EMOTIONAL AROUSAL AND MODULATION OF MULTIPLE MEMORY SYSTEMS:

EFFECTS OF UNCONDITIONED AND CONDITIONED STRESSORS

A Dissertation

by

KAH-CHUNG LEONG

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Mark Packard
Committee Members,	Stephen Maren
	Paul Wellman
	Ursula Winzer-Serhan
Head of Department,	Douglas Woods

December 2014

Major Subject: Psychology

ABSTRACT

Recently studies have uncovered a wealth of evidence supporting the theory that different areas of the mammalian brain mediate different types of memory. Specifically, evidence suggests that memory does not exist as a single construct and, more importantly, that different types of memory are mediated by different neuroanatomical regions. Two primary memory systems are the hippocampus-dependent cognitive memory system and the dorsolateral striatum-dependent habit memory system. Interestingly, there are a number of factors that can influence the relative use of these memory systems. One important factor that influences the relative use of multiple memory systems is emotional arousal. Emotional arousal, as defined in this body of work, is defined as a state of heightened emotion, particularly in reference to states of anxiety or stress. Decades of research have uncovered a role of emotional arousal in modulating memory. Despite the wealth of literature regarding the role of emotional arousal on cognitive function, only recently have studies investigating the role of emotional arousal in the context of the multiple memory systems begun to surface.

The present experiments were developed to explore the role of ethologically and physiologically relevant stressors in modulating multiple memory systems. In addition, this set of experiments also introduces the idea that a fear-conditioned stimulus may modulate memory. There were three specific aims. The first aim was to determine if an ethologically-relevant stressor such as trimethylzoline, an odor component of red fox feces, can influence the relative use of multiple memory systems and modulate

dorsolateral striatum-dependent memory. Additionally, this set of experiments also aimed to implicate the basolateral amygdala (BLA) in mediating this effect. The second aim was to administer the endogenous stress hormone corticosterone, as a physiologically-relevant stressor, to modulate dorsolateral striatum-dependent memory. Furthermore, these experiments attempted to implicate noradrenergic activity as necessary in mediating the glucocorticoid effect on habit memory. Finally, the last aim of this dissertation was to employ a fear-conditioned stimulus to modulate memory in the same manner as a stressful unconditioned stimulus. Specifically, these experiments determined if a tone, when paired with a shock, can influence the relative use of memory and modulate dorsolateral striatum-dependent memory and further investigated the role of noradrenergic activity in mediating this effect.

The findings suggest that emotional arousal, in various forms, influences the relative use of memory in similar fashion. Additionally, various forms of emotional arousal also modulate dorsolateral striatum-dependent habit memory. These results extend previous work investigating the role of emotional arousal on memory, while expanding on research specific to the dorsolateral striatum-dependent memory system. These studies also strongly suggest that the modulation of memory through emotional arousal relies on a noradrenergic activity. Overall, this body of work suggests that emotional arousal, both through unconditioned and conditioned stimuli, facilitates dorsolateral-striatum dependent habit memory.

DEDICATION

I dedicate this body of work to my wonderful family. I hold a special feeling of gratitude to my loving wife, Andrea C. Leong, who offered words of encouragement through this entire process. She will always be my greatest cheerleader. I also dedicate this dissertation to my parents, Siew-Tin Loo and Foo-Weng Leong. This dissertation exists, in part, due to their endless support and sacrifice. Finally, I dedicate this dissertation to the Lord, who has provided me with a foundation of strength to complete this work.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Mark Packard, for his guidance and wisdom throughout my years spent under his mentorship. The experience and knowledge I have acquired as his student has been invaluable. I would also like to acknowledge my committee members, Dr. Stephen Maren, Dr. Paul Wellman, and Dr. Ursula Winzer-Serhan, for their input and advice throughout the course of this research.

I would also like to express gratitude to my friends and colleagues for making my time at Texas A&M University a great experience. I would like to specifically thank Jarid Goodman for his involvement and support throughout this research. Finally I would like to thank Dr. Maren and Travis Goode who generously offered their resources and time to make some of this work possible.

TABLE OF CONTENTS

Page

LIST OF FIGURES

CHAPTER I

GENERAL INTRODUCTION

Introduction to Multiple Memory Systems Hypothesis

The last few decades have presented a wealth of evidence supporting the theory that different areas of the mammalian brain mediate different types of memory. Early evidence supporting this theory stems from amnesic patients, such as patient H.M., who received a partial temporal lobectomy as a last-resort treatment for his uncontrollable seizures. The procedure was a relative success in that it reduced the frequency of seizures. However, the procedure had also effectively removed his hippocampus and portions of his amygdala which left him suffering from severe anterograde amnesia (Scoville & Milner, 1957). While numerous tests found that H.M.'s memory was severely impaired, it was later discovered that his long-term memory of events prior to his procedure were mostly intact. Furthermore, H.M.'s motor and procedural learning remained unimpaired as he was able to acquire new motor tasks, such as the mirror-tracing task, although he could not consciously recollect memories of this task at a later time (Milner, Corkin, & Teuber, 1968). This early evidence suggested that memory did not exist as a single construct and, more importantly, that different types of memory may be mediated by different neuroanatomical regions. In sum, this was one of the first studies that suggested the possibility that the mammalian brain is comprised of multiple memory systems.

When discussing the neurobiology of multiple memory systems, it is first important to define a memory system. Sherry and Schacter (1987) define a "memory

system" as an "interaction among acquisition, retention, and retrieval mechanisms that is characterized by certain rules of operation". "Multiple memory systems", in turn, are defined as "two or more systems characterized by fundamentally different rules of operation". As seen from patient H.M., his unfortunate circumstance provided clear evidence for the existence of multiple memory systems, based on the definition put forth by Sherry and Schacter (1987). His inability to form, store, and recollect certain types of memories following damage to his hippocampal formation, while being able to form, store, and recollect other forms of memories, suggested that he suffered an impairment in a memory system defined by one set of rules of operation, but not another.

This idea was not necessarily novel even for the time. In fact, researchers as far back as a century ago argued that there was a distinction between "memory" and "habit" based on the fact that retention of certain tasks required repetitive learning trials while other memories could be acquired in a single event (e.g. pictures) (Smith & McDougall, 1920). However, it wasn't until researchers began to explore the dissociations of memory in neuropsychological patients (e.g. patient H.M.) later in the 20th century that acceptance of multiple memory systems began to take hold. Various other researchers often found similar results to these neuropsychological patients, in that they displayed normal levels of motor or procedural learning but impaired memory for experiences and events (e.g. Brooks & Baddeley, 1976; Eslinger & Damasio, 1985; Cohen & Squire, 1980). Since then, there has been a wealth of experimental evidence supporting the existence of these multiple memory systems. Studies using non-human primates found that lesions of the hippocampus and areas of the limbic system resulted in severe

impairment in matching and nonmatching to sample tasks, which require animals to recollect a specific episode (Mahut, 1985; Zola-Morgan & Squire, 1985). However, these non-human primates displayed no impairment of discrimination learning of either patterns or objects (Mishkin, 1954; Orbach, Milner, & Rasmussen, 1960) nor did they display impairments of during learning of a motor-skill (Zola-Morgan & Squire, 1985). Similar dissociations in memory were also discovered in rats (O'Keefe & Nadel, 1978; Olton, Becker, & Handelmann, 1979).

During the time that the hippocampus and limbic areas had appeared as a strong candidate that mediated a more "episodic" form of memory, research had also accumulated suggesting a role for the dorsal striatum in modulating the memory of pattern/object discrimination (Divac, Rosvold, & Szwarcbart, 1967) and rewarded behavior (Carr & White, 1984; Viaud & White, 1987). Furthermore, a group of researchers found that consumption of sucrose in a conditioned emotional response task (Messier & White, 1984) could be impaired through lesions of the dorsal striatum, but not the hippocampus (for review see White, Packard, & McDonald, 2013). Alternatively, lesions of the hippocampus, but not dorsal striatum, impaired memory of a partial reinforcement effect. These findings from various memory researchers eventually culminated in an elegant study that first demonstrated a double dissociation between the fornix (a major input-output pathway of the hippocampus) and caudate nucleus, a region of the dorsal striatum, in the mediation of spatial and habit memory, respectively (Packard, Hirsh, & White, 1989; for review see White, Packard, & McDonald, 2013). After decades of research, it was then established that there are at least two neural

systems that mediate two distinct memory systems, and that these systems can process information interactively or independently of each other, depending on a given task (for review see White and McDonald, 2002). These two primary memory systems include the hippocampus-dependent and dorsal striatum-dependent memory system. Over the years, additional memory systems have been uncovered through a number of experiments (for review see Squire, 2004) including several studies that have implicated the amygdala in the acquisition of memories that contain an affective stimulus, such as fear conditioning or conditioned place preference (Davis, 1992; LeDoux, 1993; McDonald & White, 1993). The present set of experiments were proposed to further investigate the factors influencing the neurobiology of two primary memory systems (hippocampus-dependent and dorsal striatum-dependent) through the use of several behavioral tasks that have previously been employed to examine this dissociation.

Use of the Radial-Arm Maze

Clear evidence for the existence of separate memory systems has come from studies that uncovered a double dissociation between two memory systems in the radial-arm maze (Packard, Hirsh, & White, 1989; McDonald and White, 1993). These researchers lesioned one of two primary structures in this model (i.e. fimbria-fornix or caudate nucleus) and then tested animals on the acquisition of two separate memory tasks in the radial arm maze (Figure 1). In the first task, animals were required to enter an arm to obtain a food pellet and to avoid re-entering the same arm after the food pellet had already been attained (i.e. win-shift). This task requires a form of "spatial" or

"cognitive" memory (O'Keefe & Conway, 1978; Eichenbaum, Stewart, & Morris, 1999). Lesions of the fimbria-fornix, a major input-output pathway to the hippocampus, resulted in the impairment of the win-shift form of the radial arm maze task. Therefore, lesions to the hippocampus impair the ability for animals to acquire or retain the spatial memory required for this task. Interestingly lesions to the caudate nucleus, a region of the dorsal striatum, produced no effect in the acquisition of this task. However, lesions to the caudate nucleus did impair animals' ability to acquire the win-stay version of this task. In this version, animals had to enter a lit arm to obtain a food reward twice. Upon receiving the food pellet a second time, the light in that arm was turned off. Once the arm is no longer lit, animals learn that food is no longer available. Animals learn a stimulus-response (S-R) association in this task (Hull, 1943). Some researchers have coined this form of learning a "habit of its automatic and unconscious nature (Mishkin & Petri, 1984). Additionally, if the food reward is devalued through conditioned taste aversion, animals will continue to enter the lit arms without consuming the food reward, indicating that the learned behavior is habitual and not goal-directed (Sage & Knowlton, 2000). Several studies have suggested that control over performance following reinforcer devaluation shifts to habitual action mediated by the dorsolateral striatum-dependent processes (for review see Balleine & O'Doherty, 2010). Dorsal striatum lesions impair learning in this particular task suggesting that this structure is involved in the formation of S-R or habit memories. Fornix lesions produced no deficit in this task.

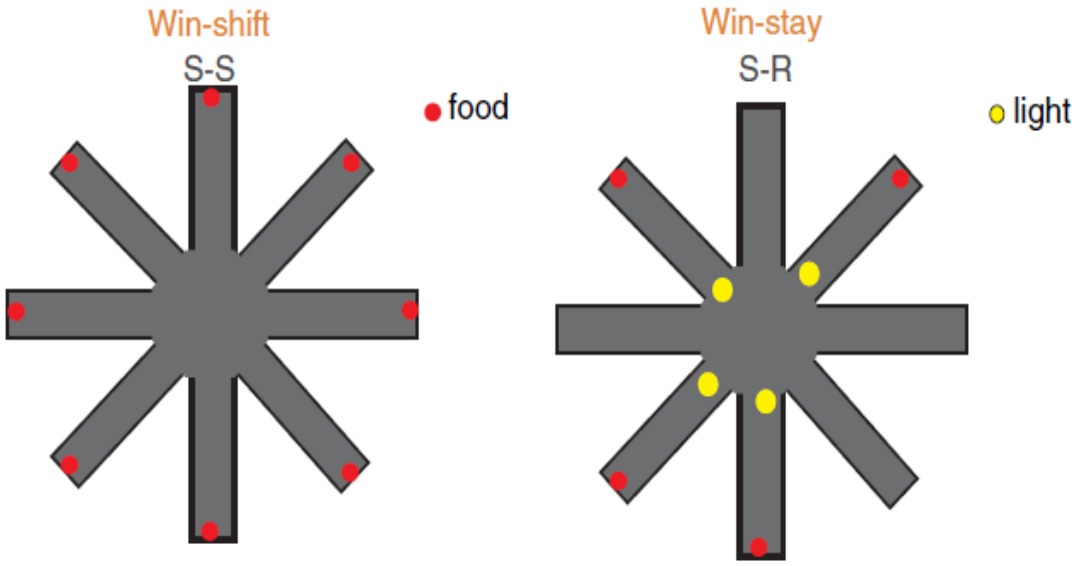

Figure 1. Diagram illustrating the win-shift and win-stay radial-arm maze task. Correct performance in the win-shift task required rats to obtain food pellets from the end of each arm without entering previously-entered arms. The win-stay task required rats to associate the presence of a visual cue (e.g. light) and the entry of the arm, reinforced by the food pellet at the end of the lit arm. The light was turned off when the arm no longer contained food. Adapted from White (2008).

Use of the Plus-Maze

The single-solution appetitive and aversive plus-maze tasks have been used to further distinguish the hippocampus-dependent cognitive memory system and the striatum-dependent habit memory system (Wingard & Packard, 2008). This task essentially combines two T-mazes to form a four-arm "plus" structure. In this particular task animals can be trained to approach the "goal" arm (e.g. east or west) from either "start" arm (e.g. north or south). In the "place" version of this task, rats are trained to consistently enter a "goal" arm (i.e. west) while starting from varying "start" arms (i.e. north or south) (Figure 2). Rats purportedly learn this forced-place task by acquiring the spatial location of the reinforcer relative to the extra-maze cues. Alternatively, in the

stimulus-response or "habit" version of this task, rats can be trained to enter a "goal" arm through the reinforcing of a particular approach response (i.e. a specific body turns at the choice point) regardless of the "start" arm location (Figure 3). The acquisition of this "forced-response" task is dependent on the striatal-dependent habit memory system.

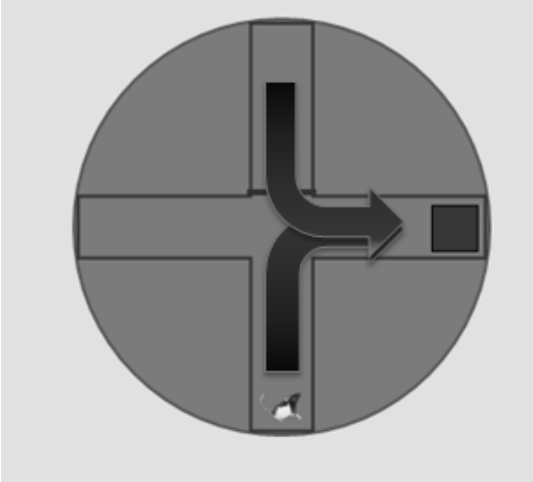

Figure 2. Diagram illustrating the single-solution place water plus-maze task. Rats are placed into alternating start arms over several trials and the hidden platform is located in the same target arm such that rats must always enter the same "place" as the previous trial (e.g. east arm).

