at the very least clearly indicate that DLS inactivation does not *impair* extinction of place learning, when examining extinction as an increase in latency to reach the previously correct food well.

6.4 Discussion

6.4.1 Summary of Findings

The present findings indicate a single dissociation regarding the role of the DLS in extinction of different kinds of memory. Post-training DLS inactivation completely blocked consolidation of extinction in the response learning task, but did not negatively

influence consolidation of extinction in the place learning task. The present findings are the first to identify a neural substrate that selectively mediates extinction of response learning. Although previous findings have implicated the DLS in extinction, the present findings are the first to indicate that the DLS may be specifically required for extinction of DLS-dependent habit memory and not hippocampus-dependent cognitive memory.

Following initial acquisition in the response learning task (experiment 3), animals given one day of extinction training and then immediate post-training inactivation of the DLS with bupivacaine displayed a higher number of perseverative trials and lower latencies to reach the previously correct food well, relative to animals in the saline group, on the subsequent day of extinction training. This suggests that DLS inactivation impaired extinction of response learning. In contrast, animals given the post-training intra-DLS injection of bupivacaine two hours after extinction training on day 1 (i.e. presumably after the consolidation phase has ended) did not display an impairment in extinction. This suggests that the impairing effect of immediate post-training DLS inactivation may be attributed to an impairment in *consolidation* of the extinction memory, rather than to some pro-active effect of DLS inactivation. In addition, the use of post-training drug administration rules out the possibility that the ostensible impairing effect of DLS inactivation may be readily attributed to disruptions in non-mnemonic processes (e.g. sensory or motoric processes) that transpired during extinction training. Moreover, within-group analyses revealed that animals given post-training DLS inactivation did not show a significant change in extinction behavior across the two days of extinction training, whereas saline control animals and delayed-

bupivacaine animals demonstrated a reduction in perseverative trials and an increase in latency. This suggests that immediate post-training DLS inactivation completely blocked extinction of response learning. Thus, the DLS may be considered a critical neural substrate for extinction of this kind of memory.

In contrast, in experiment 4, immediate post-training DLS inactivation did not impair extinction of place learning. The reduction in the number of perseverative trials and the increase in latency across the two days of extinction training were about the same regardless of whether animals received intra-DLS bupivacaine or saline. An interesting finding however was that, when examining performance on the second day of extinction training by itself, animals given post-training DLS inactivation displayed a significantly lower number of perseverative trials, relative to the saline control animals, suggesting that DLS inactivation might have *enhanced* extinction in the place learning task. However, this finding is contradicted by the observation that there was no significant difference between groups in the *decrease* in perseverative trials across Extinction Days 1 and 2; however, it could be argued that there was a trend toward a significant difference ($p = .091$). The suggestion that DLS inactivation enhanced extinction of place learning is further undermined by the observation that there was no significant difference in latency between the two groups, both when examining the increase in latency across Extinction Days 1 and 2 or when examining Extinction Day 2 by itself. Thus, although the present findings show some evidence that DLS inactivation might have enhanced extinction of place learning, other findings from the present study do not support this suggestion.

It is worth emphasizing that the purpose of the present study was not to examine whether there would be an enhancing effect of DLS inactivation on place learning. Indeed, the design for the present experiments was not ideal for examining *enhancements* in extinction, and this may be part of the reason why the findings in experiment 4 suggesting an enhancement in extinction of place learning were equivocal. It would be useful for future studies to replicate this experiment, while using fewer extinction trials so that control animals would show slower extinction, thereby creating conditions more conducive to examining a potential drug-induced enhancement of extinction learning. Although the findings of experiment 4 do not provide conclusive evidence that DLS inactivation enhanced extinction of place learning, the findings provide strong evidence that DLS activity is not *required* for extinction of place learning, which was the principal purpose of the present experiment.

6.4.2 A Role for the DLS in Extinction

The present findings provide cogent evidence that the DLS may be an important neural structure mediating extinction of specific kinds of memory. The DLS has been implicated in extinction in a variety of tasks, but the specific kind of memory that was being extinguished in each of these previous tasks remains unknown. For instance, post-training inactivation of the DLS impairs consolidation of extinction in a simple water T-maze task (Campus et al., 2015). Either DLS-dependent habit memory or hippocampus-dependent cognitive strategies can be employed to guide successful acquisition in the T-maze (e.g. Packard and McGaugh, 1996). Therefore it is difficult to determine whether

the impairing effect of DLS inactivation on consolidation of extinction in the T-maze may be attributed to an impairment in extinction of DLS-dependent response learning or hippocampus-dependent place learning. However, DLS-dependent response learning strategies are acquired quickly in the water maze version of the T-maze, and thus it is possible that the impairing effect of DLS inactivation on extinction in the water T-maze (Campus et al., 2015) may be attributed to an impairment in extinction of response learning. The DLS has also been implicated in extinction of straight alley maze performance (Dunnett and Iversen, 1981; Thullier et al., 1996; Gabriele, 2008), and like the T-maze, the straight alley maze may be acquired using DLS-dependent response learning or hippocampus-dependent place learning strategies (Kirkby, Polgar, and Coyle, 1981; Dunnett and Iversen, 1981; Rawlins, Feldon, Ursin, and Gray, 1985). In light of the present findings, it is likely that the extinction impairing effect of DLS inactivation in the T-maze and straight alley maze may be attributed to selective disruption of extinction of the DLS-dependent response learning memory rather than the hippocampus-dependent place learning component of these tasks.

A noteworthy finding of the present study was that DLS inactivation completely blocked extinction of DLS-dependent response learning. This was not observed in a previous study examining the effect of DLS inactivation on extinction of straight alley maze performance (Gabriele, 2008). In this previous study, following acquisition in the straight alley maze, DLS inactivation was only associated with an attenuation in extinction of the running approach response. Considering the observation that initial acquisition of straight alley maze performance may involve both hippocampus- and

DLS-dependent memory mechanisms, it is possible that during extinction in the straight alley the DLS is only involved in mediating extinction of the DLS-dependent memory component of the running approach response, while the hippocampus may be involved in extinguishing the place learning component of the original memory. Thus, DLS inactivation may have only blocked extinction of the response learning memory but not the place learning memory in the straight alley maze, thereby merely attenuating overall extinction behavior. The hippocampus may be involved in mediating extinction of the place learning component of straight alley maze performance, consistent with previous evidence implicating the hippocampus in extinction of spatial memory (e.g. Toumane et al., 1987, 1988; Lattal, Mullen, and Abel, 2003; Gabriele and Packard, 2006; Goodman et al., 2016). DLS inactivation in the present study may have been able to completely block extinction of response learning, because response learning only involves DLS-dependent memory, not hippocampus-dependent memory.

As discussed previously, some evidence from the present study indicates that DLS inactivation may have *enhanced* extinction of hippocampus-dependent place learning. While the absolute veracity of this finding has yet to be rigorously examined, a potential mechanism behind this presumed effect may be related to a competitive interaction between DLS- and hippocampus-dependent memory systems (Poldrack and Packard, 2003). Competitive interactions between memory systems become evident when, for instance, lesioning a part of the brain that mediates one kind of memory enhances function of another intact memory system. For example, in some learning situations lesions of the hippocampal system enhance acquisition in DLS-dependent

memory tasks (Packard, Hirsh, and White, 1989; McDonald and White, 1993; Schroeder, Wingard, and Packard, 2002; Chang and Gold, 2003a), whereas dorsal striatal lesions facilitate acquisition in some hippocampus-dependent spatial memory tasks (Mitchell and Hall, 1988; Lee, Duman, and Pittenger, 2008; Kosaki et al., 2015). Thus, in the intact brain, memory systems may sometimes interfere with one another, and lesioning one system may block this interference. For instance, spatial memory processes may compel the animal to seek a spatial location that contained food reward on a previous trial, which would lead to errors in a task where animals must make a consistent turning response to spatially opposed goal locations, as in the DLS-dependent response learning task. Lesioning the hippocampus-dependent memory system may thus enhance acquisition in the response learning task by blocking this spatial interference (Schroeder et al., 2002; Chang and Gold, 2003).

Competitive interactions between memory systems have been demonstrated primarily during acquisition and consolidation of memory, whereas these interactions have not been examined extensively during extinction. The present observation in experiment 4 suggesting that DLS inactivation may have enhanced extinction of place learning may be attributed to a competitive interaction between memory systems during extinction learning. That is, in the intact brain, DLS activity may interfere with extinction of place learning, whereas removing this interference through DLS inactivation may enhance extinction in the place learning task. It is difficult to speculate what the nature of this presumed DLS interference may be. One possibility is that the DLS subserves a kind of extinction learning that is not optimal for producing a response

decrement in the place learning task. Removal of this sub-optimal kind of extinction learning through DLS inactivation may allow another brain region (e.g. the hippocampus) to seize control and apply a more effective kind of extinction learning.

Interestingly, similar to the present study, a previous experiment indicated that DLS lesions enhanced extinction in a spatial alternation task (Moussa et al., 2011). The authors suggested that in the intact brain the DLS might be involved in S-R associations that interfere with extinction of the spatial alternation. Thus, lesioning the DLS may remove the ability of stimuli in the learning environment to activate turning behavior, thus enhancing extinction in this task. Moreover, the authors speculated that removal of DLS-mediated S-R interference may lead to greater sensitivity to changes in the action-outcome contingency (see Yin, Knowlton, and Balleine, 2006), which might allow for faster extinction. This previous finding (Moussa et al., 2011), as well as the potential mechanism involving DLS interference, provides some precedent for the current, albeit inconclusive, finding that DLS inactivation might enhance extinction of place learning.

6.4.3 DLS Mechanisms of Extinction

There are multiple potential learning mechanisms that the DLS could subserve in mediating extinction of response learning. As discussed at length in previous sections of this dissertation, multiple investigators have proposed response-produced inhibition theories of extinction learning, including the hypothesis that extinction may be achieved through acquisition of a novel inhibitory S-R association (Hull, 1943; Rescorla, 2001). According to this view, during extinction training, cues in the learning environment may

become associated with an inhibition of the original response. This proposed S-R mechanism is at least consistent with observations from experiments 1 and 2 indicating that in order for extinction of response learning to occur, the animal must have the opportunity to make the previously acquired response (see previous chapter). Given that the DLS has been critically implicated in acquisition of S-R learning, the DLS may be needed for extinction to the extent that S-R learning mechanisms are involved. Importantly, the DLS may not mediate changes in expectation during extinction training given that DLS inactivation does not influence latent extinction (Gabriele, 2008).

Another potential mechanism involves learned avoidance. According to the frustration theory of extinction (Amsel, 1962), animals become frustrated when they no longer receive a palatable food reinforcer during extinction training and therefore learn to avoid a certain place or behavior in order to avoid frustration. This proposed mechanism might be similar to the kind of learning underlying inhibitory avoidance, whereby the animal learns to avoid crossing a boundary into another compartment to avoid footshock. Acquisition of passive avoidance and active avoidance is partially mediated by DLS function (Salado-Castillo et al., 1996; Wendler et al., 2014), and it is possible that during extinction training the DLS may subserve a similar kind of avoidance learning, in which the turning response is suppressed in order to avoid the frustrating consequence of not receiving reinforcement.