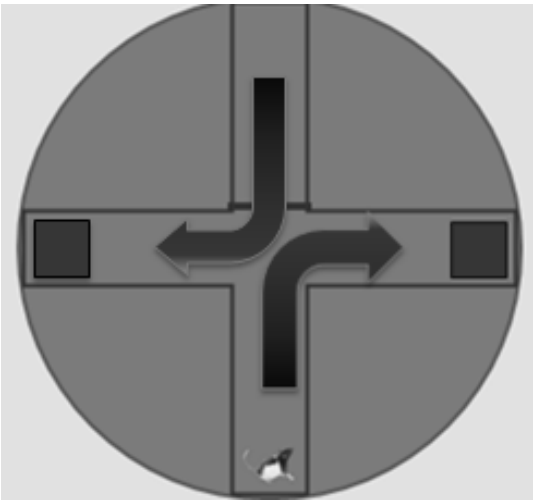

Figure 3. Diagram illustrating the single-solution response water plus-maze task. Rats are placed into alternating start arms over several trials and the hidden platform is located in the target arm such that rats must make the same body "response" at the choice point to locate the platform (e.g. right body turn).

The plus-maze can also be set up as a dual-solution task such that either the cognitive or habit memory system can provide an adequate solution to learning the task (Packard & McGaugh, 1996; Packard & Wingard, 2004). Over a series of trials, rats are trained to enter the same "goal" arm (i.e. west) while starting from the same "start" arm (i.e. north). Rats can learn this task either through acquiring the spatial information of the "goal" arm (hippocampus-dependent memory system) or through acquiring information that a specific body turn (i.e. right turn) leads to the "goal" arm (dorsolateral striatal-dependent memory system). To determine which memory system is accessed in this task a probe trial is given to the rats after a period of training. The probe trial consists of placing the rats in the opposite arm from the "start" arm during training (Figure 4). For example, if the "start" arm was located in the north arm during training, the "start" arm during the probe trial would be located in the south arm. Animals are then scored on whether they enter the same spatial location ("place" learners) of the goal arm during training (i.e. west) or whether they make the same body turn ("response" learners) into the opposite "goal" box (i.e. east).

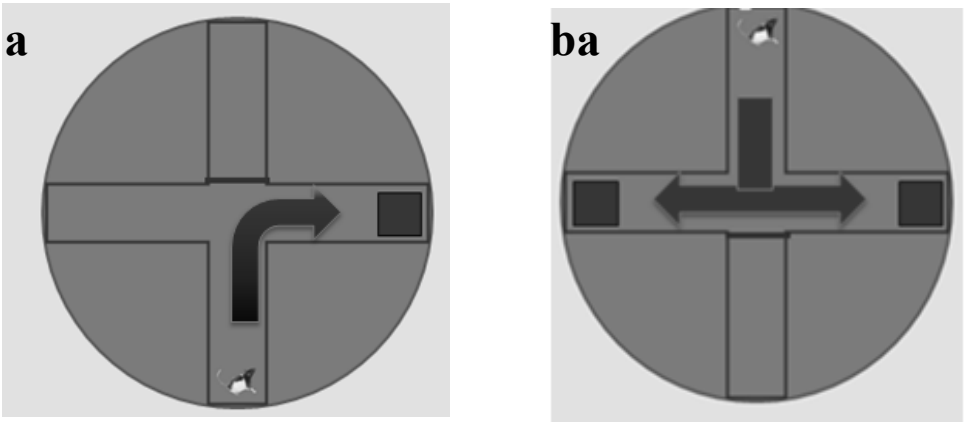

Figure 4. Diagram illustrating the dual-solution response water plus-maze task. (a) Training trial. Rats are placed in the same start arm (e.g. south arm) and hidden platform is located in same target arm (e.g. east arm). (b) Probe trial. Rats are placed in the opposite arm (e.g. north arm). The arm in which the rat swims into determines the type of strategy employed. Entry into the east arm suggest a place strategy, and entry into the west arm suggest a response strategy.

Interaction between Memory Systems

Previous studies have observed an interaction, or competition, between the hippocampus-dependent cognitive memory system and the striatal-dependent habit memory system (for review see Poldrack & Packard, 2003). Indeed, studies have found that with extended training on a dual-solution task, which can be acquired with either memory system, animals tended to shift from a more cognitive-based behavior to more habit-based behavior (Packard & McGaugh, 1996). Here, animals are trained in a plus maze to approach food at the end of a goal arm (i.e. west) from the same start arm (i.e. south). During the probe trial, which occurs after several days of training, animals are placed in the north arm and the direction in which they turn at the choice point dictates whether they learned the task using their spatial memory system (i.e. runs to the same spatial location as during training) or whether they learned this task using their habit memory system (i.e. make the same body turn at the choice point) (as discussed above).

While animals that are probed after just 7 days of training are predominantly place learners (i.e. spatial memory system), with extended training (i.e. 14 days), animals become more habit-like in their behavior during the probe trial. It has previously been shown that this shift from the predominant use of cognitive memory to habit memory can be mediated by infusions of glutamate (Packard, 1999). When animals receive post-training hippocampal infusions of glutamate early during training in this task they display place learning both in the early and late probe trials. However, if animals received post-training infusions of glutamate into the caudate-putamen they displayed response learning in both early and late probe trials. These results suggest that not only do animals naturally shift from cognitive to habit memory over the course of time, but that this shift may be dependent on glutamatergic mechanisms.

While it has been shown that there is a natural shift in preference from cognitive memory to habit memory with extended training (Packard & McGaugh, 1996), few studies have shown a shift from habit memory back to cognitive memory. There have been studies that have shown that inhibition of the dorsal striatum results in the enhancement of spatial learning (Mitchell & Hall, 1988) and vice versa (Packard, Hirsh & White, 1989). However, these studies allude to a functional 'competition' between both memory systems. That is, during learning of tasks that require just one of the two memory systems, there is a degree of "on-line" processing occurring in which both memory systems might be attempting to acquire a new behavior. In line with this, previous studies have shown that lesions of either the hippocampus or striatum facilitates acquisition of dorsolateral striatal-dependent memory or hippocampal-dependent

memory, respectively (e.g. Lee, Duman, & Pittenger, 2008), suggesting that the competition between memory systems works in both directions.

Factors Influencing Memory Systems

In order to examine the neural mechanisms underlying multiple memory systems it is important to consider the factors that influence the relative use of these systems. Over the course of several decades, researchers have uncovered a role of several experimental factors such as reinforcement parameters, amount of training, the sensory environment, and emotional state in influencing the relative use of multiple memory systems. For example, as previously discussed, the amount of training an animal receives influences the memory system that is engaged during retrieval of the memory. Specifically, it is well established that with extended training in a dual-solution plus-maze task that can be acquired using a hippocampus-dependent place strategy or a dorsolateral striatum-dependent response strategy, extended training results in predominant use of response learning (Packard & McGaugh, 1996; Packard, 1999). From these studies, researchers have concluded that early learning of this task is predominantly acquired through hippocampus-dependent cognitive processes while expression of learning following extended training is predominantly controlled by the dorsolateral striatum-dependent habit memory system.

Given that the hippocampus and the dorsolateral striatum mediate rapid and slower forms of learning, respectively, it was also hypothesized that temporally-distinct reinforcement parameters may also play a role in the relative use of multiple memory

systems. Indeed, previous studies have found that when learning trials are separated by 15 minute inter-trial intervals (ITI), rats acquired a response task in a plus-maze more quickly than place task (Thompson & Thompson, 1949). These results have been replicated in a water version of a plus-maze (Wingard, Goodman, Leong, & Packard, unpublished data). Here, researchers found that shorter ITIs (30 seconds) facilitated hippocampus-dependent place learning while longer ITIs (30 minutes) facilitated dorsolateral striatum-dependent response learning.

Effects of Emotional Arousal on Memory

An important factor that influences the relative use of multiple memory systems is emotional arousal. Emotional arousal, as defined in this body of work, is a state of heightened emotion, particularly in reference to states of anxiety or stress. States of anxiety and stress are typically adaptive responses that occur in response to perceived dangers that prepares the animal to engage or disengage from the hostile environment (Gutierrez-Garcia & Contreras, 2013). Emotions can be considered transient events or transient states that produce changes in a variety of behaviors (Critchley, 2003). During dangerous or hostile situations, emotional arousal may give rise to expression of strategies that enhance the chances of survival. However, dysregulation of emotions can often be attributed as a major influence in a wide range of psychiatric disorders (Dolan, 2002) and produce deleterious effects on cognitive function.

The effects of emotional arousal on cognitive function are two-fold. The adverse effects of stress and anxiety on cognitive function are well documented (Maier &

Seligman, 1976). Furthermore, uncontrollable and excessive emotional arousal produces severe impairments in learning and memory as well as cell death (for review, see McEwen & Sapolsky, 1995). Several studies indicate that exposure to stress may impair memory under certain situations. Exposure to stress prior to training in a spatial water maze task produced impairments in spatial memory (Kim, Koo, Lee & Han, 2005). In addition, footshocks given 30 minutes prior to retention testing following spatial water maze training also resulted in impaired spatial memory (de Quervain, Roozendaal, McGaugh, 1998). Alternatively, it is also well-recognized that emotional arousal plays an important role in memory modulation (for review see McGaugh, 2004). Indeed, early evidence has shown that emotionally arousing experiences produced during training may aide the consolidation of memory of these training experiences (Gold & McGaugh, 1975). Extensive work has now discovered that the emotionally-driven enhancements in modulation of memory for these experiences are mediated by stress-hormones. For example, post-training administration of epinephrine and corticosterone, and respective adrenergic and glucocorticoid agonists/antagonists, has produced various enhancements of memory (Oitzel & de Kloet, 1992; Lupien & McEwen, 1997; Sandi & Rose, 1994; Gold & van Buskirk, 1975). Interestingly, both the adrenergic system and glucocorticoid system interact to influence memory consolidation (Borrell et al., 1983). This will be discussed in further detail in later chapters. Studies have also found that rats show enhanced memory for spatial locations in which a footshock was received (Morris, Anderson, Lynch & Baudry, 1986), or enhanced spatial memory following a stressful, aggressive encounter with a dominant male (Buwalda et al., 2005). Therefore, emotional

arousal can modulate memory in a bi-directional manner such that both enhancing and impairing effects of stress can be observed (Joels, Pu, Wiegert, Oitzl & Krugers, 2006; Shors, 2006).

Despite some inconsistencies with regard to the specific findings, it is nevertheless certain that stress plays an influential role in the acquisition, consolidation, and retrieval of various types of memory. Few studies, however, have observed the effect of stress in the context of striatum-dependent habit memory, and even fewer studies have observed the effect of stress within the framework of relative use of multiple memory systems.

The Role of Emotional Arousal in the Relative Use of Multiple Memory Systems

Despite the wealth of literature regarding the role of emotional arousal on cognitive function, only recently have studies investigating the role of emotional arousal in the context of the multiple memory systems begun to surface. One early experiment explored the role of acute stress on retrieval of a dual-solution water maze task (Kim et al., 2001). Here, rats were trained to swim to a visibly cued water platform that was located in the same spatial location on each trial. From this task, rats could acquire information about the spatial location of the platform and stimulus (platform cue)-response (approach) associations simultaneously. The relative use of learning strategy was assessed during a probe trial in which the cued platform is relocated in a new quadrant of the water-maze. Therefore, rats that swam to the spatial location in which the cued platform had been located during the probe trial were classified as having

learned a spatial strategy, while rats that swam to the cued platform in its new location were designated as response learners, having acquired a stimulus-response association during training. It is well understood that the hippocampus-dependent memory system is involved in the acquisition of spatial information during this task as lesions of the hippocampal system resulted in rats swimming to the cued platform, whereas the dorsolateral striatum is an important structure in acquiring stimulus-response associations as lesions to this structure resulted in rats predominantly swimming to the previous spatial location of the platform (McDonald & White, 1994). Rats that received an acute stress regimen (consisting of restraint and tail-shocks) prior to training in this dual-solution water maze task displayed predominant use of a dorsolateral striatum-dependent response strategy during the subsequent probe trial (Kim et al., 2001). The experiment described here was one of the first studies suggesting that emotional arousal through acute stress produces a bias towards the use of a dorsolateral striatum-dependent response strategy in a task that may be acquired using either learning strategy. This corresponds to previous findings suggesting that exposure to stress resulted in perseverative behavior in a T-maze, regardless of reinforcement contingencies, suggesting use of a nonassociative learning strategy (Mitchell, Osborne, & O'Boyle, 1985)

The effect of emotional arousal on the relative use of multiple memory systems has been further demonstrated in studies employing injections of anxiogenic drugs prior to training in a dual-solution water plus-maze task (Packard & Wingard, 2004). Through pre-training peripheral injections of the α-2 adrenoceptor antagonists yohimbine or RS

79948-197 at doses previously found to produce anxiogenic-like behaviors (Handley & Mithani, 1984; White & Birkle, 2001), rats displayed predominant use of the dorsolateral striatum-dependent response strategy over the hippocampus-dependent place strategy relative to vehicle-injected rats. Similarly, peripheral injection of RS 789948-197 prior to the probe trial (i.e. memory retrieval) produced a bias towards use of a response strategy (Elliott & Packard, 2008)

In addition to acute stress/anxiety, a recent study also found that trait anxiety in rats can also influence the relative use of multiple memory systems (Hawley, Grissom, & Dohanich, 2011). Here, investigators first measured trait anxiety levels in rats through behavior on an open-field maze. Lower trait anxiety corresponded to greater amounts of time spent in the open area of the maze. They then trained rats in a dual-solution water plus maze task and found that rats with lower trait anxiety positively correlated with preference in the hippocampus-dependent place strategy during the probe trial.

Research regarding the role of emotional arousal in the relative use of multiple memory systems has remained consistent when translated to humans (for review see Schwabe & Wolf, 2013). In one study, human participants were exposed to psychosocial stress (i.e. public speaking) before being trained in three-dimensional win-card location task, in which a participants had to locate a card associated with a reinforcer within a particular environment (Schwabe et al., 2007). Following exposure to stress, human participants displayed use of a strategy that employed use of a proximal cue to the win-card, instead of integrating contextual information around the room in order to locate the win-card. This indicated that pre-training stress produced bias in predominant use of a

stimulus-response strategy. Similar results were obtained when observing performance in a two-dimensional version of this task in participants that scored high on a chronic stress questionnaire (Schwabe et al., 2008). Furthermore, administration of hydrocortisone resulted in more habit-like responding at the expense of goal-directed responding in humans trained in a food reinforcer devaluation paradigm (Schwabe et al., 2010). It was suggested that emotional arousal, through administration of hydrocortisone, may have produced habit-like responding in human participants by engaging the striatal-dependent habit memory system as the food reinforcer devaluation paradigm has been associated with increased activity in the dorsal striatum (Tricomi et al., 2009). Overall, considerable evidence suggests that emotional arousal biases both lower animals and humans towards predominant use of the dorsolateral striatum-dependent habit memory system over the hippocampus-dependent cognitive memory system.

Emotional Arousal and Multiple Memory Systems: The Role of the Basolateral Amygdala

Over the last few decades considerable work has been carried out to determine the neuroanatomical structure(s) involved in mediating the ability of emotional arousal to influence the relative use of memory systems. A number of studies have focused on the potential role of the basolateral amygdala (BLA). The BLA has historically been linked to emotional behavior in animals (Kluver & Bucy, 1939). Furthermore, direct infusions of various drugs into the BLA induce anxiety-like behaviors in rats (e.g.

Sanders & Shekhar, 1991; Scheel-Kruger & Peterson, 1982). More recently, the BLA has been implicated as an important structure in mediating the memory modulatory processes of several neurotransmitter systems that are activated by emotional arousal (for review see McGaugh, 2004). It is hypothesized that the BLA functions as a memory modulation "center" in which projections from the BLA influences memory consolidation at target brain structures. Consistent with this hypothesis, evidence has found that the BLA modulates memory occurring in both the hippocampus and dorsal striatum (Packard et al., 1994; Roozendaal & McGaugh, 1997; Packard & Teather, 1998).

As a follow-up to the observation that peripheral anxiogenic drugs bias rats towards the use of dorsolateral striatum-dependent habit strategy, rats were trained in a dual-solution plus-maze and infused with the same anxiogenic drug (RS 79948-197) directly into the BLA. Rats displayed predominant use of response learning relative to controls on the drug-free probe trial (Packard & Wingard, 2004), suggesting that the BLA was mediating the effect of emotional arousal on shift in preference towards a dorsolateral striatum-dependent learning strategy. A subsequent study attempted to determine if intra-BLA injections biased rats towards use of the dorsolateral striatum-dependent learning strategy by *directly* facilitating striatal-dependent response learning or *indirectly* influencing response learning by impairing hippocampus-dependent place learning. Rats were trained in "single-solution" water plus-maze that could only be acquired using either a hippocampus-dependent place strategy or dorsolateral striatum-dependent response strategy (as earlier described). Rats were trained in either of these

tasks and received post-training intra-BLA injections of RS 79948-197. Rats that received anxiogenic drug injections displayed enhanced acquisition of the single-solution response task relative to controls while separate groups receiving drug injections displayed impaired acquisition of the place learning task (Wingard & Packard, 2008). This pattern of results suggest that the anxiogenic drug may be facilitating response learning through by impairing hippocampus-dependent place learning, which frees the dorsolateral striatum-dependent memory system from competition (as described earlier; for review see Poldrack & Packard, 2003).

Most recently, our lab conducted a set of experiments to determine whether the BLA is involved in both place and response learning if RS 79948-197 was administered peripherally. Indeed, both the enhancing and impairing effects of peripheral administration of RS 79948-197 on response and place learning, respectively, were blocked by neural inactivation of the BLA through intra-BLA injections of bupivacaine (Packard & Gabriele, 2009). This further supports the hypothesis that the BLA mediates the influence of emotional arousal on various types of memory. Taken together, there is considerable evidence that the BLA is a key neuroanatomical structure that mediates the influence of emotional arousal on the relative use of multiple memory systems.

Goals of the Present Experiments

Considerable evidence suggests that emotional arousal is an important factor influencing cognitive processes, particularly memory. It is important to continue the quest in fully understanding the role of emotional arousal within the context of the

multiple memory systems hypothesis. While it has been widely shown that emotional arousal produces a shift away from hippocampus-dependent memory towards predominant use of the dorsolateral striatum-dependent memory system, there remains a large number of uncovered topics within this area of interest that have yet to be fully explained. The present set of experiments aimed to uncover and elucidate some of these ideas. The general theme of the present set of experiments were to broaden the scope of emotional stimuli employed in these studies to encapsulate a more relevant model to both day-to-day behavior as well as stress-mediated psychopathology. In other words, the present experiments were developed to explore the role of ethologically and physiologically relevant stressors. In addition, these set of experiments also introduce the idea that a fear-conditioned stimulus may modulate memory. The studies examine whether these relevant stressors would influence multiple memory systems in a fashion similar to standard laboratory stressors, such as foot/tail-shock or administration of pharmacological drugs. Specifically these experiments employed the use of three separate emotionally arousing stimuli:

1) An ethologically-relevant stressor, to 2,5-Dihydro-2,4,5-trimethythiazoline (TMT), a sulfur-containing compound that is specific to red fox feces (a natural predator of the rat).
2) A physiologically-relevant stressor, corticosterone, the primary stress hormone released endogenously following exposure to a hostile or stressful stimulus/situation.

3) Fear-conditioned stimuli. Here we examine the effect, if any; exposure to fear-related conditioned stimuli may have on memory modulation, particularly with regards to the dorsolateral striatum-dependent habit memory system. The studies employed a relatively novel paradigm of exposing rats to a fear-related conditioned stimulus as a method of producing emotional arousal to influence memory modulation. Rats were not exposed to the unconditioned emotionally-arousing stimulus in a manner that would influence memory modulation. Therefore, any effect on memory modulation would be attributed to the emotional properties acquired by the neutral stimulus associated to the emotional unconditioned stimulus.

The present sets of experiments were designed to explore the role of these relevant stressors in modulating multiple memory systems. Through the use of these stressors, when presented in relation to behavioral tasks designed to illuminate the mechanisms of multiple memory systems, the present set of experiments hoped to build on the understanding of the role of emotional arousal on the relative use of multiple memory systems and the mechanisms of individual memory systems.

CHAPTER II

ETHOLOGICALLY-VALID STRESSOR: PREDATOR ODOR[*]

Introduction

The vast majority of previous research has investigated the role of emotional arousal on the relative use of memory systems through employment of manipulations such as pharmacologically anxiogenic drugs (Packard & Wingard, 2004) or foot/tail-shocks (Kim et al., 2001). While these studies have expanded our understanding on the role of emotional arousal on influencing memory, these emotionally arousing stimuli are not ethologically relevant. Animals rarely, if ever, experience footshock or infusions of exogenous pharmacological agents in their natural environment. Recently there has been a rise in interest towards understanding the behavioral responses to ethologically-relevant stressors. That is, stressors that may occur in the animals natural environment. Therefore it is of interest to determine if an ethologically relevant stressor, such as predator odor stress, can influence memory in similar ways. It is well documented that exposure to a predator odor induces stress and anxiety-like behavioral responses in rats (Griffith, 1919, 1920). Exposure to predator odor results in a range of fear/anxiety-like behaviors such as freezing, hiding, and decreased stimulus contact (Blanchard, Blanchard, Wiess, & Meyers, 1990; Dielenberg & McGregor, 2001).

[*] Reprinted with permission from "Exposure to predator odor influences the relative use of multiple memory systems: Role of basolateral amygdala" by Leong, K.-C. & Packard, M. G., 2014. *Neurobiology of Learning and Memory,* 109, 56-61, Copyright 2014 Elsevier.

2,5-Dihydro-2,4,5-Trimethythiazoline (TMT) as an Ethologically Valid Stressor

The odor compound 2,5-Dihydro-2,4,5-trimethythiazoline (TMT), a sulfur-containing compound that is found in red fox feces (a natural predator of rats), has been found to induce fear/anxiety in rats (Vernet-Maury, 1980; Vernet-Maury, Polak & Demael, 1984; Morrow, Elsworth, & Roth, 2002; Burwash, Tobin, Woolhouse & Sullivan, 1998; for review see Fendt, Endres, Lowry, Apfelbach, & McGregor, 2005). Corticosterone levels in rats' blood following acute single exposure to TMT correlated to the strength of fear-like behaviors (Vernet-Mauray et al., 1984). Similarly, TMT exposure increases secretion of adrenocorticotropin and corticosterone (Day et al., 2004) suggesting that exposure to this predator odor elicits physiological stress response. Furthermore, exposure to TMT in the presence of a conditioned fear stimulus induces freezing and analgesia (Hotsenpiller & Williams, 1997). More recently studies have found that TMT induces fear-like effects through a variety of behaviors such as freezing, defecation, approach latency, etc. (Morrow et al., 2002; Burwash et al., 1998; for review see Fendt et al., 2005). The neural basis of acute TMT-induced anxiety has yet to be fully established. Electrolytic lesions of the anterior hypothalamic nucleus (AHN) and dorsomedial part of the ventromedial hypothalamic nucleus (VMHdm) (Pagani & Rosen, 2009) and lateral amygdala (Wallace & Rosen, 2001) reduced and blocked TMT-induced freezing, respectively. However, these effects were not seen with neurotoxic lesions (Pagani & Rosen, 2009; Wallace & Rosen, 2001). Additionally, temporary inactivation of the medial amygdala and BLA blocks TMT-induced freezing (Muller & Fendt, 2006). Animals exposed to TMT showed increased activity in the bed nucleus of

the stria terminalis (BNST) as well as the central nucleus of the amygdala (CeA) (Day et al., 2004), providing further support for the role of the amygdala in TMT-induced anxiety.

The fear/stress-inducing properties of TMT are inherent to the compound of the odor and not simply due to its salience. This is evidenced in studies in which rats are differentially exposed to TMT and butyric acid, a rancid and noxious odor (Wallace & Rosen, 2001). Here rats exposed to TMT exhibited freezing at higher levels compared to a neutral odor. However, rats exhibited no freezing when exposed to butyric acid. This suggests that the fear-like behaviors elicited by TMT is not due to the unpleasant odor per se, but rather the inherent familiarity of the odor to a predator.

The Effect of TMT Exposure on Memory

Exposure to predator stress can produce various effects of learning and memory. Previous research has found that exposure to a predator (presence of a cat), can impair hippocampus-dependent spatial learning (Park, Campbell & Diamond, 2002; Park et al., 2008) and retrieval (Diamond et al., 2006). Cat exposure also impaired spatial working memory in a delayed alternation version of the elevated T-maze task (Williams, Baker, Gress, & Givens, 1998) and the radial-arm water maze task (Diamond, Park, Heman, & Rose, 1999) compared to rats that did not receive cat exposure.

Exposure to a predator odor can influence memory in ways comparable to direct predator exposure. Several studies have examined the effect of exposure to TMT on performance of various learning and memory tasks (for review see Takahashi

Nakashima, Hong & Watanabe, 2005), and TMT has been used extensively as an unconditioned stimulus (US) in fear conditioning tasks (for review see Takahashi, Chan & Pilar, 2008). The effectiveness of TMT as a US is seen particularly in contextual fear conditioning, in which presentation of TMT during contextual fear conditioning facilitated subsequent freezing responses in the same context the next day (Rosen, 2004). Similarly, rats presented with TMT for seven days in a two-compartment chamber subsequently avoided the TMT-paired compartment, displaying place preference for the chamber not previously paired with TMT (Endres and Fendt, 2007). Additionally, TMT exposure resulted in the impairment of short-term working memory in a delayed nonmatching-to-sample task (Morrow, Roth & Elsworth, 2000). That is, when exposed to TMT, rats failed to selectively explore a novel object compared to a familiar one. Finally, exposure to TMT impaired spatial working memory in a spatial-alternation task and impaired spatial reference memory in a Morris water maze task (Williams, Baez, Hladky, & Camacho, 2005; but see Gaillot et al., 2010). Interestingly, this impairment in spatial reference memory can be blocked by the anxiolytic benzodiazepine agonist, midazolam.

Specific Goals

The following set of experiments aimed to investigate the role of an ethologically-relevant stressor in modulating both the relative use of multiple memory systems. Experiment 1a examined the role of a predator odor, 2,3,5-trimethyl-3-thiazoline (TMT), in modulating the relative use of the hippocampus and striatal-

dependent learning strategies through training in a dual-solution task that could be acquired using either a hippocampus- or dorsolateral striatum-dependent strategy. Experiment 1b, similarly, examined the role of pre-training exposure to TMT in a strictly dorsolateral striatum-dependent task. Experiment 2 then attempted to implicate the BLA in mediating the effect of pre-training TMT on the modulation of dorsolateral-striatum dependent learning, if any. Here, animals received pre-training exposure to TMT prior to training and immediately post-training the BLA is inactivated through local infusion of bupivacaine. The experimental paradigm in which rats receive pre-training TMT exposure remains more ethologically-relevant as animals may often encounter the presence of a predator prior to engaging in various behaviors.

Methods

Experiment 1a

Subjects

Subjects were 30 experimentally naïve adult male Charles River Long-Evans rats (300-400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle. Handling and care of all rats in the studies reported in this dissertation adhered to the standards and guidelines set by the Institutional Animal Care and Use Committee (IACUC) of Texas A&M University.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Water Plus-Maze

The water plus maze used was identical to that used in previous studies from our lab (e.g. Leong, Goodman, & Packard, 2012; Packard & Gabriele, 2009). A clear Plexiglas plus-maze (43 cm height, arm-width of 27 cm, and arm-length of 60 cm) was inserted in a black circular water maze (180 cm diameter, 45 cm height). The water maze was filled to a water level of approximately 21 cm and water temperature was maintained at 25ºC. An invisible clear Plexiglas escape platform (15 x 14 x 20 cm) is submerged inside of the plus maze at the end of the designated goal arm, 1 cm below water level. The arm opposite to the start arm is blocked by a piece of clear Plexiglas so that the animals are trained in a T-maze configuration. The maze is located in a room containing several extra-maze cues.

Odorant Exposure Chamber

The method of exposure to the predator odor 2,3,5-trimethyl-3-thiazoline (TMT) is similar to previous studies (Endres and Fendt, 2008 and Fendt et al., 2003). Animals are placed inside PVC holding containers (45 x 30 x 25 cm) located underneath a ventilation hood during odor exposure. Consistent with studies examining the behavioral

effects of TMT exposure (e.g. Galliot, Levaillant, Beard, Millot, & Pourie, 2010; Hacquemand et al., 2012 and Morrow et al., 2000) distilled water is used for the control group. TMT (5 µl) or distilled water (5 µl) is deposited onto circular filter paper (4.7 cm diameter) and placed on the wall of the holding container 10 cm from the bottom. Rats are placed into the appropriate TMT or control (distilled water) container for 5 min immediately prior to training. Different containers were used for TMT exposure and distilled water exposure to control for any lingering odors that may persist.

Behavioral Procedures

Dual-Solution Water Plus-Maze Task

Immediately following pre-training exposure to TMT (n = 15) or distilled water (n = 15) rats are transported to the behavioral testing room. Animals are trained in a dual-solution water plus-maze task for 2 consecutive days (6 trials/day). On each trial, animals are placed into the start-arm of the maze (i.e. south arm), facing the maze wall and are given 60 s to swim to a hidden platform located in the goal-arm (i.e. east arm). The start-arm and goal-arm remain fixed throughout the training period. The opposite arm from the start-arm is blocked off with a Plexiglas barrier. After reaching the platform rats remain there for 10 s before being removed and placed in an adjacent opaque holding container for a 30 s inter-trial interval. If the rat makes a full body turn into the incorrect arm (i.e. west arm) the trial was scored as an error. Following two days of training, rats receive a probe trial on the third day to determine whether they use place or response learning. No exposure to predator odor is given prior to the probe trial. On

the probe trial, the start-arm is shifted to the opposite arm (i.e. north arm), with the arm directly opposite blocked off (i.e. south arm). Rats that turn left at the choice point and enter the east arm on the probe trial (i.e. approach the same spatial location that the hidden platform was located in during training) are designated as place learners. Rats that make a right turn at the choice point and enter the west arm on the probe trial (i.e. make the same body turn to swim to the hidden platform as during training) are designated as response learners.

Statistical Analysis

A two-way one-repeated measures ANOVA (Group x Day) was carried out to examine if groups in both the TMT-exposure and distilled water-exposure conditions learned the task and that there were no differences between groups at the end of training. A chi-square analysis was performed to compare the number of animals that used a place strategy vs. a response strategy between TMT and distilled water-exposure conditions during the probe trial on day 3.

Experiment 1b

Subjects

Subjects were 33 experimentally naïve adult male Charles River Long-Evans rats (300-400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h

light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Water Plus-Maze

The water plus-maze apparatus used was identical to that use in Experiment 1

Odorant Exposure Chamber

The odor exposure chamber was identical to that used in Experiment 1.