By the same token, it is also possible that as the animal learns to avoid making the original response, the animal may also learn to execute a new response that does not produce frustration (e.g. the opposite body-turn response). To the extent that the DLS

has been critically implicated in initial acquisition of response learning, one possibility is that during extinction training this brain region may also be involved in acquiring a novel response that competes with the old response. Indeed, DLS function may be required for reversal learning in a T-maze task (Rueda-Orozco et al., 2008b), and the DLS has also been implicated in acquiring quick changes in strategy following omission of reinforcement (Skelin et al., 2014).

6.4.4 Conclusion

The present findings indicate that DLS inactivation completely blocked extinction of response learning, but not extinction of place learning. This is the first demonstration of a neural substrate that selectively mediates extinction of habit memory. The actual learning mechanisms subserved by the DLS that promote extinction of response learning remain unexamined, but there is some reason to believe that mechanisms involving response-produced inhibition, such as those discussed above, might play a role. In addition, the present experiments have not considered what precise neural mechanisms occur in the DLS to support extinction of response learning. Future research should examine potential neurotransmitter systems (see next chapter), as well as the changes in DLS synaptic plasticity that accompany extinction learning in this task.

Multiple investigators have suggested that the habit-like behavioral features of some human psychopathologies (e.g. drug addiction and relapse) may reflect heightened engagement of the DLS-dependent habit memory system (Goodman and Packard, 2016). The present findings might be useful in understanding what neural systems mediate the

successful extinction of maladaptive habit memory. Indeed, the same brain region involved in *initial acquisition* of habit memory (i.e. the DLS) may also be involved in *extinction* of habit memory, and therefore behavioral and pharmacological treatments that target this brain region might be used to effectively alleviate bad habits in human psychopathology.

CHAPTER VII

AIM 3: A NEUROTRANSMITTER SYSTEM OF HABIT MEMORY EXTINCTION

7.1 Introduction

The previous experiments described in this dissertation have suggested some important behavioral and neurobiological mechanisms supporting extinction of habit memory. However, these previous experiments have not considered what neurotransmitter systems might be involved. As reviewed at length in Chapter IV, multiple neurotransmitter systems in the DLS have been associated with *initial acquisition* and *consolidation* of response learning, including glutamate, acetylcholine, estrogen, and cannabinoid systems. To the extent that these neurotransmitter systems have been implicated in mnemonic functions of the DLS related to acquisition and consolidation, they might also comprise some of the DLS mechanisms supporting *extinction* of response learning.

Glutamate is the major excitatory neurotransmitter in the mammalian brain, and extensive research has indicated that the glutamatergic N-methyl-D-aspartate (NMDA) receptor in particular is critical for mediating multiple types of learning and memory (Morris, Anderson, Lynch, and Baudry, 1986; Miserendino, Sananes, Melia, and Davis, 1990). The role of NMDA receptor activity in learning and memory has been attributed to its role in experience-dependent modification of brain function, i.e. synaptic plasticity (Morris, 2013). NMDA receptors are required for induction, but not maintenance, of long-term potentiation and long-term depression in the hippocampus (Harris, Ganong,

and Cotman, 1984; Dudek and Bear, 1992), as well as long-term potentiation in the DLS (Li, Li, and Han, 2009). These synaptic changes have been hailed as likely neural underpinnings of learning and memory (Hebb, 1949; Bliss & Collingridge, 1993; Martin, Grimwood, and Morris, 2000; Kandel, 2001).

Several studies have indicated that initial acquisition and consolidation of DLS-dependent memory also depends on glutamatergic mechanisms (Packard and Teather, 1997; Corbit, Nie, and Janak, 2014). Specifically, acquisition/consolidation in a response-learning plus-maze task is disrupted following pre-training or post-training DLS infusions of the NMDA receptor antagonist AP5 (2*R*)-amino-5-phosphonopentanoate (AP5; Palencia and Ragozzino, 2005; Leong and Packard, 2013). In addition, systemic infusions of MK-801 (Mackes and Willner, 2006) or deletion of NMDA receptors from nigrostriatal dopaminergic neurons (Wang et al., 2011) may also influence the relative use of place and response learning in the plus-maze. Thus, multiple studies have linked glutamatergic NMDA receptor activity with initial acquisition or consolidation of response learning, but have not examined their role in *extinction* of this kind of memory.

Although the connection between NMDA receptor activity and extinction of response learning has yet to be made, extensive prior evidence has associated NMDA receptor activity with extinction in other learning and memory tasks. NMDA receptors are required for extinction of fear-potentiated startle (Falls, Miserendido, and Davis, 1992), Pavlovian fear conditioning (Baker and Azorlosa, 1996), and eyeblink conditioning (Kehoe, Macrae, and Hutchinson, 1996; Thompson and Disterhoft, 1997).

Given the role of NMDA receptors in both initial acquisition of response learning and extinction of other kinds of memory, it is reasonable to hypothesize that NMDA receptor activity within the DLS might also be required for extinction of response learning.

7.1.1 Experiment 5

The present aim examined whether NMDA receptors in the DLS play a role in extinction of response learning. Specifically experiment 5 examined whether NMDA receptor activity in the DLS is *required* for consolidation of extinction in the response learning task. This involved running animals in the response learning version of the plus-maze and then giving them extinction training. Immediately following the first day of extinction training, animals received intra-DLS infusions of AP5. AP5 is an NMDA receptor antagonist that inhibits NMDA receptor activity by competitively blocking the ligand binding site for glutamate. AP5 was chosen for the present experiment for several reasons. For one, this drug has been classically employed to show that various forms of synaptic plasticity and memory depend on NMDA receptor activation (Collingridge et al., 1983; Harris et al., 1984; Morris, 1986). In addition, this drug has been employed to demonstrate that NMDA receptors in the DLS are needed for initial acquisition/consolidation in the response learning versions of the plus-maze and Morris water maze (Packard and Teather, 1997; Palencia and Ragozzino, 2005; Leong and Packard, 2013).

Like experiments 3 and 4, the present experiments employed post-training drug administrations. The use of post-training administration presumably targets the

consolidation phase of the memory and also precludes drug administration from influencing online non-mnemonic processes that occur during acquisition. A role for NMDA receptors in the post-training consolidation phase of memory may be surprising given that NMDA receptors might only be needed during *induction* of synaptic plasticity, which may be considered the neural analogue for initial acquisition of memory. Nonetheless, previous research has demonstrated that post-training AP5 administration influences consolidation of different kinds of memory, including consolidation of DLS-dependent response learning (Packard and Teather, 1997; Leong and Packard, 2013). It is possible that plastic changes dependent on NMDA receptor activation occur in the DLS following initial acquisition, allowing the memory to be consolidated into long-term storage.

7.1.2 Experiment 6

Whereas experiment 5 examined whether NMDA receptor activity in the DLS is *required* for extinction of response learning, experiment 6 examined whether increasing activation of NMDA receptors might *enhance* extinction of response learning. Animals received training in the response learning task and then extinction training, similar to experiment 5. However, in experiment 6, animals were given fewer extinction trials to prevent behavioral extinction from occurring too fast, giving the animals more room to show enhancement of extinction following drug administration.

Similar to the other experiments in this dissertation, experiment 6 also employed post-training administration of drugs immediately after the first day of extinction

training. Animals received post-training administration of D-cycloserine (DCS). Historically, DCS was originally used to treat tuberculosis, but was later discovered to have agonist properties in the central nervous system. DCS is a partial NMDA receptor agonist that facilitates opening of the NMDA receptor by acting at the glycine binding site (Hood et al., 1989; Watson et al., 1990).

Consistent with the observation that NMDA receptors are critically involved in learning and memory, administration of DCS is associated with enhancement of different kinds of learning and memory. Notably, extensive research indicates that increasing NMDA receptor activation with DCS enhances extinction across a variety of tasks, including fear conditioning (Walker et al., 2002; Ledgerwood et al, 2003), conditioned taste aversion (Mickley et al., 2012), cocaine self-administration (Thanos et al., 2011), and latent extinction in the straight alley maze (Gabriele and Packard, 2007). Considering that these memory tasks serve as animal models of maladaptive memory formation in humans, DCS has been considered as a potential treatment for numerous human psychopathologies, to the extent that it might suppress or *extinguish* maladaptive memory in these disorders (for review, see Davis et al., 2006). Finally, it is worth mentioning that, as observed with NMDA receptor antagonists, post-training administration of DCS may also influence the *consolidation* of memory (Rodgers et al., 2011). Based on previous evidence, it was predicted that in the present study post-training intra-DLS administration of DCS would enhance consolidation of extinction in the response learning task.

7.2 Method

7.2.1 Subjects

The subjects were 52 male Long-Evans rats weighing 250-320 g upon arrival. Animals were subsequently food-restricted and maintained at 85% of the their ad lib weight throughout all behavioral procedures. Water was provided ad libitum. Animals were housed individually in a temperature-controlled vivarium with a 12 h light-dark cycle (lights on at 7AM), and all behavioral procedures were conducted during the light phase of this cycle. Age, weight, and housing conditions did not differ between animals in experiments 5 and 6. Animal use in this study was carried out in accordance with the ethical guidelines of the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University. The protocol was approved by IACUC.

7.2.2 Apparatus

The apparatus was the plus-maze used for experiments 1-4 (see previous two chapters).

7.2.3 Surgical Procedures

Animals were implanted with bilateral guide cannulae targeting the DLS, as described in experiments 5 and 6 (see previous chapter).

7.2.4 Histology

Histological procedures were employed to examine the actual target regions of the cannula implants, as described in experiments 5 and 6 (see previous chapter). Histologies for animals in experiment 5 are depicted in Figure 20. Histologies for animals in experiment 6 are depicted in Figure 23.

7.2.5 Drug and Injection Procedures

Drugs were administered with the same injection procedures used in our previous studies (Packard and Wingard, 2004; Wingard and Packard, 2008; Goodman and Packard, 2014). The NMDA receptor antagonist AP5 (Santa Cruz Biotechnology) was diluted with physiological saline at a dose of 2 μg/0.5 μl. This dose was selected based on previous evidence that intra-DLS administration of 2 μg AP5 impaired consolidation of DLS-dependent memory in the response learning plus-maze task and cued Morris water maze task (Packard and Teather, 1997; Leong and Packard, 2013). The NMDA receptor partial agonist DCS was diluted with physiological saline at a 10 and 20 μg/0.5 μl dose. These doses were selected based on previous evidence that intracerebral injections of DCS at these doses enhance extinction (Walker et al., 2002; Akirav et al., 2009; Peters and De Vries, 2013). Control animals received intra-DLS infusions of physiological saline.

Intra-DLS infusions were administered bilaterally over a period of 52 s via a microsyringe pump using 10μl Hamilton syringes connected to polyethylene tubing.

Following infusions, the injection needles (16mm) were left in the guide cannulae for an additional 60s to allow for diffusion of drug from the needle tip.

7.2.6 Behavioral Procedures

In experiments 5 and 6, animals received habituation, training in the response learning task, and extinction training using behavioral procedures identical to those described in experiments 3 (see previous chapter). The only exception was that in experiment 6, animals only received 6 trials per day of extinction training, in order to provide more room to see an enhancement of extinction following drug administration.

In experiment 5, immediately after the first day of extinction training, animals received post-training intra-DLS administration of 2 μg AP5 (n = 8) or physiological saline (n = 7). A third group (n = 7) received intra-DLS administration of 2 μg AP5 two hours following training on Extinction Day 1 to control for potential proactive effects of drug administration.

In experiment 6, immediately after extinction training, animals received post-training intra-DLS administration of 10 μg DCS (n = 7), 20 μg DCS (n = 7), or physiological saline (n = 8). A third group (n = 8) received intra-DLS administration of 20 μg DCS two hours after training on Extinction Day 1, to control for potential proactive effects of drug administration. Whether post-training drug infusions influenced consolidation of the extinction memory would be determined by looking at average latency and number of perseverative trials on the *second* day of extinction training, i.e. 24 hours after drug administration.

7.3 Results

7.3.1 Experiment 5: Intra-DLS AP5

Initial acquisition: Initial acquisition of the response learning task is depicted in Figure 21. A two-way repeated measures 3 X 7 ANOVA (Group X Day) computed on percentage of correct turning responses over the course of training (Figure 21A) indicated a significant main effect of Day ($F(6, 114) = 21.90$, $p < .001$), but no effect of Group ($F(2, 19) = .10$, $p = .908$) and no Group X Day interaction ($F(12, 114) = .45$, $p = .940$). Likewise, a 3 X 7 ANOVA (Group X Day) computed on latency (Figure 21B) indicated a significant effect of Day ($F(6, 114) = 89.47$, $p < .001$), but no effect of Group ($F(2, 19) = .02$, $p = .979$) and no Group X Day interaction ($F(12, 114) = .29$, $p = .991$). Together, these results indicate that all groups acquired the task about equally over the course of training, and any subsequent differences between groups during extinction may not be readily attributed to differing rates of initial task acquisition.

Extinction: Number of perseverative trials across extinction training is depicted in Figure 22A. The rationale for comparing extinction performance with the first 10 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 3 X 3 ANOVA (Group X Day) was computed for number of perseverative trials across acquisition day 7 (first 10 trials), extinction day 1, and extinction day 2 (Figure 22A). Results indicated no significant main effect of Group ($F(2, 19) = .55$, $p = .583$), but there was a significant main effect of Day ($F(2, 38) = 80.16$, $p < .001$) and a significant Group X Day interaction ($F(4, 38) = 6.49$, $p < .001$). These findings suggest that an effect of Group may only be observable on certain days.

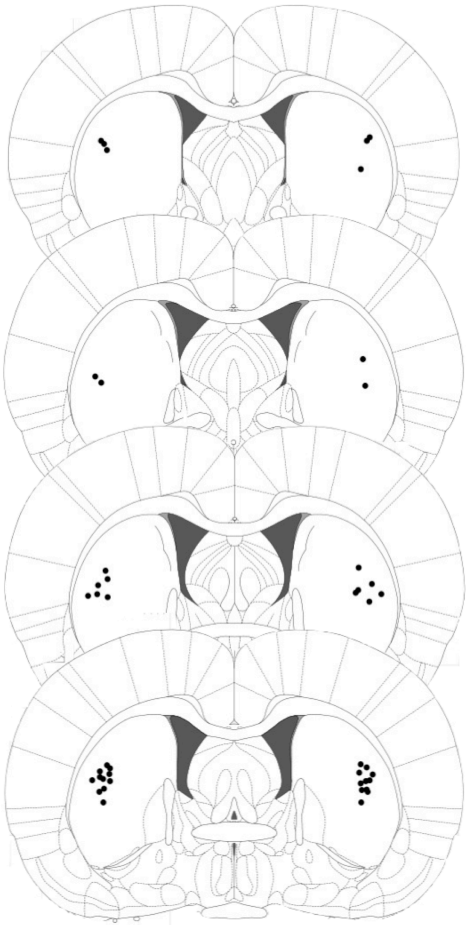

Figure 20. Injection needle placements in the DLS. Dorsolateral striatum cannula placements for experiment 5 showing the anterior/posterior extent of needle tip locations at 40-µm sections. Placements ranged from +0.24 to –0.36 mm from bregma. Images were adapted from Paxinos and Watson, 2007.

A one-way ANOVA indicated that there was no main effect of Group on number of perseverative trials during the last acquisition day (i.e. Training Day 7; first 10 trials; $F(2, 19) = 1.32$, $p = .292$) or on Extinction Day 1 ($F(2, 19) = .36$, $p = .705$). These findings suggest that all groups had similar baseline measures of perseveration during

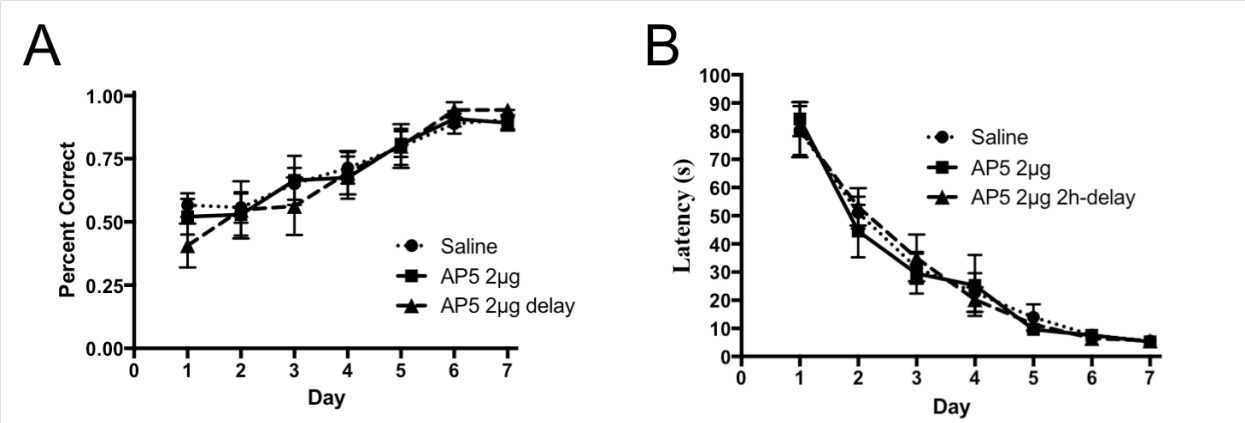

Figure 21. Acquisition of DLS-dependent response learning in the plus-maze. The percentage of correct turns increased (**A**) and the latency to reach the correct food well decreased (**B**) over the course of training, with no differences between groups.

Training Day 7 and Extinction Day 1, i.e. before drugs were administered. A two-way 3 X 2 (Group X Day) ANOVA computed on perseverative trials across Training Day 7 and Extinction Day 1 indicated an effect of Day ($F(1, 19) = 27.04$, $p < .001$), but no main effect of Group ($F(2, 19) = .83$, $p = .450$) and no Group X Day interaction ($F(2, 19) = .27$, $p = .768$). Thus, all groups displayed a comparable decrease in the number of perseverative trials from Training Day 7 to Extinction Day 1. This indicates that all groups extinguished about equally before drugs were administered.

A one-way ANOVA computed on number of perseverative trials during Extinction Day 2 (i.e. 24h *after* post-training drug administration) indicated a main effect of Group ($F(2, 19) = 4.45$, $p = .026$). Fisher's LSD test indicated that animals given immediate post-training 2 μg AP5 displayed a higher number of perseverative trials ($M = 6.63$) than control animals given saline ($M = 4.14$), $p = .027$. In contrast,

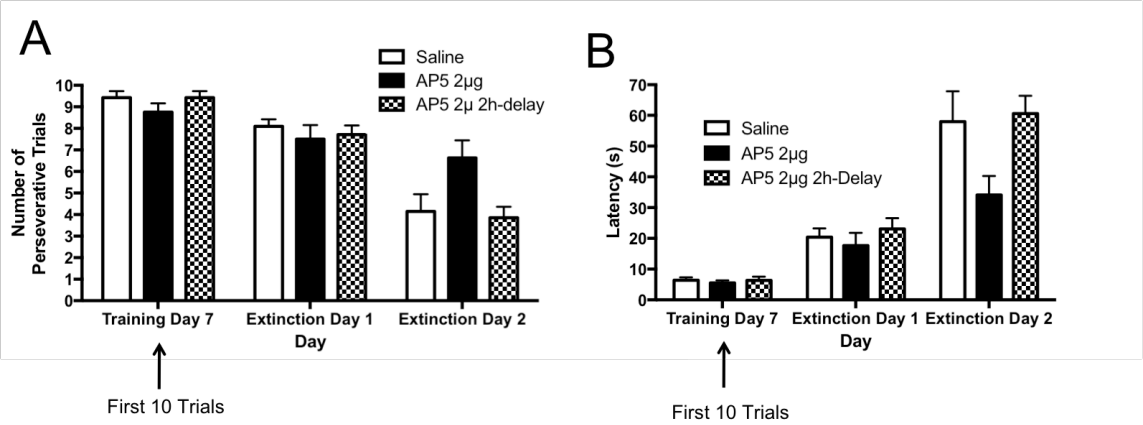

Figure 22. The influence of intra-DLS AP5 on consolidation of extinction in the response learning task. There were no differences between groups in perseveration (**A**) or latency (**B**) during the first ten trials of the last acquisition day (i.e. training day 7) or during the first day of extinction training (i.e. extinction day 1). This indicates that there were no differences between groups before drugs were administered. On the second day of extinction training (Extinction Day 2, i.e. 24h after post-training drug administration), intra-DLS NMDA receptor blockade with AP5 increased number of perseverative trials and reduced latencies relative to animals given saline or delayed bupivacaine. Results indicate that the DLS NMDA receptors play an essential role in the consolidation of extinction in the response learning task.

animals given 2 µg AP5 after a 2h delay displayed a comparable number of perseverative trials (M = 3.86) relative to animals given saline, p = .792. These findings suggest that immediate post-training blockade of NMDA receptors with AP5, but not AP5 administered after a 2h delay, impaired extinction of response learning, as measured by number of perseverative trials.

A two-way 3 X 2 (Group X Day) ANOVA computed on number of perseverative trials across Extinction Day 1 and Extinction Day 2 indicated no main effect of Group ($F(2, 19) = 1.37$, $p = .278$), but there was a main effect of Day ($F(1, 19) = 92.24$, $p < .001$) and a significant Group X Day interaction ($F(2, 19) = 11.74$, $p < .001$). Fisher's

LSD test indicated that the saline group displayed a significant decrease in number of perseverative trials from Extinction Day 1 (M = 8.10) to Extinction Day 2 (M = 4.14), $p < .001$. Animals given delayed 2 μg AP5 also displayed a significant decrease in perseverative trials from Extinction Day 1 (M = 7.71) to Extinction Day 2 (M = 3.86), $p < .001$. However, animals given immediate post-training 2 μg AP5 did not show a significant reduction in the number of perseverative trials from Extinction Day 1 (M = 7.50) to Extinction Day 2 (M = 6.63), but it could be argued that there was a trend toward significance, $p = .096$. These findings suggest that blocking NMDA receptor activity in the DLS with AP5 may have blocked consolidation of extinction in the response learning task, when measuring extinction as a reduction in the number of perseverative trials.