Behavioral Procedures

Single Solution Place Plus-Maze Task

Immediately following pre-training exposure to TMT (n = 6) or distilled water (n = 5) (days 1 through 3) rats are transported to the behavioral testing room. Animals are then trained in a single-solution response water plus-maze task. In this task rats were trained for 5 consecutive days (6 trials/day). On each trial, rats were placed in the start arm (north or south) facing the maze wall and are given 60 s to swim to a hidden escape platform located in the same arm across all training trials (west). The sequence of the

start arm varied depending on day. On odd days, the start arm sequence is NSSNNS and on even days, the start arm sequence is SNNSSN. The escape platform is always placed in the same such that the body turn at the choice point varies to reach the goal arm. If the rat fails to find the escape platform in 60 s, the experimenter manually guides the rat to the platform. Upon climbing onto the platform, rats remains there for 10 s before being removed from the maze and placed in an opaque holding container for a 30 s inter-trial interval. On each trial, a correct response is scored if the rat made a full body turn into the correct arm in which the escape platform was located. A full body turn into the wrong arm results in the trial being scored as an error.

Single Solution Response Plus-Maze Task

Immediately following pre-training exposure to TMT (n = 11) or distilled water (n = 11) (days 1 through 3) rats are transported to the behavioral testing room. Animals are then trained in a single-solution response water plus-maze task. In this task rats are trained for 5 consecutive days (6 trials/day). On each trial, rats are placed in the start arm (north or south) facing the maze wall and are given 60 s to swim to a hidden escape platform located in another arm (east or west). The sequence of the start arm varied depending on day. On odd days, the start arm sequence is NSSNNS and on even days, the start arm sequence is SNNSSN. The escape platform is always placed in the arm in which a right body turn at the maze choice point leads to the platform. If the rat fails to find the escape platform in 60 s, the experimenter manually guides the rat to the platform. Upon climbing onto the platform, rats remain there for 10 s before being

removed from the maze and placed in an opaque holding container for a 30 s inter-trial interval. On each trial, a correct response was scored if the rat makes a full body turn into the correct arm in which the escape platform was located. A full body turn into the wrong arm results in the trial being scored as an error.

Statistical Analysis

A two-way one-repeated measures ANOVA (Group x Day) was carried out to examine whether there was any difference in acquisition of the task between animals receiving either TMT-exposure or distilled-water exposure.

Experiment 2

Subjects

Subjects were 41 experimentally naïve adult male Charles River Long-Evans rats (300-400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Surgery

Rats were anesthetized with isoflurane gas anesthesia (Vedco) and draped with sterile drapes before being placed in the stereotaxic. The top of the skull is shaved and scrubbed with povidone-iodine (Betadine) before rinsing with 70% alcohol. This process is repeated three times before initial incision. Bilateral guide cannulae (23 gauge, 15 mm long) were inserted into the basolateral amygdala using standard stereotaxic techniques. Coordinates for the basolateral amygdala are AP = -2.2, ML = +/- 4.7, DV = -7.0 (Packard & Gabriele, 2009). Cannulas were anchored with dental acrylic and anchored to the surface of the skull with jeweler's screws. Following surgery rats were given analgesic (Children's Tylenol/Acetaminophen) in their water supply (6 mg/ml) for 3 days. Animals were allowed to recover for one week following surgery.

Infusions

Bilateral intra-BLA infusions (0.5 µl/side) of bupivacaine (1% solution, Abbott Laboratories) or saline were administered via a microsyringe pump with an electronic timer (Sage Instruments) through 10 µl Hamilton syringes connected to an polyethylene tubing (PE 10) and injection needle (16 mm length, 30 gauge). Bupivacaine is a sodium channel blocker, hence providing temporary inactivation of the region via the blockade of action potential conductance. Infusions were administered over a period of 52 seconds. Following this period, injection needles were left in the guide cannula for an additional 60 seconds to allow for diffusion. This infusion procedure is identical to that of a previous study from our lab indicating a role for BLA in mediating the effect of an

anxiogenic drug on multiple memory systems (Packard & Gabriele, 2009). Following infusions injection needles are swabbed with 70% alcohol and allowed to dry.

Histology

Following the completion of behavioral procedures rats were sacrificed with a 1 ml injection of pentobarbital sodium (Euthasol Euthanasia Solution, Virbac Corporation, Texas). Rats were then perfused in the heart with physiological saline followed by 10% formaldehyde-saline solution. Brains were removed and post-fixed in formalin. Brains were sectioned at 20 μm through the cannula tract region using a cryostat, and were subsequently mounted on slides and stained with cresyl violet. The location of the injection needle tips were confirmed using a standard rat brain atlas (Paxinos & Watson, 1997), and were located in the basolateral amygdala ranging from -1.80 to -3.14 mm from bregma (Figure 5). Only animals displaying bilateral cannula placements were including in this study.

Although needle tips were located within the BLA, it is still possible that infusions of bupivacaine may have spread into surrounding amygdala nuclei (CeA), such as the central nucleus. However, the dose of bupivacaine chosen for this study was based off earlier studies that employed the same dose of bupivacaine to implicate the BLA in memory modulation (Packard & Gabriele, 2009). Furthermore, numerous studies have found converging evidence to suggest that the BLA mediates the memory modulatory effects following emotional arousal. For example, BLA lesions, but not CeA lesions, blocked modulatory effects on hippocampus-dependent memory (Roozendaal &

McGaugh, 1996a). Additionally, intra-BLA, but not intra-CeA, post-training drug administration modulated memory (Roozendaal & McGaugh, 1997). Finally, researchers found that hippocampal long-term potentiation (LTP) is mediated by the BLA, but not CeA (Akirav & Richter-Levin, 2002).

Apparatus

Water Plus-Maze

The water plus-maze apparatus used was identical to that use in Experiment 1

Odorant Exposure Chamber

The odor exposure chamber was identical to that used in Experiment 1.

Behavioral Procedures

Dual Solution Water Plus-Maze Task

Experiment 2 replicated the behavioral procedure used for the dual-solution water maze task in Experiment 1a with the exception that animals were trained for 4 trials/day (a sufficient number of trials to produce learning). Following exposure to TMT (n = 29) or distilled water (n = 12), rats were trained to swim from the same start arm to the same goal arm on all trials (4 trials/day) on days 1 and 2. Immediately following training on days 1 and 2 rats received post-training intra-BLA infusions of either bupivacaine (0.5 µl/side) or saline, depending on group. Rats were then given a probe trial on day 3 and were designated as either "place" or "response" learners based on their

probe trial behavior. Previous work from our lab has demonstrated that bupivacaine, when infused intra-BLA, does not influence hippocampus-dependent place learning or dorsolateral striatum-dependent response learning by itself (Packard & Gabriele, 2009).

Statistical Analysis

A two-way one-repeated measures ANOVA (Group x Day) was carried out to examine if groups in both the TMT-exposure and distilled water-exposure conditions learned the task and that there were no differences between groups at the end of training. A chi-square analysis was performed to compare the number of animals that used a place strategy vs. a response strategy between rats receiving intra-BLA bupivacaine or vehicle following TMT and distilled water-exposure conditions during the probe trial on day 3.

Results

Experiment 1a

TMT Exposure Biases Rats towards Response Learning in a Dual Solution Plus-Maze Task

A two-way repeated measures ANOVA (Group x Day) revealed a main effect of Day ($F(1,28) = 8.29$, $p < 0.05$), indicating that animals in both groups learned the task over two days. There was no significant difference between groups ($F(1,28) = 0.25$, n.s.), indicating that there was no effect of pre-training exposure to TMT on task acquisition.

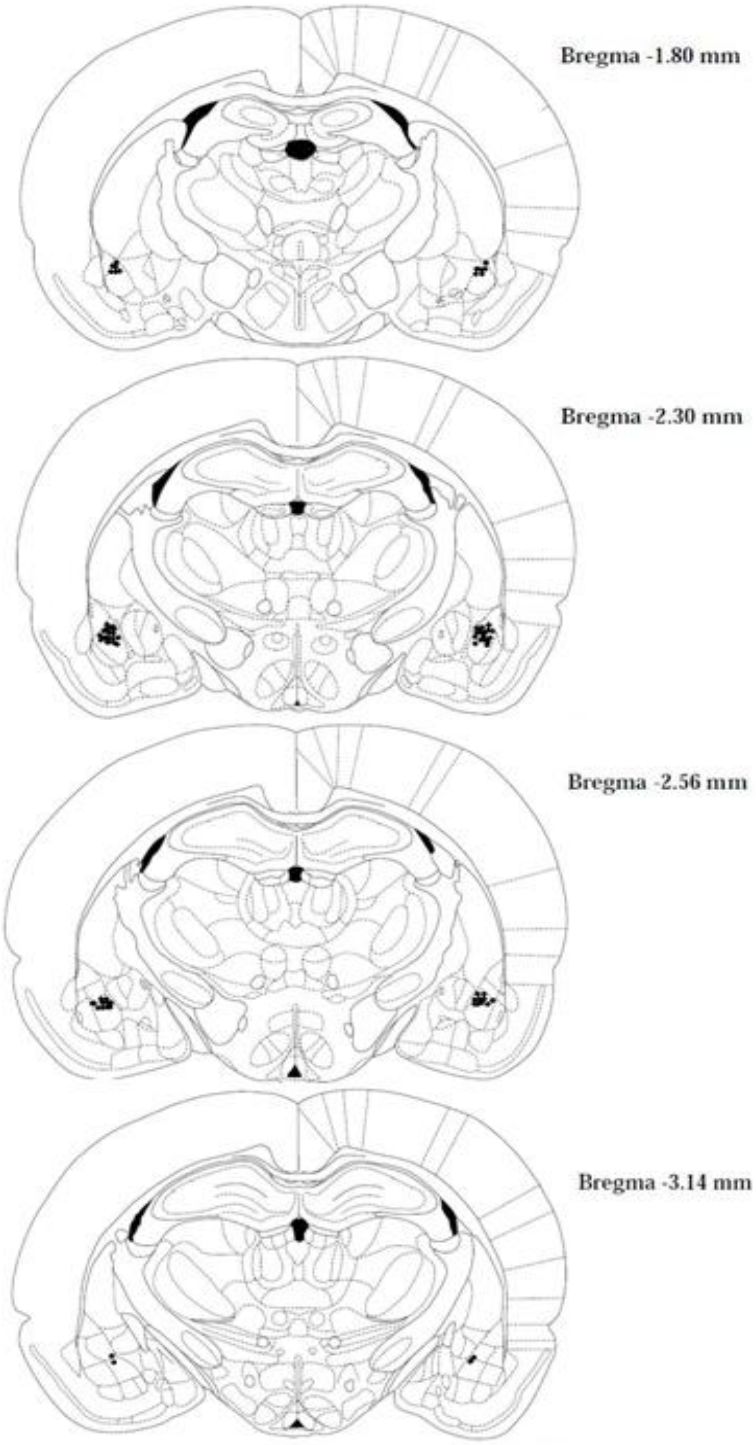

Figure 5. Illustrated brain sections from rats indicating the location of bupivacaine infusion needle placements in the BLA (filled circles shown with overlap). Diagram displays BLA ranging from -1.80 mm to -3.14 mm anterior-posterior from bregma (unlabelled diagrams from Atlas of Paxinos and Watson, 1997). Adapted from Leong & Packard, 2014.

The effect of pre-training exposure to TMT on the use of "place" or "response" learning on the subsequent day 3 probe trial is shown in Figure 6. A χ^2 analysis was performed to determine if there was a difference in the relative use of place or response learning on the probe trial. Rats exposed to distilled water displayed an absolute preference for the use of place learning on the probe trial (11 place rats, 4 response rats with the analysis showing a significant trend ($\chi^2 = 3.27$, $p = 0.07$). In sharp contrast, rats exposed to TMT pre-training displayed a significant use of response learning strategy on the probe trial (3 place rats, 12 response rats; $\chi^2 = 5.4$, $p < 0.05$). These findings indicate that in the dual-solution water plus-maze task in which place and response learning can both provide an adequate solution, pre-training exposure to TMT influenced the type of strategy adopted by rats during the probe trial. Specifically, relative to control rats, rats that had received pre-training TMT exposure were biased towards the use of response learning (Figure 6).

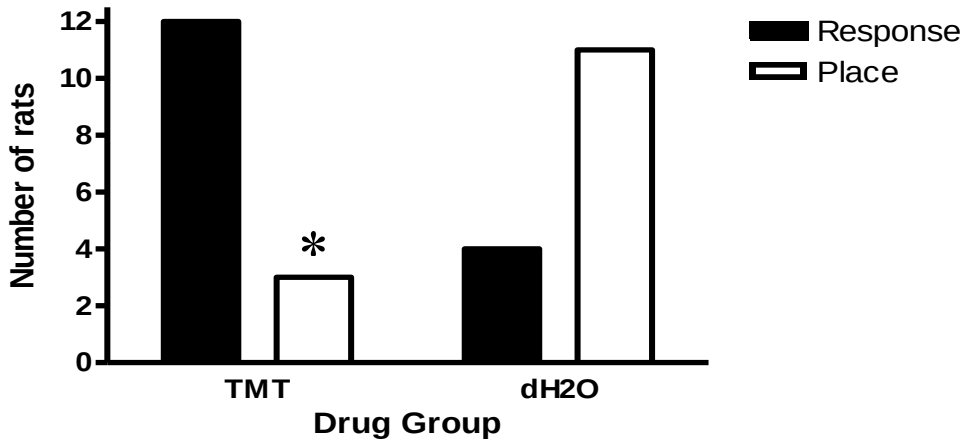

Figure 6. Effect of pre-training TMT exposure on learning strategy in a dual-solution probe trial. Number of rats in each experimental group that exhibited place or response learning on the day 3 probe trial. Rats received pre-training exposure to TMT or distilled water (dH2O) on training days but no odor exposure prior to the day 3 probe. Asterisks (*) denotes statistical significance at $p < 0.05$.

Experiment 1b

Exposure to TMT Has No Effect on Place Learning in a Single-Solution Plus Maze Task

The effect of pre-training exposure to TMT on acquisition of the single-solution place learning task is shown in Figure 3. A two-way repeated measures ANOVA (Group X Day) computed on percentage correct on days 1-5 revealed no main effect of Group, ($F(1,9) = 0.93$, n.s., but a significant effect of Day ($F(4,36) = 12.33$, $p < 0.01$). There was no significant Group x Day interaction ($F(4,36) = 0.40$, n.s.). These results indicate that rats from both groups displayed learning of the single-solution place task over the 5 day training period. However, there was no difference between both groups over 5 days suggesting that pre-training exposure to TMT did not influence learning of this place task (Figure 7).

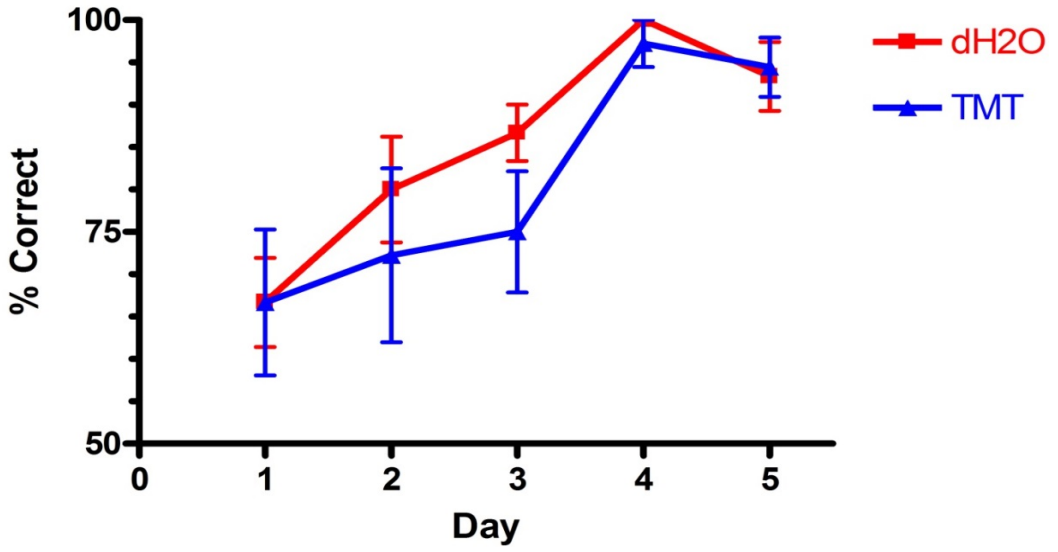

Figure 7. Effect of pre-training exposure on acquisition of a single-solution place task. Pre-training exposure to TMT has no effect on acquisition of place learning in the forced-place water plus-maze task. Adapted from Leong & Packard, 2014.