Average latency across extinction training is depicted in Figure 22B. The rationale for comparing extinction performance with the first 10 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 3 X 3 ANOVA (Group X Day) was computed for latency across acquisition day 7 (first 10 trials), extinction day 1, and extinction day 2 (Figure 22B). Results indicated that there was a trend toward a significant main effect of Group ($F(2, 19) = 2.98$, $p = .075$), a main effect of Day ($F(2, 38) = 93.38$, $p < .001$), and a significant Group X Day interaction ($F(4, 38) = 3.65$, $p = .013$). Thus, an effect of Group on latency may only be observable on certain days.

A one-way ANOVA indicated that there was no main effect of Group on latency during the last acquisition day (i.e. Training Day 7; first 10 trials; $F(2, 19) = .23$, $p =$

.799) or on Extinction Day 1 ($F(2, 19) = .58$, $p = .568$). These findings suggest that all groups had similar baseline measures of latency before drugs were administered. A two-way 3 X 2 (Group X Day) ANOVA computed on latency across Training Day 7 and Extinction Day 1 indicated an effect of Day ($F(1, 19) = 58.47$, $p < .001$), but no main effect of Group ($F(2, 19) = .57$, $p = .574$) and no Group X Day interaction ($F(2, 19) = .53$, $p = .597$). Thus, all groups displayed a comparable increase in latency from Training Day 7 to Extinction Day 1. This indicates that all groups extinguished about equally before drugs were administered.

A one-way ANOVA computed on latency during Extinction Day 2 (i.e. 24h *after* post-training drug administration) indicated a main effect of Group ($F(2, 19) = 4.00$, $p = .036$). Fisher's LSD test indicated that animals given immediate post-training 2 μg AP5 displayed lower running latencies ($M = 34.12$) than control animals given saline ($M = 57.96$), $p = .034$. In contrast, animals given 2 μg AP5 after a 2h delay displayed comparable latency ($M = 60.56$) relative to animals given saline, $p = .811$. These findings suggest that immediate post-training blockade of NMDA receptor activity in the DLS with AP5, but not administration of AP5 after a 2h delay, impaired extinction of response learning, as measured by latency to reach the previously correct food well.

A two-way 3 X 2 (Group X Day) ANOVA computed on latency across Extinction Day 1 and Extinction Day 2 did not reveal a significant main effect of Group, although there was a trend toward significance ($F(2, 19) = 3.16$, $p = .065$). There was also a significant main effect of Day ($F(1, 19) = 68.29$, $p < .001$) and a significant Group X Day interaction ($F(2, 19) = 3.77$, $p = .042$). Fisher's LSD test indicated that the saline

group displayed a significant increase in latency from Extinction Day 1 (M = 20.41) to Extinction Day 2 (M = 57.96), $p < .001$. Animals given 2 μg AP5 also displayed a significant increase in latency from Extinction Day 1 (M = 17.65) to Extinction Day 2 (M = 34.12), $p = .014$. Finally, animals given delayed post-training AP5 also showed a significant increase in latency from Extinction Day 1 (M = 23.10) to Extinction Day 2 (M = 60.56), $p < .001$. These findings suggest that all groups displayed an increase in latency across Extinction Days 1 and 2, regardless of drug treatment. Thus, immediate post-training blockade of NMDA receptor activity in the DLS with AP5 did *not* appear to block consolidation of extinction in the response learning task, when extinction is operationalized as an increase in latency.

7.3.2 Experiment 6: Intra-DLS DCS

Initial acquisition: Initial acquisition of the response learning task is depicted in Figure 24. A two-way repeated measures 3 X 7 ANOVA (Group X Day) computed on percentage of correct turning responses over the course of training (Figure 24A) indicated a significant main effect of Day ($F(6, 156) = 105.40$, $p < .001$), but no effect of Group ($F(3, 26) = .53$, $p = .664$) and no Group X Day interaction ($F(18, 156) = 1.31$, $p = .191$). Likewise, a 3 X 7 ANOVA (Group X Day) computed on latency (Figure 24B) indicated a significant effect of Day ($F(6, 156) = 105.40$, $p < .001$), but no effect of Group ($F(3, 26) = .53$, $p = .664$) and no Group X Day interaction ($F(18, 156) = 1.31$, $p = .191$). Together, these results indicate that all groups acquired the task about equally

over the course of training, and any subsequent differences between groups during extinction may not be readily attributed to differing rates of initial task acquisition.

Extinction: Number of perseverative trials across extinction training is depicted in Figure 25A. The rationale for comparing extinction performance with the

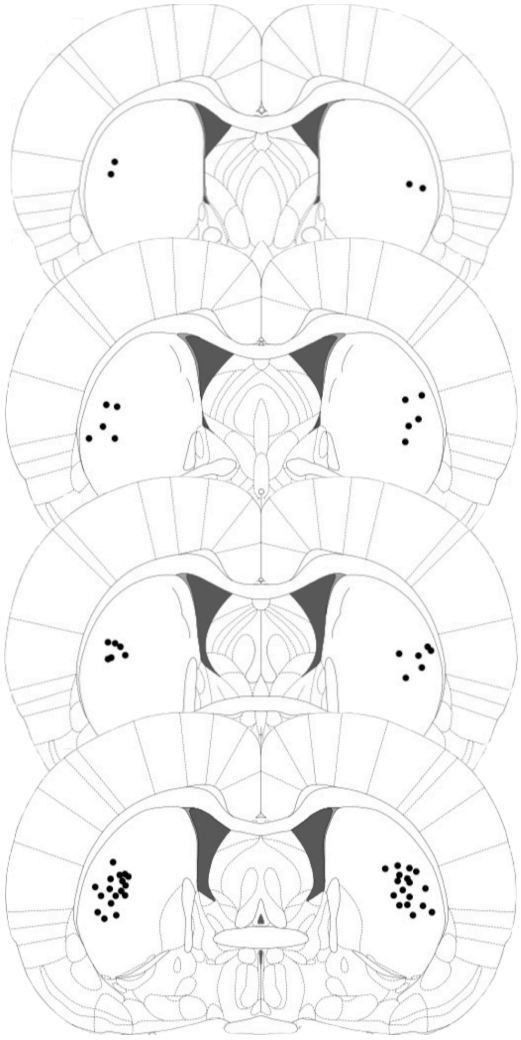

Figure 23. Injection needle placements in the DLS. Dorsolateral striatum cannula placements for experiment 6 showing the anterior/posterior extent of needle tip locations at 40-µm sections. Placements ranged from +0.24 to –0.36 mm from bregma. Images were adapted from Paxinos and Watson, 2007.

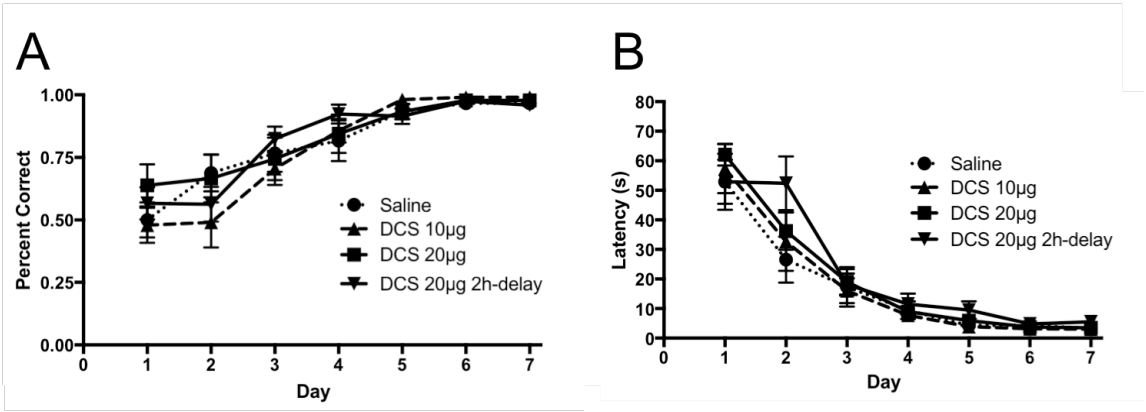

Figure 24. Acquisition in the response learning task before intra-DLS administration of DCS. The percentage of correct turns increased (**A**) and the latency to reach the correct food well decreased (**B**) over the course of training, with no differences between groups.

first 6 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 3 X 3 ANOVA (Group X Day) was computed for number of perseverative trials across acquisition day 7 (first 6 trials), extinction day 1, and extinction day 2 (Figure 25A). Results indicated no significant main effect of Group ($F(3, 26) = .83$, $p = .488$), but there was a significant main effect of Day ($F(2, 52) = 72.13$, $p < .001$) and a significant Group X Day interaction ($F(6, 52) = 2.64$, $p = .026$).

A one-way ANOVA indicated that there was no main effect of Group on number of perseverative trials during the last acquisition day (i.e. Training Day 7; first 6 trials; $F(3, 26) = .26$, $p = .854$) or on Extinction Day 1 ($F(3, 26) = .59$, $p = .629$). These findings suggest that all groups had similar baseline measures of perseveration during Training Day 7 and Extinction Day 1, i.e. before drugs were administered. A two-way 3 X 2 (Group X Day) ANOVA computed on perseverative trials across Training Day 7

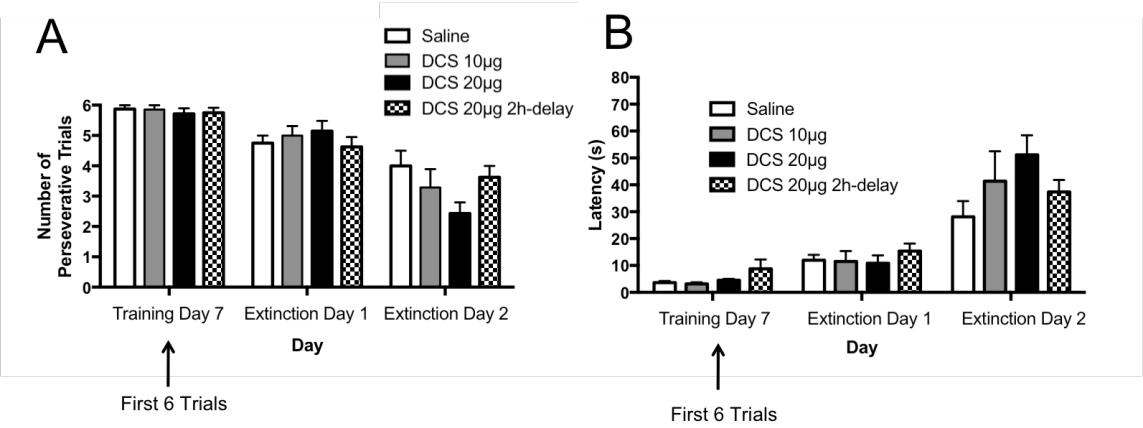

Figure 25. Influence of intra-DLS administration of DCS on extinction of response learning. There were no differences between groups in perseveration (**A**) or latency (**B**) during the first six trials of the last acquisition day (i.e. training day 7) or during the first day of extinction training (i.e. extinction day 1). This indicates that there were no differences between groups before drugs were administered. On the second day of extinction training (extinction day 2, i.e. 24h after post-training drug administration), increasing intra-DLS NMDA receptor activity with 20µg DCS, but not 10µg DCS, decreased number of perseverative trials and increased latencies relative to animals given saline or delayed 20µg DCS. Results indicate that increasing NMDA receptor activity in the DLS with the NMDA receptor partial agonist DCS enhances consolidation of extinction in the response learning task.

and Extinction Day 1 indicated an effect of Day ($F(1, 26) = 29.42$, $p < .001$), but no main effect of Group ($F(3, 26) = .44$, $p = .724$) and no Group X Day interaction ($F(3, 26) = .60$, $p = .623$). Thus, all groups displayed a comparable decrease in the number of perseverative trials from Training Day 7 to Extinction Day 1. This indicates that all groups extinguished about equally before drugs were administered.

A one-way ANOVA computed on number of perseverative trials during Extinction Day 2 (i.e. 24h *after* post-training drug administration) indicated a trend toward a significant main effect of Group ($F(3, 26) = 2.54$, $p = .078$). Fisher's LSD test

indicated that animals given immediate post-training 20 μg DCS displayed a lower number of perseverative trials (M = 2.29) than control animals given saline (M = 4.00), p = .014. In contrast, animals given immediate post-training 10 μg DCS (M = 3.29) or delayed administration of 20 μg DCS (M = 3.63) did not differ significantly from the saline group, p = .280 and p = .554, respectively. These findings suggest that increasing NMDA receptor activity in the DLS with 20 μg DCS, but not 10 μg DCS or delayed administration of 20 μg DCS, enhanced consolidation of extinction in the response learning task, as measured by number of perseverative trials.

Average latency across extinction training is depicted in Figure 28B. The rationale for comparing extinction performance with the first 6 trials of the final training day was described in the results for experiment 1 (see above). A two-way repeated measures 3 X 3 ANOVA (Group X Day) was computed for latency across acquisition day 7 (first 6 trials), extinction day 1, and extinction day 2 (Figure 28B). Results indicated that there was no main effect of Group ($F(3, 26) = 1.02$, $p = .399$), but there was a main effect of Day ($F(2, 52) = 77.58$, $p < .001$) and a trend toward a significant Group X Day interaction ($F(6, 52) = 2.05$, $p = .076$). Thus, an effect of Group on latency may only be observable on certain days.

A one-way ANOVA indicated that there was no main effect of Group on latency during the last acquisition day (i.e. Training Day 7; first 6 trials; $F(3, 26) = 1.82$, $p = .168$) or on Extinction Day 1 ($F(3, 26) = .50$, $p = .688$). These findings suggest that all groups had similar baseline measures of latency before drugs were administered. A two-way 3 X 2 (Group X Day) ANOVA computed on latency across Training Day 7 and

Extinction Day 1 indicated an effect of Day ($F(1, 26) = 24.44$, $p < .001$), but no main effect of Group ($F(3, 26) = 1.34$, $p = .282$) and no Group X Day interaction ($F(3, 26) = .13$, $p = .943$). Thus, all groups displayed a comparable increase in latency from Training Day 7 to Extinction Day 1. This indicates that all groups extinguished about equally before drugs were administered.

A one-way ANOVA computed on latency during Extinction Day 2 (i.e. 24h *after* post-training drug administration) indicated no main effect of Group ($F(3, 26) = 1.69$, $p = .193$). However, considering that the experimental design was constructed to make specific planned comparisons, the results of the ANOVA were ignored. Fisher's LSD test indicated that animals given immediate post-training 20 μg DCS displayed greater running latencies ($M = 51.19$) than control animals given saline ($M = 28.10$), $p = .035$. In contrast, animals given immediate post-training administration of 10 μg DCS ($M = 41.33$) or delayed post-training after a 2h delay displayed comparable latency ($M = 37.38$) relative to animals given saline, $p = .180$ and $p = .327$. These findings suggest that increasing NMDA receptor activity in the DLS with 20 μg DCS, but not 10 μg DCS or delayed administration of 20 μg DCS, enhanced consolidation of extinction in the response learning task, as measured by increases in latency.

7.4 Discussion

7.4.1 Summary of Findings

The present findings indicate bidirectional effects of modulating DLS NMDA receptor activity on extinction of response learning. Disrupting NMDA receptor activity in the DLS with the NMDA receptor antagonist AP5 impaired consolidation of extinction, whereas increasing NMDA receptor activity in the DLS with the NMDA receptor partial agonist DCS enhanced consolidation of extinction in the response learning task. To our knowledge, the present experiments are the first to identify a specific neurotransmitter system implicated in extinction of habit memory. In addition, the second experiment is the first to our knowledge to employ intra-DLS administrations of the NMDA receptor partial agonist DCS and demonstrate an effect on memory.

In experiment 5, following initial acquisition in the response learning plus-maze task, animals given one day of extinction training and then immediate post-training inactivation of NMDA receptors in the DLS with AP5 displayed a higher number of perseverative trials and lower latencies to reach the previously correct food well, compared to the saline group, on the second day of extinction training. These findings suggest that intra-DLS administration of NMDA receptor antagonist AP5 impaired extinction. In contrast, animals that received post-training intra-DLS administration of AP5 two hours after extinction training on day 1 (i.e. after the extinction memory has already been consolidated into long-term memory) did not show an impairment in extinction. This suggests that the memory impairment produced by immediate post-training AP5 may be attributed to an impairment in *consolidation* of the extinction

memory, rather than to some pro-active effect of the drug. The use of post-training drug administration also prevents the drug from influencing non-mnemonic processes that occur during extinction training, such as sensory, motivational, and motor processes. This makes it more likely that the drug specifically influenced *mnemonic* function. Findings from the within-group analyses revealed that, similar to control animals, animals given post-training AP5 displayed an increase in latency and a trending reduction in the number of perseverative trials across the two extinction days. Thus, in contrast to the complete blockade of extinction observed following post-training DLS inactivation, disrupting NMDA receptors in the present experiment only impaired—and did not block—extinction of response learning. It is possible that other neurotransmitter systems in the DLS may partially mediate extinction in the response learning task.

In experiment 6, animals given post-training intra-DLS administration of the NMDA receptor partial agonist DCS immediately after extinction training on day 1 displayed fewer perseverative trials and higher latencies to reach the previously correct food well on Extinction Day 2, relative to animals given saline. The effect of DCS on extinction was dose-dependent. Intra-DLS administration of DCS at the 20 μg dose, but not the 10 μg dose, enhanced extinction of response learning. In addition, the memory enhancing effect of post-training 20 μg DCS on extinction may be specifically attributed to an effect of the drug on *consolidation* of the extinction memory, given that administration of drug 2 hours after extinction training had no effect on memory. As previously noted, this is the first experiment to demonstrate an effect of intra-DLS administration of DCS. In a previous study, a drug similar in structure and function to

DCS (i.e. D-serine) was administered into the nucleus accumbens and DLS (Seif et al., 2015). Intra-accumbens injections of D-serine reduced compulsive alcohol drinking, whereas intra-DLS administration of D-serine was without effect (Seif et al., 2015). Another study indicated that intra-accumbens injections enhanced social reinforcement learning in prairie voles, whereas intra-amygdala or intra-DMS injections had no effect (Modi and Young, 2011).

7.4.2 Potential Mechanisms

The proposed mechanisms behind the role of the DLS in extinction of response learning have already been discussed ad nauseum. In the present dissertation, the possibility that the DLS mediates Hullian inhibitory S-R associations to promote the response decrement in extinction has itself received excessive attention. Thus, the potential behavioral mechanisms of the DLS's role in extinction of response learning will not be presently discussed. Of note, however, is that the present experiments provide important insight into the specific neurotransmitters systems arising in the DLS that are involved in extinction of response learning.

NMDA receptors are ligand-gated ionotropic glutamate receptors. During the period of resting potential, magnesium and zinc ions block activation of the receptor until the cell depolarizes, in which case these ions are released from the receptor. Following the removal of magnesium and zinc ions, the simultaneous activation of glutamate and glycine binding cites of the NMDA receptor opens the ion channel, allowing positively charged calcium ions to flow into the cell. The influx of calcium

ions promulgates a retinue of signaling cascades leading to neurotransmitter release and activation of various molecular substrates that promote gene expression and protein synthesis. These changes allow for long-lasting changes in synaptic plasticity, which may serve as a neural underpinning of learning and memory (Hebb, 1949; Bliss & Collingridge, 1993; Martin, Grimwood, and Morris, 2000; Kandel, 2001).

The present finding that intra-DLS administration of AP5 impaired extinction of response learning indicates that NMDA receptors may be critically implicated in this kind of memory. It is possible that the role of NMDA receptors in extinction of response learning may be attributed to their role in DLS synaptic plasticity. Activation of NMDA receptors is required for induction of long-term potentiation in the DLS (Li, Li, and Han, 2009), leading to the possibility that synaptic plasticity mechanisms may be involved in extinction of response learning. Synaptic plasticity in glutamatergic corticostriatal synapses could strengthen extinction of response learning by leading to greater glutamate release in the DLS, whereas blockade of synaptic plasticity with the NMDA receptor antagonist AP5 could impair this kind of memory. On the other hand, the NMDA receptor partial agonist DCS could enhance extinction of response learning by increasing corticostriatal plasticity.

Alternatively, NMDA receptors may participate in other mechanisms unrelated to synaptic plasticity in mediating consolidation of response learning. Indeed, glutamate has been associated with the release of multiple neurotransmitters, including norepinephrine (Navarro, Cabrera, & Donoso, 1995), dopamine (Krebs et al., 1991; Whitton, Maione, Biggs, & Fowler, 1994), and acetylcholine (Giovannini et al., 1995;

Login, Borland, Harrison, Ragozzino, & Gold, 1995), all of which have been associated with consolidation of DLS-dependent memory (Packard and White, 1989; Packard, Introini-Collison, and McGaugh, 1996; Packard and Gabriele, 2009).

7.4.3 Clinical Applications

DLS-dependent memory may be related to some behavioral symptoms in human psychopathology, in particular symptoms characterized by automatic, uncontrollable behaviors, i.e. habits. Specifically, multiple investigators have suggested that dysfunctional DLS-dependent memory function may be related to compulsive drug taking behaviors in drug addiction and relapse (Everitt and Robbins, 2005, 2013; Goodman and Packard, 2016), the compulsive behaviors in obsessive compulsive disorder (Gillan and Robbins, 2014), the repetitive and stereotypes behaviors in autism spectrum disorders (Goh and Peterson, 2012; Goodman et al., 2014), the behavioral tics in Tourette syndrome (Marsh et al., 2005), the avoidance behaviors in post-traumatic stress disorders (Goodman et al., 2012), and so forth. Extinction learning may serve as a model of memory suppression, including the suppression of maladaptive memories underlying the habit symptoms in psychopathology. Thus, the present findings may lead to a better understanding of the neural mechanisms underlying the suppression of maladaptive habits. NMDA receptors in the DLS and the consequent synaptic plasticity arising in this brain region may constitute some of the mechanisms through which habit-like symptoms in human psychopathology may be combated.