Exposure to TMT Enhances Response Learning in a Single-Solution Plus Maze Task

The effect of pre-training exposure to TMT on acquisition of the single-solution response learning task is shown in Figure 8. A two-way repeated measures ANOVA (Group X Day) computed on percentage correct on days 1-5 revealed a main effect of Group, ($F(1,20) = 5.83$, $p < 0.05$), and of Day ($F(4,80) = 12.33$, $p < 0.01$). There was no significant Group x Day interaction ($F(4,80) = 0.13$, n.s.). These results indicate that rats from both groups showed significant improvement in response learning over the 5 day training period, and relative to rats that received pre-training exposure to distilled water, rats that received pre-training exposure to TMT displayed facilitated task acquisition (Figure 8).

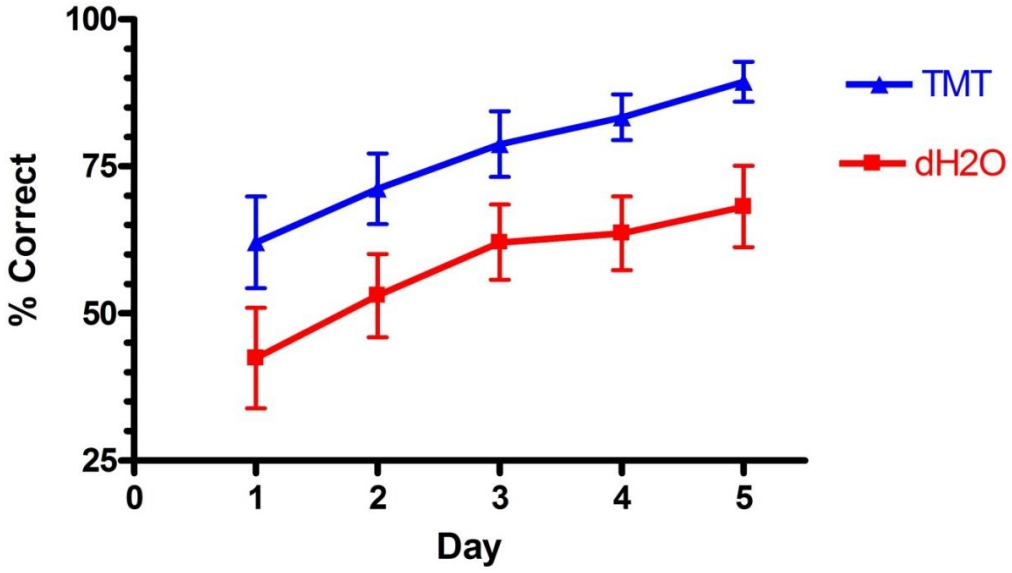

Figure 8. Effect of pre-training exposure on acquisition of a single-solution response task. Enhancing effects of pre-training exposure to TMT on acquisition of response learning in the forced-response water plus-maze task. Adapted from Leong & Packard, 2014.

Experiment 2

Intra-BLA Bupivacaine Infusions Block the TMT-Induced Bias towards Response Learning in a Dual Solution Plus-Maze Task

Similar to experiment 1a, a two-way repeated measures ANOVA (Group x Day) revealed a main effect of Day ($F(1,35) = 23.53$, $p < 0.01$), indicating all groups of animals learned the task over two days. Additionally, there was no significant difference between groups ($F(1,35) = 0.40$, n.s.), indicating that there was no effect of pre-training exposure to TMT on learning of this task. The effect of post-training intra-BLA infusions of bupivacaine on the ability of TMT exposure to influence the relative use of place and response learning on the day 3 probe trial is shown in Figure 3. χ^2 analyses revealed that control rats exposed to distilled water prior to training and receiving post-

training intra-BLA infusions of saline displayed a significant trend towards the predominant use of place learning on the probe trial, (9 place rats, 3 response rats; $\chi^2 = 3.00$, $p = 0.08$). In contrast, χ^2 analysis revealed that rats exposed to TMT prior to training and receiving post-training intra-BLA infusions of vehicle saline displayed a significant use of response learning on the day 3 probe trial (2 place rats, 11 response rats; $\chi^2 = 6.23$, $p < 0.05$). This finding replicates the bias towards the use of response learning that was produced by pre-training TMT exposure in Experiment 1a. However, when bupivacaine was infused post-training into the BLA immediately after TMT exposure, there was no significant difference in the type of learning strategy used on the subsequent day 3 probe trial (7 place rats, 6 response rats; $\chi^2 = 1.00$, n.s.). Taken together, these findings indicate that pre-training TMT exposure biases rats towards the use of response learning on a subsequent probe trial, and that intra-BLA infusions of bupivacaine attenuates this effect (Figure 9).

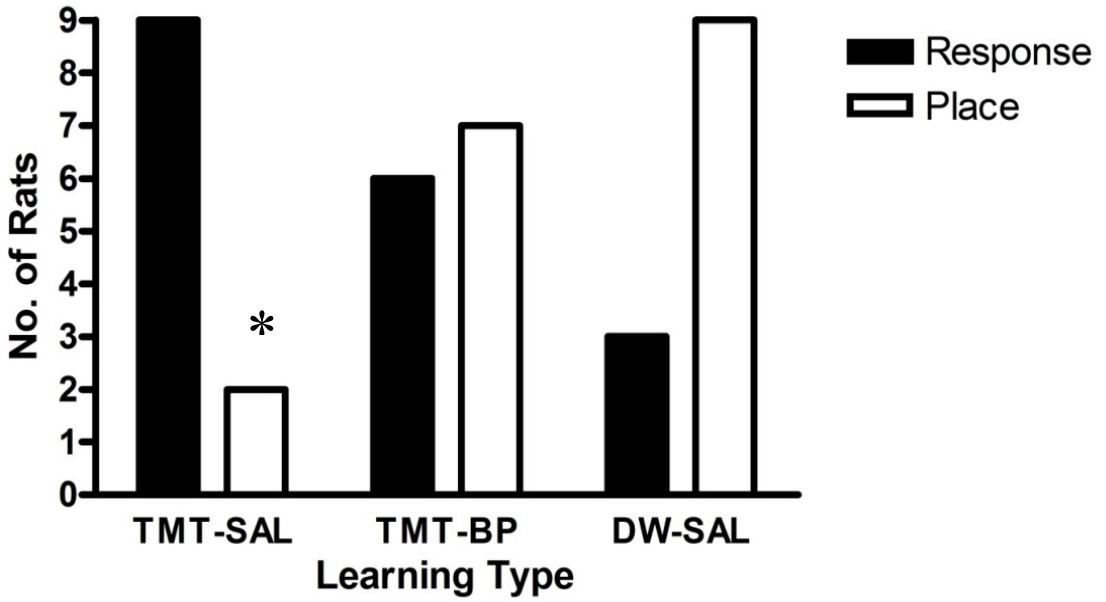

Figure 9. Effect of BLA inactivation on learning strategy in a dual-solution task. Number of rats in each experimental group that exhibited place or response learning on the day 3 probe trial. Rats received pre-training exposure to TMT or distilled water (DW) and received either post-training injections of bupivacaine or saline into BLA. Asterisks (*) denotes statistical significance at $p < 0.05$. Adapted from Leong & Packard, 2014.

Summary

The present findings indicate that in a dual-solution plus-maze task that can be acquired using both place and response learning, pre-training exposure to the predator odor 5-Dihydro-2,4,5-trimethythiazoline (TMT) biases rats towards the use of response learning. In addition, in a single solution plus-maze task that *requires* the use of response learning, pre-training exposure to TMT enhances task acquisition. Extensive evidence indicates that exposure to TMT is negatively emotionally arousing to animals, inducing anxiety/fear-like effects that have been assessed via a variety of behavioral measures including freezing, defecation, and approach latency (e.g. Vernet-Mauray et al., 1984; Morrow et al., 2002; Hotsenpiller & Williams, 1997; Burwash et al., 1998; for

review see Fendt et al., 2005). Thus, as has been previously observed following drug-induced anxiety (e.g. Packard & Wingard, 2004; Elliot & Packard, 2008; Packard & Gabriele, 2009), exposure to a putatively ethologically valid stressor (i.e. predator odor) can also influence the relative use of multiple memory systems. Overall, the findings provide further evidence supporting the hypothesis that robust emotional arousal induced by stress/anxiety leads to the facilitation and preferential use of habit memory (for review see Packard & Goodman, 2012).

The precise neural basis of TMT-induced anxiety/stress has yet to be fully established, although several studies suggest a potential role for the amygdaloid complex. For example, temporary inactivation of the BLA and medial amygdala blocks TMT-induced freezing behavior (Muller & Fendt, 2006). Exposure to TMT increases activity in the bed nucleus of the stria terminalis as well as the central nucleus of the amygdala (Day, Masini & Campeau, 2004), providing further support for the role of the amygdaloid complex in TMT-induced anxiety. Although electrolytic lesions of the lateral amygdala (Wallace & Rosen, 2001) blocked TMT-induced freezing behavior, this effect was not observed following cell-body sparing neurotoxic lesions of this area (Pagani & Rosen, 2009; Wallace & Rosen, 2001).

We have previously observed that the BLA mediates the ability of the anxiogenic drug RS 79948-197 to bias rats towards the use of response learning in a dual-solution plus-maze task, as well as facilitate response learning in a single-solution plus-maze task (Packard and Wingard, 2004; Wingard and Packard, 2008; Packard and Gabriele, 2009). These previous plus-maze findings are consistent with extensive evidence implicating

the BLA in mediating the modulatory effects of emotional arousal on memory (for review see McGaugh, 2004). Experiment 2 found that neural inactivation of the BLA prevented the bias towards response learning that is produced by pre-training TMT exposure in the dual-solution plus-maze task. However, as discussed earlier, the possibility remains that intra-BLA infusions of bupivacaine may spread into surrounding amygdala nuclei. Future studies may control for this by adding a condition in which bupivacaine is also infused into surrounding nuclei (e.g. CeA) to determine if infusions into these areas produce similar or different effects. Taken together, these findings suggest that TMT exposure and anxiogenic drug administration influence the relative use of place and response learning via a common mechanism that likely involves the BLA.

CHAPTER III

PHYSIOLOGICALLY-VALID STRESSOR: CORTICOSTERONE

Introduction

Stress can be characterized by a physiological change that results from exposure to threatening stimuli. While exposure to TMT provides a method to investigate the role of an ethologically-relevant threatening stimulus in modulating learning and memory, it is also of interest to investigate the resulting effect of increased plasma levels of endogenous stress hormones in modulating memory. The next set of experiments aimed to determine whether a physiologically-relevant stressor such as the adrenal stress hormone corticosterone can modulate the use of multiple memory systems. Previous findings indicate that anxiogenic pharmacological agents such as the α2-adrenoceptor antagonist RS 79948-197 (Packard & Wingard, 2004; Wingard & Packard, 2008) have influenced the relative use of memory systems through blocking reuptake of norepinephrine, thus artificially inducing heightened states of emotional arousal. Additionally, there is often an increase in levels of endogenous plasma corticosterone levels following stressful experience (de Kloet, Harst, & Joels, 2008). Therefore, administration of corticosterone mimics the natural physiological response to stress exposure. Similar to other stressors, corticosterone administration also modulates memory processes (for review see Roozendaal, 2006).

Corticosterone as a Physiologically-Relevant Stressor

Emotional arousal, particularly stressful experiences, results in the activation of the hypothalamic-pituitary-adrenalcortical (HPA) axis. Activation of the HPA axis results in release of hypothalamic corticotropin releasing hormone (CRH), which then lead to release of pituitary adrenocorticotropin releasing hormone (ACTH), and ultimately culminates in the secretion of corticosterone into the bloodstream from the adrenal cortex (Herman & Cullinan, 1997). It is this response of the HPA axis and subsequent corticosterone release that allow animals to respond to acute stressors in an adequate way. However, when experimentally investigating the role of stress on behavior it is important to consider that experimenter-applied stressors fails to control for individual differences in activation of the HPA axis in response to stress. That is, stressful experiences may result in different levels of corticosterone secretion depending on whether animals perceive a stressor to be as threatening as intended. Indeed, studies have found that the degree to which the HPA axis activates these stress hormone systems depend on various factors such as age, gender, and severity/type of stressor (Kopin, 1995; Korte, 2001). This would suggest that rats exposed to the same stressor, regardless of its ethological-relevance, may produce varied levels of stress responses. Therefore, administration of exogenous corticosterone would provide a good opportunity to not only measure the role of a physiologically-relevant stressor on modulating memory, but would also control individual variability of responses to experimenter-applied stress.

Administration of exogenous corticosterone has been found to mimic physiological responses to stress. For example, corticosterone injections have been

found to decrease weight gain and sexual behavior in male rats (Karten et al., 1999; Gorzalka & Hanson, & Brotto, 1998). Furthermore, corticosterone administration resulted in moderate increase of anxiety-related behaviors (Stone, Egawa, & McEwen, 1988). Finally, chronic administration of corticosterone leads to down regulation of hippocampal glucocorticoid receptors (GRs) and impairment of HPA axis negative feedback control (Vyas, Mitra, Rao & Chattarji, 2002), similar to patients suffering from chronic stress and depression (Barden, Reul, & Holsboer, 1995; Chekley, 1996).

Corticosterone readily enters the brain and binds to two intracellular adrenal steroid receptor subtypes, the low-affinity glucocorticoid receptors (GRs) and the high-affinity mineralocorticoid receptors (MRs) (Reul & De Kloet, 1985; de Kloet, 1991). Due to the high-affinity of MRs for corticosterone it is postulated that these receptors are typically saturated under basal conditions (Reul & de Kloet, 1985). Alternatively, GRs are only occupied at levels of high stress and circadian peaks. Therefore, the influence of corticosterone on memory processes likely involves a selective activation of GRs. Several studies have discovered that post-training injections of GR antagonists, but not MR antagonists, affected memory in several tasks (Oitzl & de Kloet, 1992; Roozendaal & McGaugh, 1997).