Considering that (1) extinction of DLS-dependent habit memory may serve as a model of habit memory suppression and that (2) DCS enhanced extinction of DLS-

dependent habit memory in the present study, DCS may be used as a pharmacological treatment to extinguish habit-like symptoms in psychopathology. Indeed, there is remarkable precedent for suggesting DCS may be used in suppressing maladaptive memory in human neuropsychiatric disorders. DCS enhances extinction in a multitude of animal learning paradigms that model maladaptive memory in human psychopathology, including extinction of conditioned fear (Walker et al., 2002; Ledgerwood et al, 2003) and cocaine self-administration (Thanos et al., 2011). DCS has also been implicated in extinction of straight alley maze performance (Gabriele and Packard, 2007) and conditioned taste aversion (Mickley et al., 2012). Investigators have also found some success in using DCS to treat maladaptive memory formation in humans. DCS enhances exposure therapy in patients with obsessive compulsive disorder (Kushner et al., 2007), post-traumatic stress disorder (Heresco-Levy et al., 2002), phobia disorders (Ressler et al., 2004), and drug addiction (Oliveto et al., 2003). Consistent with the view that the neurobiological profile of these disorders may include maladaptive DLS-dependent memory processes, it is possible that the role of DCS in these experiments may be partially attributed to DCS enhancing extinction of habit memory through activation of NMDA receptors in the DLS.

7.4.4 Conclusion

The present findings indicate that modulation of NMDA receptor activity in the DLS may have bidirectional effects on extinction of response learning. Decreasing DLS NMDA receptor activity impairs extinction, whereas increasing DLS NMDA receptor

activity enhances extinction of response learning. These effects may be attributed to modulation of NMDA-receptor dependent forms of synaptic plasticity arising in the DLS. The present findings may be useful in understanding the neurobiological mechanisms through which habit memory might be suppressed, and DCS may be used as a pharmacological adjunct to exposure therapy in disorders characterized by strong, habit-like behavioral features.

CHAPTER VIII

SUMMARY AND GENERAL DISCUSSION

8.1 Summary of Dissertation Findings

The experiments of the present dissertation project have revealed novel findings pertaining to the behavioral and neurobiological mechanisms guiding extinction of habit memory in a response learning task. The experiments in the first aim indicated that in order for extinction of response learning to occur, the animal must be given the opportunity to perform the original to-be-extinguished behavior. The experiments in the second aim indicated that the DLS selectively mediates extinction of response learning, and experiments in the third aim indicated that the role of the DLS in extinction of response learning may be more specifically attributed to NMDA receptor activity in this brain region. The findings from each aim will be summarized in more detail presently.

8.1.1 Aim 1: Behavioral Mechanisms Underlying Extinction of Response learning

The findings from the first aim indicated that not all kinds of extinction training are effective at targeting habit memory in the response learning task. The latent extinction protocol, in which an animal is placed in the original goal location without reinforcement, was not effective at extinguishing memory in a DLS-dependent response learning task, but was effective at extinguishing memory in a hippocampus-dependent place learning task. Typical "response extinction," in which the animal is given the

opportunity to make the original response to the unreinforced goal location was effective in both place and response learning tasks. These findings suggest that the animal must be afforded the opportunity to perform the original response during extinction training for extinction of DLS-dependent habit memory to occur. In contrast, giving the animal the opportunity to form a new expectation that the goal location no longer contains food (as during latent extinction) is not effective at targeting DLS-dependent habit memory.

These experiments provide some insight into the behavioral mechanisms underlying extinction of response learning. During typical response extinction training, it could be argued that the response decrement is achieved through Hullian S-R mechanisms (i.e. the animal forms a new inhibitory response). There are, however, a few alternative mechanisms that could be at play during response extinction: (1) the animal could learn that the spatial goal location no longer contains food; the animal could acquire a new Pavlovian association between cues readily viewable in the goal box and the absence of reinforcement; or the animals could learn that the turning response no longer results in a favorable outcome (this would suggest a change in the response-outcome contingency). The observation that confining animals to the empty goal box (i.e. latent extinction) was not effective suggests that the alternative spatial and Pavlovian explanations might not account for extinction in the response learning task.

The possibility that changes in the response-outcome contingency guided extinction of response learning was not directly examined in the present study; however, there is reason to believe that this kind of mechanism was not involved. DLS-dependent memory performance in the radial maze, operant chamber, and T-maze remains

insensitive to devaluation of the outcome (Sage and Knowlton, 2000; Yin, Knowlton, and Balleine, 2004; Smith et al., 2012). In addition, the ineffectiveness of latent extinction might also indicate insensitivity to outcome devaluation. Latent extinction has often been viewed as a protocol that *informs* the animal that the goal location no longer contains food. This might be analogous to verbally instructing human participants that a previous response no longer results in the same outcome (i.e. outcome devaluation). In such studies, human participants using habitual-responding will continue to perform the response even though they have been instructed that it no longer results in a favorable outcome (Gillan et al., 2011). In other words, participants are not able to use the cognitive information (i.e. that the original response no longer results in the outcome) to modify their response, because their behavior is governed by habit. By the same token, in the response learning task, informing the animal that the goal location does not contain food (i.e. latent extinction) may not work due to the habitual nature of memory performance in the response learning task.

It is important to emphasize that the present experiments did not directly examine whether Hullian S-R mechanisms are involved in extinction of response learning. However, the present findings suggest that the animal needs to perform the original response for extinction of response learning to occur, which rules out Tolmanian cognitive mechanisms, while remaining consistent with Hullian S-R mechanisms. In addition, the selective effectiveness of latent extinction in the place learning task, but not the response learning task, suggests that extinction of place and response learning may depend on distinct behavioral mechanisms.

8.1.2 Aim II: Neural Substrate Mediating Extinction of Response Learning

Experiments in the second aim revealed that the DLS is selectively involved in extinction of response learning. Inactivation of the DLS completely blocked extinction of response learning, but not place learning. Thus, the same brain region that is involved in initial acquisition of response learning is also involved in extinction of this kind of memory.

These findings are consistent with the hypothesis that extinction of response learning may be partially achieved through Hullian S-R mechanisms (Hull, 1943; Rescorla, 1993, 2001). That is, cues in the learning environment may acquire the capacity to inhibit the original behavior, thus forming an inhibitory S-R association that produces the response decrement in this task. The DLS may be needed for extinction of response learning to the extent that this brain region mediates S-R associations, including those involving passive avoidance behaviors (Salado-Castillo et al., 1996; Wendler et al., 2014). An alternative, yet related, possibility is that during extinction of response learning the animal acquires new *excitatory* S-R associations that compete with the original turning behavior. These novel S-R associations may involve acquisition of a no-go behavior or acquisition of the opposite turning response at the choice point, and these behaviors could be reinforced by (a) relief from the frustration produced by not receiving the reward (Amsel, 1962) or (b) relief from being taken out of the maze. Consistent with the view that the role of the DLS in extinction of response learning involves acquisition of a novel excitatory S-R association, previous evidence has

implicated the DLS in reversal learning (Rueda-Orozco et al., 2008b) and acquisition of a novel response following omission of reinforcement (Skelin et al., 2014).

Unexpectedly, some evidence from the second aim of experiments suggests that DLS inactivation might have enhanced extinction of place learning. This is consistent with a prior study in which DLS lesions were associated with faster extinction in a spatial alternation task (Moussa et al., 2011). One possible mechanism underlying this finding invokes the idea that in some learning situations memory systems may compete with one another (Poldrack and Packard, 2003). That is, during extinction of place learning, a sub-optimal kind of extinction learning mediated by the DLS may have been activated, whereby DLS inactivation may have blocked this kind of extinction learning allowing another more effective memory system (e.g. the hippocampus) to take control of extinction learning in the task. It is also possible that, as suggested by other investigators (Moussa et al., 2011) inactivation of the DLS may have enhanced sensitivity to changes in the response-outcome contingency (see Yin, Balleine, and Knowlton, 2004), thereby enhancing extinction.

It will be useful for future research to identify the precise nature of the extinction mechanisms mediated by the DLS. In addition, future research should examine more closely whether DLS inactivation indeed enhances extinction of place learning and whether the mechanisms behind this proposed effect involve competition between memory systems or changes in the response-outcome contingency.

8.1.3 Aim III: Neurotransmitter System Mediating Extinction of Response Learning

Whereas the second aim indicated a brain region critically implicated in extinction of response learning, the third aim identified a specific neurotransmitter system involved in this kind of memory. Disrupting endogenous NMDA receptor activity in the DLS with AP5 impaired, but did not completely block, extinction of response learning. The failure of AP5 to completely block extinction of response learning may be attributed to the weakness of the AP5 dose or the possibility that other intact neurotransmitter systems in the DLS may also be partially involved in producing the response decrement. In the second experiment, increasing NMDA receptor activity using intra-DLS administration of DCS enhanced extinction of response learning. Thus, extinction of habit memory may be either impaired or enhanced following modulation of NMDA receptor activity in the DLS.

One possible neural mechanism behind the present findings suggests a role for NMDA receptor-dependent forms of synaptic plasticity. Indeed multiple investigators have endorsed synaptic plasticity as a candidate neural mechanism underlying learning and memory (Hebb, 1949; Bliss & Collingridge, 1993; Martin, Grimwood, and Morris, 2000; Kandel, 2001), and NMDA receptor activity is needed for long-term potentiation in the DLS (Li, Li, and Han, 2009). Thus, NMDA receptor activity may be involved in extinction of response learning to the extent that activation of these receptors mediates the plastic changes supporting extinction of habit memory.

Considering that animal models of extinction may be adapted to treat human psychopathology in humans, the present findings may be relevant to treating neuropsychiatric disorders characterized by maladaptive habitual behaviors (e.g. drug addiction, obsessive-compulsive disorder, etc.). One reasonable prediction is that administration of DCS may be used to effectively enhance the therapeutic benefit of exposure therapy in alleviating habit-like symptoms in human psychopathology. Indeed, DCS has already been successfully employed to treat a range of behavioral disorders in humans (Heresco-Levy et al., 2002; Oliveto et al., 2003; Ressler et al., 2004; Kushner et al., 2007).

8.2 A Role for the DLS in Extinction

The findings in the present dissertation project suggest that the DLS may be an important structure in memory extinction. Indeed, there is extensive previous evidence indicating that the dorsal striatum mediates extinction in a variety of learning and memory tasks. Nevertheless, contemporary views regarding the neurobiology of extinction, which have been shaped primarily by studies examining extinction of conditioned fear, have focused on roles of the hippocampus, medial prefrontal cortex, and amygdala, while the potential role of the dorsal striatum remains ignored.