The Role of Corticosterone in the Modulation of Memory

Early evidence found that HPA axis hormones could influence learning and memory in various ways. For example, administration of ACTH and corticosteroids affected extinction of avoidance behavior in rats (de Wied & Bohus, 1966; Bohus & de

Wied, 1981). Furthermore, the deleterious effects on cognition following sustained stress (McEwen & Sapolsky, 1995) have been attributed primarily to the release of glucocorticoids (Conrad et al., 1996; Dachir et al., 1993). More recently, stress hormones administered post-training have been implicated in the consolidation of memory processes, suggesting that hormones released following a stressful event may affect memory of that event (Kovacs et al., 1977; Flood et al.. 1978). Specifically, acute administration of corticosterone enhances memory in an inhibitory avoidance task (Kovacs et al., 1977; Roozendaal & McGaugh, 1996a). Similarly, corticosterone administration affects memory in various tasks including cued-fear conditioning (Pugh et al., 1997; Cordero & Sandi, 1998) and spatial water maze learning (Sandi, Loscertales, & Guaza, 1997). Additionally, glucocorticoid administration also facilitates memory consolidation of fear extinction while blocking glucocorticoid release impairs consolidation of these memory processes (Bohus & Lissak, 1968; Barrett & Gonzalez-Lima, 2004; Cai et al., 2006). Similarly, blockade of glucocorticoid synthesis with the synthesis-inhibitor metyrapone impaired memory consolidation (Maheu et al., 2004). It is important to note that typically the effect of corticosterone administration on memory adheres to an inverted-U shape curve (Akirav et al., 2004). That is, high and low levels of corticosterone may modulate memory in a facilitative or impairing manner while moderate levels of corticosterone may have the opposite effect. This inverted U shape relationship between corticosterone and memory might account for the biphasic effect of corticosterone administration of various spatial memory tasks (Sandi et al., 1997; Williams et al., 2005) and contextual fear memory tasks (Pugh et al., 1997; Cordero &

Sandi, 1998). In addition to the effect of glucocorticoids on acquisition and consolidation of memory, numerous studies have also observed an effect of glucocorticoid hormones on the retention and retrieval of memories. Elevated levels of glucocorticoids during retention testing at various tasks have led to significant impairment (de Quervain et al., 1998; Wolf et al., 2001; Roozendaal et al., 2004). For example, administration of a glucocorticoid agonist shortly prior to a probe test in a hippocampus-dependent water spatial maze task resulted in impaired retrieval of spatial memory (Roozendaal et al., 2003). Similar effects have been observed following administration of glucocorticoids in humans in impairing delayed recall on episodic tasks (de Quervain et al., 2000). In addition, several studies have also found similar impairing effects of glucocorticoids on working memory performance (Lupien et al., 1999; Wolf et al., 2001). The studies described above suggest an important role of glucocorticoids in modulating memory processes at various memory systems. However, there are only limited studies indicating that glucocorticoids play a similar role in the modulation of dorsolateral striatum-dependent memory processes (Quirarte et al., 2009; Medina et al., 2007).

The Interaction between the Glucocorticoid and the Noradrenergic System in Modulating Memory

While it has been well established that the glucocorticoid and noradrenergic neurotransmitter systems both play important roles in modulating memory (for review see McGaugh, 2004), recent evidence suggests that these two systems interact together

to influence memory consolidation and that the interaction between these two systems may be necessary for modulation of memory through emotional arousal. Early studies reported that the degree to which epinephrine can modulate memory consolidation may be altered by glucocorticoids in adrenalectomized rats (Borrell et al., 1983). More recently, a study found that attenuation of corticosterone release through administration of metyrapone, blocked the memory enhancing-effects of post-training peripheral injections of epinephrine (Roozendaal, Carmi, & McGaugh, 1996). There is also evidence to suggest that stress hormones may not necessarily modulate consolidation of all types of learning tasks, but rather stress hormones modulates memory for predominantly emotionally arousing experiences. One study employed the use of an object recognition task to test this hypothesis (Okuda et al., 2004). Previous research has shown that training in the object recognition task produces novelty-induced arousal (de Boer et al., 1990). As expected, immediate post-training corticosterone administration enhanced retention performance of rats in this task. However, when rats were extensively habituated (thus reducing arousal levels during training) to the training apparatus (without objects present), this diminished the effect of post-training corticosterone on retention performance the following day. Again, this provides evidence that the glucocorticoid effect on memory consolidation requires concurrent activation of the arousal-induced noradrenergic system. Indeed, a number of studies have found that attenuating the noradrenergic system pharmacologically can block the memory modulatory effects of glucocorticoids. For example, when rats were concurrently administered corticosterone and propranolol, the β-adrenoceptor antagonist,

this blocked the memory enhancing effect of post-training corticosterone in the object recognition task (Roozendaal, Okuda, Van der Zee, & McGaugh, 2006). Similarly, administration of either propranolol or atenolol (a β_1-adrenoceptor antagonist) blocked the memory modulatory effect of the glucocorticoid agonist dexamethasone in an inhibitory avoidance task (Quirarte, Roozendaal, & McGaugh, 1997). Despite evidence supporting the interaction between the glucocorticoid and noradrenergic system in modulating memory, there has been a shortage of studies conducted to investigate this interaction with regards to the dorsolateral striatum-dependent habit memory system.

The Role of the Glucocorticoid-Noradrenergic Interaction in the BLA

It is unsurprising that the BLA has been linked as an important neuroanatomical structure in mediating the glucocorticoid-noradrenergic interaction (for review see Roozendaal, McEwen, Chattarji, 2009). Studies have found that noradrenaline, when administered directly into the BLA immediately post-training modulates memory consolidation, while β-adrenoceptor antagonists block this effect (Hatfield & McGaugh, 1999; Liang, McGaugh, & Yao, 1990). It is hypothesized that the role of glucocorticoids and adrenaline on the modulation of memory relies on the noradrenergic system within the BLA (for review see McGaugh, 2000). Indeed, attenuation of the BLA noradrenergic system through administration of a β-adrenoceptor antagonist blocks the memory-modulatory effect of systemically administered adrenaline (Liang, Juler, & McGaugh, 1986). While adrenaline itself does not readily cross the blood-brain barrier, it is well understood that adrenaline activates β-adrenoceptors located on the vagus nerve

afferents that terminate in the nucleus of the solitary tract. From there, noradrenergic cell groups project directly into the amygdala (Clayton & Williams, 2000). Additionally, noradrenergic projections from the nucleus of the solitary tract indirectly influence noradrenergic activity within the BLA through projections into the locus coeruleus (LC), which then project into the BLA (for review see Roozendaal et al., 2009).

Glucocorticoids, on the other hand, readily passes through the blood-brain barrier and bind directly onto GRs and MRs located within the BLA and various other brain regions (Reul & de Kloet, 1985). The glucocorticoid binding within the BLA is also thought to play a key role in the influence of emotional arousal on memory modulation. Indeed, GR agonists administered into the BLA following inhibitory avoidance training and contextual fear conditioning enhances memory consolidation, while GR antagonists produce the opposite effect (Roozendaal & McGaugh, 1997; Donley, Schulkin, & Rosen, 2005). Previously, we discussed the phenomenon in which glucocorticoid effects on memory modulation required concurrent activation of noradrenergic systems (Okuda et al., 2004; Roozendaal et al., 2006). Sufficient evidence indicates that the BLA is a key structure in mediating this interaction. For example, post-training BLA infusions of a GR antagonist blocked the memory modulatory effect of a β-adrenoceptor agonist in the retention of an inhibitory avoidance task. Furthermore, β-adrenoceptor antagonists administered into the BLA block the memory enhancing effect of peripheral administration of glucocorticoids (Quirarte et al., 1997; Roozendaal et al., 2006). Therefore, given the evidence presented here, there is a clear indication that the noradrenergic system plays an important role in mediating the effects of emotional

arousal on memory modulation. Furthermore, the BLA is an important structure in mediating this noradrenergic effect (and interaction with the glucocorticoid system) on memory modulation. Therefore, it is reasonable to hypothesize that the effect of exposure to fear-conditioned stimuli on memory modulation, if any, may be, in part, due to the effects of noradrenergic activity, particularly within the BLA.

Specific Goals

The second set of experiments expanded on previous work investigating the role of a physiologically-relevant stressor (i.e. corticosterone) in modulating memory, specifically pertaining to the dorsolateral striatum-dependent habit memory system. In order to do this, the effect of corticosterone administration was examined in two separate dorsolateral striatum-dependent memory tasks. Experiment 3a and 3b employed two different tasks (cued-platform water maze task and single-solution response water maze task) aimed to examine the role of corticosterone in modulating the dorsolateral striatum-dependent memory consolidation. Experiment 4a and 4b aimed to further expand on work regarding the interaction between the glucocorticoid and noradrenergic neurotransmitter systems in modulating memory, again specifically related to the dorsolateral striatum-dependent memory system. Specifically, rats were concurrently injected with the β-adrenoceptor antagonist propranolol to block the memory-modulating effect of corticosterone in the single-solution response water maze task.

Methods

Experiment 3a

Subjects

Subjects were 22 experimentally naïve adult male Charles River Long-Evans rats (300-400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Water Plus-Maze

The water plus-maze apparatus used was identical to that described in Experiment 1.

Drugs and Injection Procedures

Corticosterone hydrochloride (1.0 and 3.0 mg/kg) was dissolved in 8% ethanol saline (similar to procedures in Quirarte et al., 2009). The peripheral doses are selected based on previous research of their memory modulatory properties in rats (Quirarte et

al., 2009). Peripheral injections were administered sub-cutaneous (s.c.) in a volume of 1 ml/kg.

Behavioral Procedures

Single Solution Response Plus-Maze Task

Training in the single solution response plus-maze task was identical to the procedures described in Experiment 2. Immediately following the last training trial on days 1-3 rats were removed from the water maze and received post-training injections (s.c.) of corticosterone hydrochloride at 3 mg/kg (n = 8) or 1 mg/kg (n = 6) or vehicle (n = 8).

Statistical Analysis

For analysis of the single-solution forced-response water plus-maze task a two-way one-repeated measures ANOVA (Group x Day) from days 2-5 was carried out to examine if rats in both the drug and vehicle conditions learned the task and if there were no differences between groups at the end of training. A separate one-way ANOVA was carried out on data from Day 1 in order to determine that there were no differences in acquisition of the task between all groups prior to the first drug injection. A post-hoc Fisher's Least Significant Difference (LSD) test was run to determine the specific differences between groups on days 2-5.

Experiment 3b

Subjects

Subjects were 26 experimentally naïve adult male Charles River Long-Evans rats (300-400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Cued-Platform Water Maze

In this setup the water maze did not contain the Plexiglas plus-maze. Instead, an invisible clear Plexiglas escape platform (15 x 14 x 20 cm) was placed inside the water maze, submerged by 1 cm of water. A white flag was attached to the submerged platform and protruded 6 cm above the water surface. The water maze remained in the same room with the same extra-maze cues. Four starting positions were equally spaced around the perimeter of the water maze, effective dividing the maze into four separate quadrants (Figure 10).

Drugs and Injection Procedures

Corticosterone hydrochloride (1.0 and 3.0 mg/kg) (Sigma-Aldrich) was dissolved in 8% ethanol saline (similar to procedures in Quirarte, Ledesma de la Teja, Casillas, Serafin, Prado-Alcala & Roozendaal, 2009). The peripheral doses are selected based on previous research of their memory modulatory properties in rats (Quirarte et al., 2009). Peripheral injections were administered in a volume of 1 ml/kg.

Behavioral Procedures

Cued-Platform Water Maze Task

The cued-platform water maze task was adapted from previous work from this lab (Packard & Teather, 1997). The rats received one training session of four trials (i.e. swims). During the one-day training session, the rat was placed into the maze facing the wall at either one of the four designated start points/quadrants (NE/SE/SW/NW). Each starting point is used once during the entire training session. The cued-platform was submerged in a quadrant across trials to balance for the distance (i.e. proximal or distal) and direction relative (i.e. right or left) from the start point such that it was submerged in each quadrant once (Figure 10). If the rat did not escape within 60 s, it was manually guided to the escape platform. After mounting the platform, the rat remained on the platform for 10 s. Following each trial they were then removed and placed in an opaque holding container adjacent to the maze for a 30 s intertrial interval. The latency to locate and mount the platform was recorded and used to measure acquisition of the task. Immediately after the last training trial rats were removed from the water maze and

receive post-training sub-cutaneous (s.c.) injections of corticosterone hydrochloride at 3 mg/kg (n = 10) or 1 mg/kg (n = 8) or vehicle (n = 8). Rats were then returned to their home cages.

24 hours following the training session, rats were returned to the water maze room for a retention test. Here, rats were placed in two starting points (NE/SW). The cued-platform was submerged in quadrants across both probe trials to balance for distance and direction relative from the start point. Upon mounting the platform, the rat remained on the platform for 10 s. Following each trial they were removed and placed in an opaque holding container adjacent to the maze for a 30 intertrial interval. Upon completing their last retention trial rats were removed from the water maze and returned to their home cages. No post-training drug injections were given on this day.

Statistical Analysis

For analysis of the cued-platform water plus maze task a two-way repeated measures ANOVA (Group x Trial) was carried out for both the training session and retention test separately. A post-hoc Fisher's Least Significant Difference (LSD) test was run to analyze the specific difference between groups, if any.

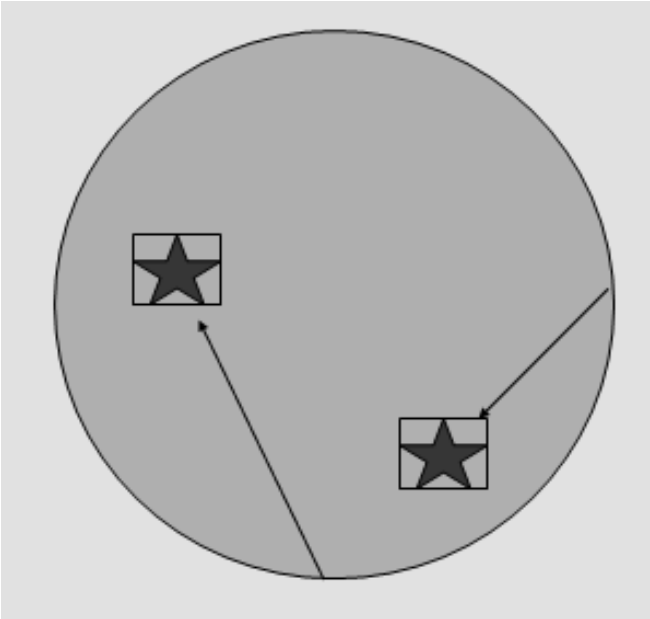

Figure 10. Diagram illustrating the visible-platform water-maze task. On each trial rats start from different locations (i.e. N, S, E, W) and must locate the hidden platform. The cued-platform was submerged in a quadrant across trials to balance for the distance (i.e. proximal or distal) and direction relative (i.e. right or left) from the start point such that it was submerged in each quadrant once.

Experiment 4a

Subjects

Subjects were 21 experimentally naïve adult male Charles River Long-Evans rats (300-400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Water Plus-Maze

The water plus-maze apparatus used was identical to that described in Experiment 1.

Drugs and Injection Procedures

Corticosterone hydrochloride (3.0 mg/kg) (Sigma-Aldrich) was dissolved in 8% ethanol saline (similar to procedures in Quirarte, Ledesma de la Teja, Casillas, Serafin, Prado-Alcala & Roozendaal, 2009). The peripheral dose was selected based on previous research of its memory modulatory properties in rats (Quirarte et al., 2009). Peripheral injections were administered sub-cutaneous in a volume of 1 ml/kg. Propranolol hydrochloride (3 mg/kg) was dissolved in physiological saline and administered intra-peritoneal (i.p). This dose was chosen based on previous research that found that this dose of propranolol blocked corticosterone effects on memory (Roozendaal et al., 2006).

Behavioral Procedures

Single Solution Response Plus-Maze Task

Training in the single solution response plus-maze task was identical to the procedures described in Experiment 2. Immediately following the last training trial on days 1-3 rats were removed from the water maze and received post-training sub-cutaneous (s.c.) injections of corticosterone hydrochloride at 3 mg/kg (n = 7) or vehicle (n = 7), or concurrent injections of corticosterone hydrochloride (s.c.) and propranolol hydrochloride (i.p.) (n = 7).

Statistical Analysis

For analysis of the single-solution forced-response water plus-maze task a two-way repeated measures ANOVA (Group x Day) from days 2-5 was carried out to examine if rats in both the drug and vehicle conditions learned the task and if there were differences between groups at the end of training. A separate one-way ANOVA was carried out on data from Day 1 in order to determine that there were no differences in acquisition of the task between all groups prior to the first drug injection. A post-hoc Fisher's Least Significant Difference (LSD) test was run to determine the specific differences between groups on days 2-5.