As mentioned previously, some investigators have suggested that extinction learning may be achieved through novel inhibitory S-R associations or through avoidance learning, both of which are mediated by the DLS (Packard et al., 1989;

Salado-Castillo et al., 1996; Wendler et al., 2014). In addition, the DLS mediates quick changes in strategy selection following omission of reinforcement (Skelin et al., 2014). These are just a few theoretical reasons why the DLS should be considered as a major player in extinction.

The view that the DLS may be a prominent neural substrate mediating extinction learning is also corroborated by extensive empirical evidence. DLS lesions impair extinction of delayed spatial alternation behavior (Butters and Rosvold, 1968; but see also, Moussa et al., 2011), water T-maze performance (Campus et al., 2014), straight alley maze performance (Dunnett and Iversen, 1981; Thullier et al., 1996), operant lever pressing (Schmaltz and Isaacson, 1972), and conditioned motor behaviors (Herz and Peeke, 1971; Denisova, 1972; Suvorov et al., 1974; Baranov, 1977; Denisova, 1981; Makarova, 2001; Shugalev et al., 2001). Electrophysiological evidence also indicates changes in neural ensemble activity of the DLS following extinction training in the conditional T-maze task (Barnes et al., 2005). Interestingly, DLS lesions also *enhance* extinction in a spatial alternation task (Moussa et al., 2011) and a two-way active avoidance task (Wendler et al., 2014), suggesting that DLS function may sometimes interfere with optimal extinction learning.

Extensive evidence also indicates a role for the DLS in extinction of drug seeking. Extinction of drug seeking has been associated with multiple changes in DLS activity including, higher proenkephalin expression (Crespo et al., 2001), adaptations in D2 and A2A receptor binding (Frankowska et al., 2013), higher NMDA receptor expression (Ghasemzadeh et al., 2009a), increased expression of metabotropic glutamate

receptors (Ghasemzadeh et al., 2009b; Schwendt et al., 2012), increased phosphorylation of peroxiredoxin 6 (Gramage et al., 2013), and higher neurotensin levels (Hanson et al., 2013). In addition, suppression of the immediate early gene Arc in the DLS impairs extinction of cocaine seeking (Hearing et al., 2011). Disrupting metabotropic glutamate receptors in the DLS also impairs extinction of cocaine seeking, which may be attributed to changes in DLS Arc expression (Knackstedt et al., 2014; Knackstedt and Schwendt, 2016).

Thus, findings indicate that the DLS is not only implicated in extinction of response learning, as demonstrated in the present dissertation experiments, but also may be involved in extinction across a range of other learning and memory tasks. Thus, the DLS should be considered as a prominent neural structure participating in extinction learning. However, the present experiments constrain the view that the DLS has a universal role in extinction learning, given that inactivation of the DLS failed to impair extinction of hippocampus-dependent place learning. Considering that on the other hand DLS inactivation impaired extinction of response learning, it is tempting to speculate that the DLS may be selectively involved in extinction of DLS-dependent habit memory. In addition, previous research implicating the DLS in extinction employed tasks that could have been solved using DLS-dependent habit memory (i.e. straight alley maze, T-maze, or operant lever pressing tasks). The drug-self administration studies in particular were most likely acquired initially using DLS-dependent habit memory given that drug reinforcement preferentially engages the habit memory system (Goodman and Packard, 2016).

8.3 Extinguishing Psychopathology

Another subject frequently considered in the present dissertation has been how the present findings may be related to treating psychopathology. Animal models of extinction are believed to be analogous to suppression of maladaptive memories in human neuropsychiatric disorders. Thus, the present findings may be relevant to understanding disorders characterized by maladaptive DLS-dependent habit memory. These disorders include drug addiction and relapse (Schwabe et al., 2011; Everitt and Robbins, 2005, 2015; Goodman and Packard, 2016), obsessive-compulsive disorder (Graybiel and Rauch, 2000; Gillan and Robbins, 2014), autism spectrum disorders (Goh and Peterson, 2012; Goodman et al., 2014), Tourette syndrome (Marsh et al., 2005), post-traumatic stress disorder (Goodman et al., 2012), and many others. However, it should be emphasized that the DLS should not be considered a central part of the neurobiological profile of each of these disorders. Instead, this brain region may be implicated in these disorders to the extent that it mediates the formation and expression of habit-like symptoms.

Considering the results of the present dissertation experiments, a few predictions regarding the treatment of habit-like behavioral symptoms can be made. One, it is possible that behavioral treatments modeled after response extinction may be more effective at combating habit-like symptoms than purely cognitive strategies. Two, the same brain region involved in the formation and expression of habit-like symptoms, the DLS, may also be involved in the suppression of these symptoms. Third, the NMDA receptor partial agonist DCS, which has been employed in the treatment of multiple

human psychopathologies, may also be employed to suppress maladaptive habitual behaviors. Future clinical studies should investigate these hypotheses.

8.4 The Multiple Memory Systems Approach to Extinction

The present dissertation project has been firmly couched in the multiple memory systems view of learning and memory. According to this view, different kinds of memory are subserved by dissociable neuroanatomical substrates. The present experiments were designed, and the findings were interpreted, within the context of this multiple memory systems approach. As indicated in the introduction of this dissertation, the multiple memory systems approach might be relevant to understanding not only initial acquisition of memory, but also extinction of memory. That is, different kinds of extinction learning may be mediated by distinct neural systems.

In the straight alley maze, the hippocampus mediates latent extinction, but not response extinction, whereas the DLS mediates response extinction, but not latent extinction. These findings suggest a double dissociation regarding the involvement of distinct neural systems in different kinds of extinction training. However, a few shortcomings to these experiments were identified. For instance, we do not know what kind of memory was initially acquired in the straight alley maze, and therefore we also do not know what kind of memory was being extinguished. Whether the kind of memory being extinguished is relevant to the multiple memory systems view of extinction leads to a few specific empirical questions, which will be reiterated here:

1. Are latent and response extinction protocols effective at extinguishing all kinds of memory, or is each protocol only effective at targeting specific kinds of memory?

2. Are the DLS and hippocampus still needed for response and latent extinction, respectively, when different kinds of memory are being extinguished?

The following section will address the former inquiry regarding the effectiveness of these protocols at extinguishing different kinds of memory, and the second inquiry will be addressed in the subsequent section.

8.4.1 Effectiveness of Latent and Response Extinction Depends on the Kind of Memory Being Extinguished

Considering that latent and response extinction protocols may invoke different kinds of extinction learning, it remains possible that each of these protocols might only be effective for certain kinds of memory. For instance, during latent extinction, animals presumably acquire an association between the original goal location and the *absence* of reinforcement. Thus, the memory acquired during latent extinction might only be effective when the spatial location of the reinforcer is relevant to the to-be-extinguished memory, as in hippocampus-dependent spatial memory tasks. In contrast, latent extinction might not be effective when the spatial location of the reinforcer is irrelevant, as in DLS-dependent S-R/habit memory tasks.

In experiment 1, animals were trained in a response learning version of the plus-maze that depends on function of the DLS and not the hippocampus (Packard and

McGaugh, 1996; Chang and Gold, 2003, 2004; Compton, 2004; Asem and Holland, 2015). In this task, animals were released from opposite starting positions, however the palatable reinforcer was rotated to different arms in such a way that in order for animals to quickly retrieve the reinforcer, they needed to make a consistent body-turn response (e.g. left turn). Following initial acquisition, animals were given response extinction, latent extinction, or no extinction. During subsequent probe trials, animals previously given response extinction demonstrated a lower number of perseverative trials and higher extinction latencies, relative to animals given no extinction. These findings suggest that response extinction was effective at targeting the original DLS-dependent memory. However, animals given normal latent extinction training (10 confinements/day) or extended latent extinction (20 confinements/day) displayed a comparable number of perseverative trials and comparable extinction latencies, compared to animals in the no extinction control group. These results suggest that response extinction, but not latent extinction, was effective at producing extinction of DLS-dependent response learning.

In experiment 2, animals were trained in a place learning version of the plus-maze that depends on function of the hippocampus and not the DLS (Packard and McGaugh, 1996; Chang and Gold, 2003a; Compton, 2004). Over the course of initial acquisition in this task, animals were released into the maze from opposite starting positions (N and S), and a palatable food reward was located in a consistent goal arm (E). This place learning protocol presumably compelled animals to acquire a spatial cognitive map of the learning environment in order to quickly and accurately guide

behavior from different starting positions to the rewarded spatial location. Following initial acquisition of the place learning task, separate groups of animals were given response extinction, latent extinction, or no extinction, and all groups were subsequently given probe trials to determine the effectiveness of these protocols. During the probe trials, animals previously given latent or response extinction displayed a lower number of perseverative trials and higher extinction latencies, relative to control animals previously given no extinction. These findings suggest that both the latent and response extinction protocols were effective at extinguishing the hippocampus-dependent place learning memory. The relative effectiveness of latent and response extinction training has also been demonstrated in water maze versions of the place and response learning tasks (Goodman et al., 2016).

These findings provide evidence for a dissociation regarding the effectiveness of extinction protocols at targeting different kinds of memory. Latent extinction was effective at producing extinction of hippocampus-dependent place learning, but not DLS-dependent response learning. The present findings suggest that within the context of the multiple memory systems view of extinction, the kind of memory being extinguished is an important factor to consider. However, these findings do not address the relative involvement of the hippocampus and DLS in extinction of different kinds of memory. In the following section, experiments are described suggesting that the involvement of a neural system in extinction learning might not only depend on the extinction protocol, but also the kind of memory being extinguished.

8.4.2 Involvement of Neural System in Extinction Depends on the Protocol and the Kind of Memory Being Extinguished

The experiments relating to the multiple memory systems view of extinction described above indicate that (1) latent and response extinction protocols tap into different kinds of extinction learning mediated by dissociable neural systems and that (2) the effectiveness of an extinction protocol may depend on the kind of memory being extinguished. The present section describes additional experiments suggesting that whether a neural system is critically involved in extinction depends on both the protocol used for extinction, as well as the type of memory that is being extinguished. In one experiment published previously (Goodman et al., 2016), the role of hippocampus NMDA receptors in latent and response extinction of place learning was examined. The other experiments reviewed below and described in the present dissertation project examined the role of the DLS in extinction of response learning and place learning, when using a response extinction protocol.

As described earlier, the hippocampus has been implicated in latent extinction, but not response extinction, in the straight alley maze. An experiment was conducted to determine whether the hippocampus is still implicated in latent extinction when a hippocampus-dependent spatial memory is being extinguished. In addition, although it had been previously demonstrated that response extinction in the straight alley maze may not depend on hippocampal function, the presently described study examined whether this might also be true when response extinction is used to target a hippocampus-dependent spatial memory. Animals were trained in a water maze version

of the place learning task. This task is similar to the appetitive version described earlier, however instead of animals learning to run to an appetitively reinforced place, animals swam to a consistent spatial location in a water plus-maze to mount an invisible escape platform. Importantly, acquisition in this water maze version of the task also depends on hippocampal function (Schroeder, Wingard, and Packard, 2002; Compton, 2004). Following initial acquisition of place learning in the water plus-maze, rats were given latent or response extinction training. Immediately before each extinction session, animals received intra-hippocampal injections of the NMDA receptor antagonist AP5 or saline. Given the role of hippocampal NMDA receptors in many forms of learning and memory, including extinction learning, it was predicted that hippocampal NMDA receptors might also be involved in extinction of place learning. Consistent with this hypothesis, animals previously given intra-hippocampal AP5 before latent extinction training demonstrated lower extinction latencies, relative to saline-treated controls, during the drug-free probe trials. However, animals previously given intra-hippocampal AP5 before response extinction training demonstrated comparable latencies to saline-treated controls on the probe trials.