Experiment 4b

Subjects

Subjects were 12 experimentally naïve adult male Charles River Long-Evans rats (300-400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Water Plus-Maze

The water plus-maze apparatus used was identical to that described in Experiment 1.

Drugs and Injection Procedures

Propranolol hydrochloride (3 mg/kg) was dissolved in physiological saline and administered intra-peritoneal (i.p). This dose was chosen based on previous research that found that this dose of propranolol blocked corticosterone effects on memory (Roozendaal et al., 2006).

Behavioral Procedures

Single Solution Response Plus-Maze Task

Training in the single solution response plus-maze task was identical to the procedures described in Experiment 2. Immediately following the last training trial on days 1-3, rats were removed from the water maze and received post-training intra-peritoneal (i.p) injections of propranolol hydrochloride (n = 6) or vehicle (n = 6).

Statistical Analysis

For analysis of the single-solution forced-response water plus-maze task a two-way repeated measures ANOVA (Group x Day) from days 2-5 was carried out to examine if rats in both the drug and vehicle conditions learned the task and if there were differences between groups at the end of training. A separate one-way ANOVA was carried out on data from Day 1 in order to determine that there were no differences in acquisition of the task between all groups prior to the first drug injection.

Results

Experiment 3a

Peripheral Administration of Corticosterone Enhanced Consolidation of Response Learning in a Single-Solution Plus Maze Task

The effect of post-training peripheral administration of corticosterone on consolidation of the single-solution response learning task is shown in Figure 11. A two-way repeated measures ANOVA (Group x Day) computed on percentage correct

responses on days 2-5 revealed a main effect of Group, ($F(1,19) = 3.75$, $p < 0.05$), and of Day ($F(3,57) = 36.30$, $p < 0.01$). There was no significant Group x Day interaction ($F(2,19) = 0.22$, n.s.). These results indicate that rats from all groups showed significant improvement in response learning over the training period. More importantly, there was a significant difference between groups, suggesting that there was a difference in consolidation of this task based on drug administration. A post-hoc Fisher's Least Significant Differences (LSD) test revealed that administration of a higher dose of corticosterone (3 mg/kg) ($M = 77.60$) produced enhanced consolidation of response learning in this task based on percentage of correct responses relative to the lower dose of corticosterone (1 mg/kg) ($M = 55.56$), $p = .019$. Furthermore, a post-hoc Fisher's Least Significant Differences test also revealed a strong trending effect in the effectiveness of the higher dose of corticosterone (3 mg/kg) ($M = 77.60$) in enhancing consolidation of response learning relative to vehicle ($M = 61.46$), $p = 0.057$. There were no differences between the effect corticosterone at a lower dose (1 mg/kg) and vehicle in consolidation of response learning. A one-way ANOVA computed on percentage correct responses on day 1 revealed no significant difference between groups following training on that day, suggesting that all groups displayed equal acquisition prior to any drug injections ($F(3, 18) = .34$, n.s.) (Figure 11). Overall, these results suggest that post-training peripheral administration of a higher dose of corticosterone (3 mg/kg) enhanced consolidation of response learning in a single-solution response water plus-maze task relative to a lower dose of corticosterone and vehicle.

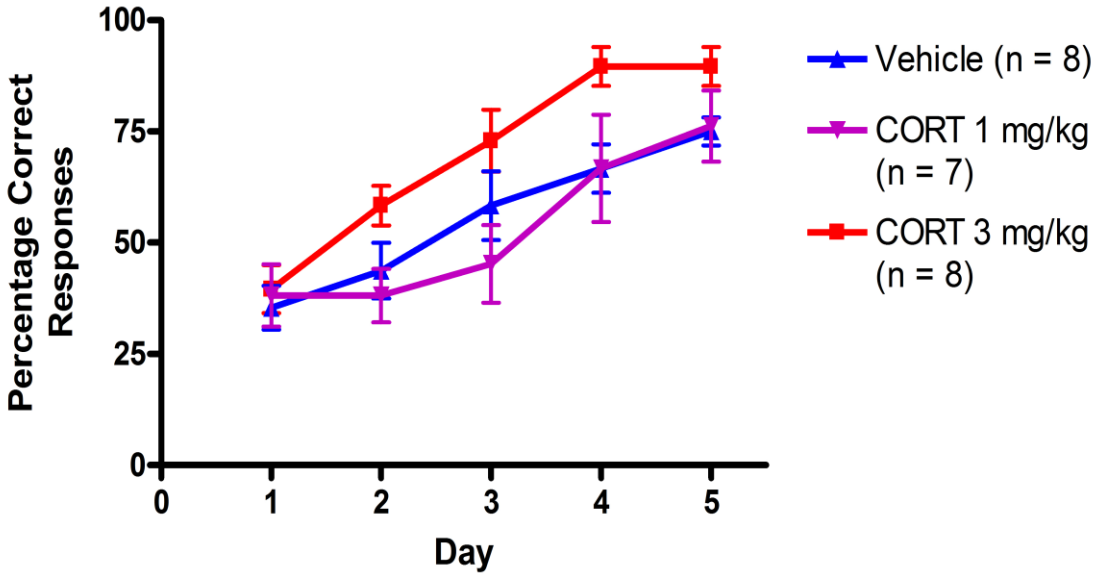

Figure 11. Effect of post-training corticosterone administration in a single-solution response task. Post-training administration of the higher dose of corticosterone (3 mg/kg) facilitates consolidation in a single-solution response water maze task.

Experiment 3b

Post-Training Administration of Corticosterone Enhances Consolidation in a Cued-Platform Water Maze Task

A two-way repeated measures ANOVA (Group x Trial) computed on latency to find the platform on trials 1-4 revealed a main effect of Trial, ($F(3,69) = 28.09$, $p < 0.05$), and no main effect of Group ($F(2,23) = 0.5$, n.s.). There was no significant Group x Trial interaction ($F(2,23) = 0.03$, n.s.). This suggests that rats from all groups acquired the task and that there were no differences between groups at the end of training prior to any drug injections (Figure 12).

A two-way one-repeated measures ANOVA (Group x Probe) was computed to determine the effect of corticosterone administration on latency to find the platform during both probe trials (Figure 13). A two-way one-repeated measures ANOVA revealed a significant main effect of Group ($F(2, 23) = 5.40$, $p < 0.05$) and a significant main effect of Probe ($F(1, 23) = 4.32$, $p < 0.05$). There was no significant Group x Probe interaction ($F(2,23) = .99$, n.s.). A post-hoc Fisher's Least Significant Differences (LSD) test revealed that administration of a higher dose of corticosterone (3 mg/kg) ($M = 8.81$) produced enhanced consolidation of this task as measured by latency to find the platform relative to the lower dose of corticosterone (1 mg/kg) ($M = 16.69$), $p = .005$. Furthermore, a post-hoc Fisher's Least Significant Differences test also revealed a that the higher dose of corticosterone (3 mg/kg) ($M = 8.81$) enhanced consolidation of this task relative to vehicle ($M = 14.85$), $p = 0.019$. There was no difference between the effect corticosterone at a lower dose (1 mg/kg) and vehicle in consolidation of this task. Overall, the results indicate that post-training administration of corticosterone at a higher dose (3 mg/kg) facilitates consolidation of a cued-platform water maze task relative to corticosterone at a lower dose (1 mg/kg) and vehicle.

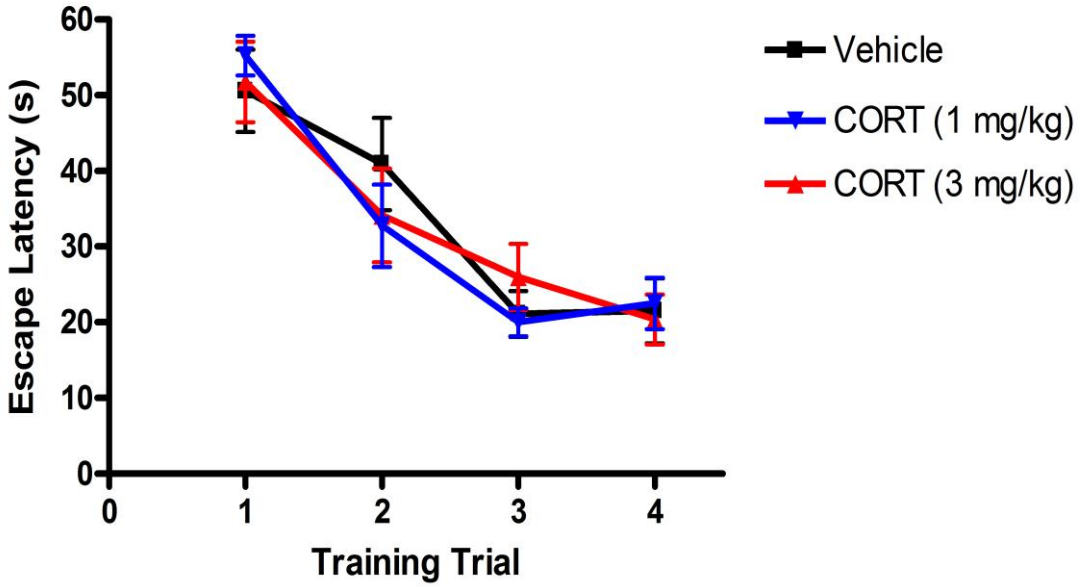

Figure 12. Escape latencies for rats during training in a visible-platform water maze task. Escape latencies for rats in all groups during training trials in the visible platform water maze task prior to any drug administration

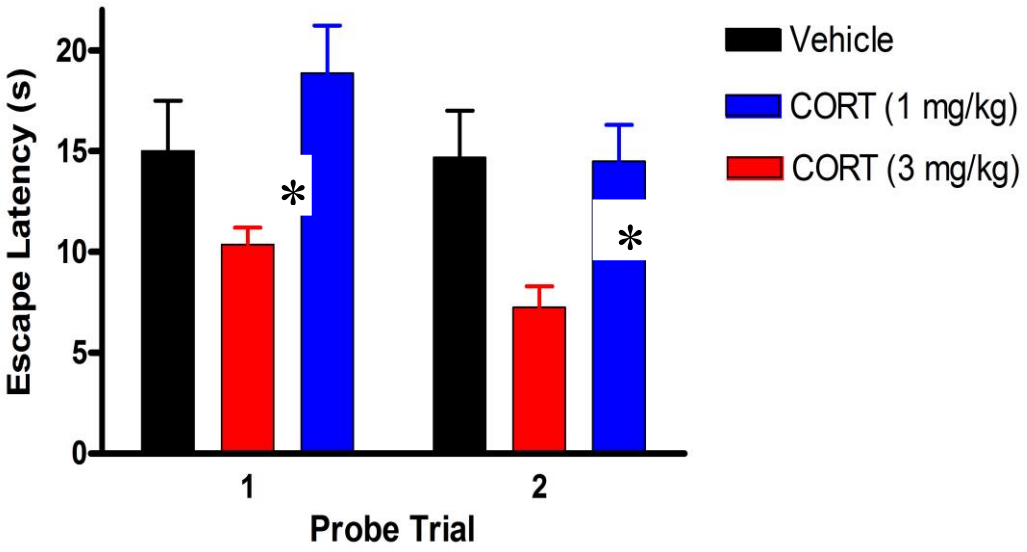

Figure 13. Effect of post-training corticosterone administration on probe trials in a visible platform water maze task. Enhancing effects of post-training peripheral administration of corticosterone on consolidation of the visible platform water maze task. Escape latencies was significantly lower on both probe trials for rats that received post-training administration of corticosterone. Asterisks (*) denote statistical significance at $p < 0.05$.

Experiment 4a

Concurrent Administration of Propranolol Blocks the Enhancing Effect of Corticosterone in a Single-Solution Response Water Maze Task

The effect of post-training concurrent peripheral administration of corticosterone and propranolol on consolidation of the single-solution response learning task is shown in Figure 14. A two-way repeated measures ANOVA (Group x Day) computed on percentage correct responses on days 2-5 revealed a main effect of Group, ($F(2,18) = 3.55$, $p = 0.05$), and of Day ($F(3,54) = 9.15$, $p < 0.01$). There was no significant Group x Day interaction ($F(2,18) = 0.13$, n.s.). These results indicate that rats from all groups showed significant acquisition of the response task over the training period. Additionally, the analyses revealed a significant difference between groups based on the drug administered. A post-hoc Fisher's Least Significant Differences (LSD) test revealed that administration of corticosterone (3 mg/kg) ($M = 77.38$) produced enhanced consolidation of response learning in this task based on percentage of correct responses relative to vehicle ($M = 61.31$), $p = .031$. Furthermore, a post-hoc Fisher's Least Significant Differences test also revealed that administration of corticosterone (3 mg/kg) ($M = 77.38$) in enhanced consolidation of response learning relative to concurrent administration of corticosterone and propranolol ($M = 61.91$), $p = 0.036$. There were no differences between concurrent injections of corticosterone and propranolol and vehicle in consolidation of response learning. A one-way ANOVA computed on percentage correct responses on day 1 revealed no significant difference between groups following training on that day, suggesting that all groups displayed equal acquisition prior to any

drug injections (F (2, 18) = .28, n.s.) (Figure 14). Overall, these results replicate the enhancing effect of post-training corticosterone on the consolidation of response learning. Furthermore, concurrent administration of propranolol with corticosterone blocks the enhancing effect of corticosterone in consolidation of this task.

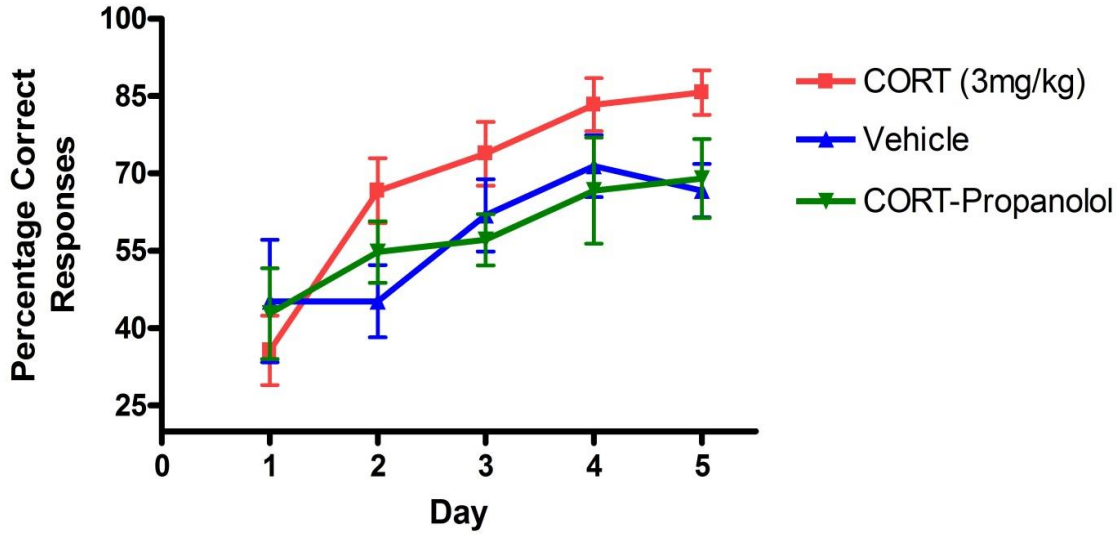

Figure 14. Effect of concurrent corticosterone and propranolol administration on response learning. Enhancing effects of post-training peripheral administration of corticosterone on consolidation is blocked by concurrent administration of propranolol in the forced-response water plus-maze task.