These findings indicate that NMDA receptor activity in the hippocampus may be required for the learning mechanisms underlying latent extinction. NMDA receptor activity may be involved to the extent that the learning mechanisms through which latent extinction operates depend on NMDA receptor-dependent forms of synaptic plasticity in the hippocampus. However, the findings of this study do not provide evidence that hippocampal NMDA receptors are needed for response extinction of place learning.

This observation is consistent with the findings in the straight alley maze, that the hippocampus is implicated in latent extinction, but not response extinction. However, it is possible that a role for the hippocampus in response extinction of place learning might be observed by using other doses of AP5 or by examining other neurotransmitter systems. Indeed, endogenous changes in hippocampal activation (e.g. decreased CREB levels, increased cholinergic activation, etc.) have been observed following response extinction in other spatial memory tasks (Toumane, Durkin, Marighetto, Galey, and Jaffard, 1987; Toumane, Durkin, Galey, and Jaffard, 1988; Topic, Huston, Namestkova, Zhu, Mohammed, and Schulz, 2008; Porte, Trifilieff, Wolff, Micheau, Buhot, and Mons, 2011).

As previously reported, the DLS is needed for response extinction in the straight alley, however whether the DLS is needed for response extinction of all kinds of memory or only some kinds of memory could not be determined using the straight alley maze. To examine the role of DLS in extinction of response learning (Experiment 3), rats with cannulas in the DLS were trained in an appetitive version of the response learning task and subsequently received two days of response extinction training. Immediately following the first day of extinction training, animals received post-training intra-DLS infusions of the sodium channel blocker bupivacaine or physiological saline. Following acquisition of response learning, animals that received post-training DLS inactivation on Extinction Day 1 displayed more perseverative trials and lower latencies on Extinction Day 2, relative to animals that received post-training intra-DLS saline. Moreover, animals that received post-training bupivacaine did not demonstrate a

significant difference in perseverative trials or latencies across Extinction Days 1 and 2, suggesting that DLS inactivation completely blocked extinction of response learning. Other findings of the present dissertation indicate that the role of the DLS in extinction of response learning may be attributed to NMDA receptor activity. In sum, these findings indicate that the DLS is critically implicated in extinction of response learning, when using a response extinction protocol.

A separate group of animals with cannulas in the DLS was trained in the appetitive hippocampus-dependent place learning task and subsequently received two days of response extinction training (Experiment 4). Immediately after training on Extinction Day 1, animals received intra-DLS infusions of bupivacaine or saline. On Extinction Day 2, animals previously given post-training intra-DLS bupivacaine did not show an impairment in extinction of place learning.

In sum, the findings reported in this section indicate that the kind of memory being extinguished and the protocol used for extinction determine what neural system will be needed for successful extinction learning. Hippocampus NMDA receptors mediate latent extinction of place learning. Whether the hippocampus might also be implicated in response extinction of place learning has yet to be rigorously examined. In contrast to the hippocampus, the DLS has been critically implicated in response extinction of a DLS-dependent response learning task, but not in response extinction of a hippocampus-dependent place learning task. The role of the DLS in extinction of response learning may be partially attributed to activation of DLS NMDA receptors. Thus, NMDA receptor-dependent forms of synaptic plasticity in the hippocampus and

DLS may be critical neural mechanisms supporting extinction of place learning and extinction of response learning, respectively.

8.4.3 The Multiple Memory Systems Approach to Extinction: a Hypothetical Model

The experiments described in the above sections provide evidence for a multiple memory systems approach to extinction. According to this approach, each extinction protocol engages a unique pattern of neural activity. Some extinction protocols might engage multiple neural systems equally, whereas other protocols might engage one neural system more than another. The term neural system should be interpreted broadly. A neural system in this model can be a group of neurobiological structures that function as a unit (e.g. the hippocampal formation), an anatomically segregated brain structure (e.g. the hippocampus proper), one component of the neurobiological structure (e.g. the dentate gyrus), and so forth. The latent and response extinction protocols might each engage one neural system more than another. Latent extinction might engage the hippocampus over the DLS, whereas response extinction might engage the DLS over the hippocampus. However, it could also be argued that response extinction protocols, especially in spatial memory tasks, might also summon function of the hippocampus, albeit to a lesser degree. Consistent with this hypothesis, measures of hippocampal activity correlate with response extinction in some spatial memory tasks. The observation that hippocampal inactivation does not influence the effectiveness of response extinction training might be attributed to another neural system (e.g. the DLS)

being sufficient to produce the response decrement. The above experiments did not directly examine whether latent and response extinction protocols invoke unique patterns of neural activity, however this has been inferred based on the results of lesion studies: the hippocampus was needed for latent extinction and not response extinction, and the DLS was needed for response extinction and not latent extinction. However, to directly examine whether response and latent extinction protocols engage distinct patterns of neural activity, it would be useful to measure expression of immediate early genes or other molecular markers of activity following training in each of these protocols.

After an extinction protocol engages a unique pattern of neural activity, the engaged neural system or systems mediate extinction learning. It is hypothesized that each neural system mediates a unique kind of extinction learning. This kind of extinction learning involves learning mechanisms that are distinct from other types of extinction learning mediated by other neural systems. When multiple neural systems are activated, multiple kinds of extinction learning could be online and potentially contribute to the response decrement. Multiple kinds of extinction learning working together could amount to a composite mechanism that could be labeled as a singular kind of extinction learning. On the other hand, some extinction protocols, as noted above, might preferentially engage one neural system over another. In this case, one kind of extinction learning becomes active, while other kinds of extinction learning mediated by other neural systems remain less active or dormant. Presumably latent extinction engages the hippocampus, which mediates a kind of extinction learning involving an association between the original goal location and the *absence* of reinforcement.

Response extinction, on the other hand, engages the DLS, which might mediate a kind of extinction learning involving an association between various stimuli in the learning situation and the inhibition of the original response. Response extinction, again, might also activate to a lesser degree another kind of extinction learning involving spatial information.

Given that each kind of extinction learning might involve distinct mechanisms, it is likely that a kind of extinction learning might only produce response decrements in some learning situations. A kind of extinction learning may be effective when it produces an extinction memory that competes with the original memory that guided behavior, whereas a kind of extinction learning may fail to be effective when the extinction memory is irrelevant to the originally acquired memory. In other words, whether a kind of extinction learning is effective may depend on the kind of memory being extinguished. Latent extinction engages the hippocampus, which promotes a kind of extinction learning, in which the original goal location is associated with absence of reinforcement. This new extinction memory may effectively compete with an original memory in which the spatial location was originally associated with presence of reinforcement. Thus, the kind of extinction learning incited by latent extinction and mediated by the hippocampus can be effective at producing extinction in spatial memory tasks (Experiment 2). However, this same kind of extinction learning may be ineffective at extinguishing memories that do not involve the spatial location of reinforcement. In the response learning task, spatial locations of the reinforcer are irrelevant to successful memory performance, and therefore the kind of extinction learning incited by latent

extinction is not effective at producing a response decrement in the response learning task (Experiment 1).

The type of memory being extinguished might also determine whether a particular neural system is required for extinction. As mentioned above, the kind of memory acquired during initial acquisition of a task may only be extinguished by specific kinds of extinction learning. Thus, we should expect that inactivating the neural system that mediates that required kind of extinction learning would prevent extinction of that particular memory. For instance, evidence suggests that the kind of extinction learning invoked by a response extinction protocol (presumably an inhibitory S-R memory) may be needed for extinction of response learning to occur. As observed in the straight alley maze, the kind of extinction learning invoked by the response extinction protocol depends on DLS activity. Thus, it makes sense that inactivation of the DLS (which disrupts the kind of extinction learning underlying the response extinction protocol) blocks extinction of response learning (Experiment 3). However, the DLS is not required when using the response extinction protocol to extinguish a place learning memory (Experiment 4). It is possible that a second kind of extinction learning might be invoked by the response extinction protocol. This other kind of extinction learning is sufficient to produce extinction of place learning and does not depend on DLS function. One possibility is that this second kind of extinction learning might involve a learned association between the original spatial location and the absence of the reinforcer and could be dependent on hippocampal function. Examination of this hypothesis would require the use of hippocampal inactivation during response extinction of place learning.

Although we did not observe a role for hippocampal NMDA receptors (Goodman et al., 2016), it is possible that targeting other neurotransmitter systems or using a sodium channel blocker could reveal a role for the hippocampus in response extinction of place learning. Indeed, early lesion studies revealed a role for the hippocampus in response extinction in spatial memory tasks, although there were a few methodological problems with these studies (e.g. the use of complete hippocampal ablations instead of neurotoxic lesions or temporary inactivation; the fact that these ablations were made before initial acquisition of the memory, etc).

To sum up the multiple systems approach to extinction, each extinction protocol engages a unique pattern of neural activity, sometimes engaging multiple neural systems equally and at other times engaging one neural system more than another. Each neural system mediates a unique kind of extinction learning involving distinct learning mechanisms. The effectiveness of a particular kind of extinction learning depends on the kind of memory being extinguished. Whether a neural system is required for extinction of a particular kind of memory depends on whether that neural system is mediating the kind of extinction learning responsible for suppression of the memory. Although this multiple memory systems approach to extinction has been discussed primarily within the context of maze learning experiments, it might also be useful in understanding extinction in other learning situations, e.g. extinction of conditioned fear.

8.5 Conclusion

In sum, the present experiments provided novel information regarding the behavioral and neurobiological mechanisms mediating extinction of habit memory in a response learning plus-maze task. The findings suggest that in order for extinction of habit memory to occur, an animal might need to perform the original to-be-extinguished behavior. In addition, extinction of habit memory may be mediated by a neural system involving the DLS, and one of the neurotransmitter systems implicated in the DLS's role in extinction may be the glutamatergic system, given that NMDA receptor blockade impaired extinction of response learning. These experiments have broached a new topic that requires further experimentation. It will be important for future research to examine the potential role of other brain regions (e.g. the DMS, medial prefrontal cortex, hippocampus, etc.) and other neurotransmitter systems (e.g. dopamine, acetylcholine, and cannabinoid systems) in extinction of habit memory, while also employing other habit memory tasks to verify these findings hold up in the face of non-mnemonic task differences. The present findings fit into a bigger picture suggesting that extinction learning should be considered within the context of a multiple memory systems approach, given that mechanisms for extinction may depend on the protocol used for extinction training and the kind of memory being extinguished. Moreover, the multiple memory systems approach may be useful for gaining a comprehensive understanding of extinction learning and also for tailoring behavioral and pharmacological treatments to alleviate specific kinds of maladaptive memory.

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