Experiment 4b

Administration of Propranolol Alone Has No Effect on Consolidation of Response Learning in the Single-Solution Water Maze Task

A two-way repeated measures ANOVA (Group x Day) computed on percentage correct responses on days 2-5 revealed no significant main effect of Group, ($F(1,10) = 2.00$, n.s.), but a significant main effect of Day ($F(1,10) = 22.75$, $p < 0.01$). These

results indicate that rats from both groups showed significant acquisition of the response task over the training period. Furthermore, post-training peripheral administration of propranolol did not produce differences in consolidation of this task relative to vehicle rats, indicating that post-training administration of propranolol alone did not influence consolidation of the single-solution response water maze task.

Summary

The current set of experiments revealed that post-training administration of systemic corticosterone enhanced consolidation of dorsolateral striatum-dependent learning in two versions of the water-maze task. Post-training administration of corticosterone produced enhanced acquisition of a single solution response plus-maze task and enhanced retention in a cued-platform water plus-maze task. Furthermore, the memory modulatory effect of corticosterone on dorsolateral striatum-dependent memory occurred in a dose-dependent manner in which a higher dose of corticosterone (3 mg/kg) produced enhancement although a lower dose of corticosterone (1 mg/kg) produced performance similar to control animals. These results are in agreement with previous studies that have found that emotional arousal produced by anxiogenic drugs enhances response learning (Wingard & Packard, 2008) and that corticosterone influences memory consolidation in a dose-dependent fashion in other tasks (Roozendaal et al., 1999; Sandi & Rose, 1997; Medina et al., 2007).

In addition, this set of experiments also expanded on prior work investigating the glucocorticoid-noradrenergic interaction underlying memory modulation following

emotional arousal. Here, concurrent administration of the β-adrenoceptor antagonist, propranolol, blocked the memory-enhancing effect of post-training corticosterone in a single-solution response plus-maze task. It is important to note that this dose of propranolol (3 mg/kg) does not affect response learning if administered by itself. In line with previous studies (Roozendaal et al., 2006; Okuda et al., 2004), the attenuation of noradrenergic function can block the memory modulatory effect of glucocorticoids. Specifically, these experiments display the importance of this glucocorticoid-noradrenergic interaction in consolidation of a strictly dorsolateral striatum-dependent task.

CHAPTER IV

EXPOSURE TO FEAR-CONDITIONED STIMULI CAN INFLUENCE MULTIPLE MEMORY SYSTEMS

Introduction

It is well understood that post-training stress or anxiety can modulate striatum-dependent memory (Wingard & Packard, 2008) and bias animals towards preferential use of a striatum-dependent learning strategy (Packard & Wingard, 2004). From an associative learning perspective, the vast majority of studies investigating the modulatory role of stress on memory have exposed animals to acute unconditioned stress-evoking stimuli. For example, exposure to acute restraint/tail-shock stress can produce a bias toward use of a striatum-dependent stimulus-response strategy over a hippocampus-dependent place strategy (Kim et al., 2001). In addition, pre-training administration of anxiogenic drugs (Packard & Wingard, 2004) produced a similar shift in preference toward the use of a striatum-dependent response strategy. Moreover, post-training administration of an anxiogenic drug enhances striatum-dependent response learning while impairing consolidation of hippocampus-dependent place learning (Wingard & Packard, 2008). A number of studies have also found that exposure to acute stress and anxiety may also impair spatial cognition on a number of spatial tasks (Conrad et al., 2004; Wingard & Packard, 2008; Diamond et al., 1996; de Quervain et al., 1998).

Interestingly, the memory-modulatory effects of these stimuli are facilitated by the innate ability of these stimuli to evoke emotional arousal, such as shock (Kim et al., 2001) and predator exposure (Diamond et al., 2006). Similarly, studies that have

investigated mechanisms of fear conditioning have also employed footshocks (Maren, Aharonov, & Fanselow, 1997) and predator exposure (Wang, Fraize, Yin, Yuan, Petsagourakis, Wann, & Muzzio, 2013) as reliable unconditioned stimuli, indicating that a neutral stimulus, when paired with these unconditioned stressors, may acquire the ability to elicit an emotional response. However, few studies have determined if an emotional state elicited by a learned stimulus can modulate memory in the same manner as an unlearned stimulus. The ability for a neutral stimulus, when paired with a stress-evoking unconditioned stimulus, to influence the relative use of learning strategy and modulate memory systems would provide an interesting phenomenon when examining the emotional arousal of multiple memory systems. Therefore, it is important to determine whether a stress-paired conditioned stimulus can influence learning strategy and modulate striatum-dependent memory in a manner similar to its associated unconditioned stress-evoking stimulus.

Fear Conditioning

Studies investigating the neurobiology of fear conditioning have often received significant interest as it combines two popular research topics of memory and emotion (for review see Maren, 2001). Therefore, it is the next seemingly logical step to integrate fear conditioning paradigms when investigating the role of emotional arousal in the modulation of multiple memory systems. In 1920, Dr. John Watson and Rosalie Rayner conducted arguably the most well-known early work in Pavlovian fear conditioning (Watson & Rayner, 1920). Here, they initially exposed a naïve male infant, Albert, to a

white rat and found that Albert did not display any negative emotion in response to the rat, thus making it an effective neutral stimulus. However, upon pairing the white rat with a loud noise (through striking a hammer onto a steel bar), Albert eventually learned to associate the rat with the loud noise and began to display strong negative emotions (e.g. crying) in response to the presentation of the rat. In sum, Albert had learned that the white rat (conditioned stimulus; CS) predicted the occurrence of a loud and unpleasant noise (unconditioned stimulus; US) and thus developed a conditioned emotional response (CER/CR) to the CS.

Through employing the basic techniques of Pavlovian conditioning (Pavlov, 1927) in a fear conditioning paradigm like the one described earlier, researchers have uncovered extensive evidence of the mechanisms and neurobiology underlying Pavlovian fear conditioning. Over the course of the next few decades, researchers began to find the amygdala to be an extremely important structure in mediating fear conditioning. For example, a group of researchers discovered that damage to the amygdala resulted in a loss of fear in monkeys (Weiskrantz, 1956; Kluver & Bucy, 1937). Further work found that functionally distinct nuclei within the amygdala, including the central nuclei (CE) and BLA, are important for Pavlovian fear conditioning (LeDoux, 1995; Maren & Fanselow, 1996). It is now well understood that these two sets of nuclei are components of two subsystems that are important for fear conditioning. Lesions to the BLA produce impairments in acquisition and expression of Pavlovian fear conditioning, regardless of stimulus modality, because of its importance in receiving sensory information about the stimuli (Campeau & Davis, 1995; Maren, Aharonov, &

Faneslow, 1996). The CE, on the other hand, is important for the performance of fear-related behaviors. For example, electrical stimulation of this area produced fear-like behaviors (Iwata et al., 1987). While lesions to the CE produces impairments in acquisition and expression of fear conditioning as well (Kim & Davis, 1993; Roozendaal, Koolhas, & Bohus, 1991), researchers have found that this is due to an inability to display fear-behaviors, and not an ability to form stimulus associations (Fanselow & Kim, 1994). Recently, the dorsal hippocampus has also been implicated as an important structure in mediating contextual information in contextual fear conditioning paradigms (Kim & Fanselow, 1992; Phillips & LeDoux, 1992), which falls in line with the idea that the hippocampus is important in the processing of spatial information (Packard et al., 1989).

Fear Conditioning Results in Stress Response to Conditioned Stimuli

The premise underlying the idea that a fear-conditioned stimulus may modulate memory in a manner similar to the more commonly-employed stress-invoking unconditioned stimuli (e.g. shock/predator exposure/anxiogenic drugs) is based on the hypothesis that a neutral stimulus, when previously paired with an unconditioned stressor, can produce a physiological emotional response similar to acute presentation of the unconditioned stimulus. That is, if exposure to a conditioned fear stimulus produces a stress-response similar to exposure to an unconditioned emotional stimulus (e.g. shock/predator exposure) it is reasonable to postulate that the fear-conditioned stimulus may be sufficient to modulate memory in a similar way to that of the unconditioned

stimulus. Indeed, in a previous study, exposure to fear-conditioned context is related to a rise in corticosterone levels (Goldstein et al., 1996; Hagewoud et al., 2011) such that the amount of fear-like behaviors displayed during exposure is positively correlated to elevation of corticosterone in rats (Cordero, Merino, & Sandi, 1998). It has been well established that freezing during the testing period increases in relation to the intensity of the shock during fear-conditioning (Young & Fanselow, 1992; Cordero et al., 1998). Similarly, there was an elevated level of plasma corticosterone after conditioning, and throughout all testing sessions (24 hours and 7 days later), in rats that received a higher intensity shock (1 mA) relative to a lower intensity shock (0.2 mA, 0.4 mA). Interestingly, during exposure to the fear-conditioned context, the rise in corticosterone levels was positively correlated to freezing behavior (Cordero et al., 1998). These results are important for two reasons. First, it indicates that the increase in corticosterone following contextual fear-conditioning may play an important role in the consolidation of these contextual fear memories. Second, it provides evidence that exposure to fear-conditioned stimuli can produce a physiological response (i.e. rise in plasma stress hormones) similar to the response to an unconditioned stimulus (e.g. foot-shock), in a manner such that the higher the shock intensity was during fear conditioning, the greater the rise of plasma corticosterone levels were during subsequent testing periods. Given that corticosterone plays an important role in memory consolidation during emotional arousal (as discussed earlier) it is theoretically possible for a fear-conditioned stimulus to modulate memory in a manner similar to that of an unconditioned stimulus.

Specific Goals

In experiment 5a we examined the effect of post-training exposure to fear-conditioned cues on the relative use of "place" and "response" learning in a dual-solution plus-maze. Rats were first exposed to a standard fear-conditioning paradigm which involves repeated tone-shock pairings. They were then trained in a dual-solution water plus-maze task on a subsequent day and exposed to the previously fear-conditioned stimuli without shock (i.e. context and tone) immediately post-training. In experiment 5b we examined the effect of post-training exposure to fear-conditioned cues on the consolidation of a dorsolateral-dependent single-solution response plus-maze task. Again, rats received fear-conditioning training in a single-solution response task on subsequent days. Following maze training sessions, rats were exposed to fear-conditioned stimuli without shock to examine the effect on the consolidation of dorsolateral striatum-dependent memory.

Experiment 6 investigated the importance of noradrenergic processes in mediating the effect of exposure to fear-conditioned stimuli on striatum-dependent memory consolidation. Specifically, all animals were fear-conditioned and then trained in a single-solution response task. Prior to post-training exposure to fear-conditioned stimuli, rats were administered propranolol.

Methods

Experiment 5a

Subjects

Subjects were 22 experimentally naïve adult male Charles River Long-Evans rats (300 - 400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Water Plus-Maze

The water plus-maze apparatus used was identical to that described in Experiment 1.

Conditioning Chamber

The conditioning chamber was located in a moderately lit and isolated room. The chamber was constructed of aluminum (walls) and Plexiglas (hinged ceiling). The floor of the chamber consisted of 19 stainless steel rods (4-mm diameter), spaced 1.5 cm

apart. The rods are wired to a shock generator for delivery of footshock (1 mA). Tone is supplied by a speaker located directly above the chamber.

Behavioral Procedures

Fear Conditioning Procedure

Fear-conditioning procedures were adapted from previous studies showing conditioned stimulus-mediated memory modulation (Holahan & White, 2002). 24 hours prior to any water-maze training, all rats were exposed to the fear-conditioning chamber. Rats were removed from the home cage and were transported to the conditioning chamber. Rats remained in the chamber for the duration of 7 minutes. During the first 3 min ("Pre-Shock" period) no tones or shocks were presented. At the start of the 4th minute a tone was presented (2 kHz, 20 dB) for 20 s. For rats receiving tone-shock parings (n = 11) ("Conditioned" group), a footshock (1 mA) was administered through the floor rods and co-terminated simultaneously with the tone during the final 2 s of the tone presentation. The tone-shock pairings occurred two additional times with a 1 min interval between tone presentations. In sum, each rat received three tone-shock pairings. Following the last tone-shock pairing, rats remained in the chamber for additional 1 min. Control rats (n = 11) received presentations of the tone only with no pairings with foot-shock using identical parameters ("Control" group). After the rat removed from the chamber the rod floor, walls, and catch pan underneath the floor of the chamber were cleaned with 70% alcohol and allowed to dry. Following fear conditioning rats were returned to their home cage.

Dual-Solution Water Plus-Maze Task

The day following fear conditioning, rats were trained in the dual solution water plus-maze task identical to Experiment 1. Following the sixth and final training trial, rats were immediately transported to the conditioning chamber and were exposed to post-training tone (CS) presentations identical to the procedures described during the fear conditioning phase. No shocks were presented at this phase for any group. Following tone presentations rats were returned to their home cages.

The following day, 24 hours after water maze training, rats received a probe trial to determine the type of strategy employed to acquire the task. No exposure to the conditioning chamber was given prior to the probe trial. During the probe trial, the start-arm was shifted to the opposite arm (i.e. north arm), with the arm directly opposite blocked off (i.e. south arm). Rats that turn left at the choice point and enter the east arm on the probe trial (i.e. approach the same spatial location that the hidden platform was located in during training) were designated as place learners. Rats that make a right turn at the choice point and enter the west arm on the probe trial (i.e. make the same body turn to swim to the hidden platform as during training) were designated as response learners. Following the probe trial, rats were returned to their home cage.

Statistical Analysis

A two-way repeated measures ANOVA (Group x Day) was carried out to examine if groups in both the tone-shock pairings condition and tone-alone conditions learned the task and that there were no differences between groups at the end of training.

A chi-square analysis was performed to compare the number of animals that used a place strategy vs. a response strategy between tone-shock and tone-alone conditions during the probe trial on day 3.

Experiment 5b

Subjects

Subjects were 21 experimentally naïve adult male Charles River Long-Evans rats (300 - 400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Water Plus-Maze

The water plus-maze apparatus used was identical to that described in Experiment 1a.

Conditioning Chamber

The conditioning chamber is identical to that described in Experiment 5a.

Behavioral Procedures

Fear Conditioning Procedure

24 hours prior to any water-maze training, all rats were exposed to the fear-conditioning chamber, identical to Experiment 5a. Two groups of rats received three tone-shock pairings. Of these two groups, one group would be exposed to fear-conditioned stimuli post-training, and one group would be returned to their home cage post-training. One control group of rats received presentations of the tone only with no pairings with foot-shock. Following fear conditioning rats were returned to their home cage.

Single-Solution Response Plus-Maze Task

The day following fear conditioning, rats were trained in the single-solution response plus-maze task identical to Experiment 2. Following the sixth and final training trial, two groups of rats ("Conditioned-Exposed"; n = 7 and "NotConditioned-Exposed"; n = 7) were immediately transported to the conditioning chamber and were exposed to post-training tone (CS) presentations identical to the procedures described during the fear conditioning phase. No shocks were presented at this phase for any group. Following tone presentations rats were returned to their home cages. Another control group of rats that received tone-shock pairings during fear-conditioning were returned to

their home cage immediately post-training ("Conditioned-NotExposed"; n = 7) and did not receive any exposure to the conditioning chamber and tone.

Statistical Analysis

For analysis of the single-solution forced-response water plus-maze task a two-way repeated measures ANOVA (Group x Day) from days 2-5 was carried out to examine if rats in all conditions learned the task and also to test for differences between groups at the end of training. A post-hoc Fisher's Least Significant Difference (LSD) test was run to determine the specific difference between groups, if any. A separate one-way ANOVA was carried out on data from Day 1 in order to determine that there were no differences in acquisition of the task between all groups prior to the first drug injection.

Experiment 6

Subjects

Subjects were 32 experimentally naïve adult male Charles River Long-Evans rats (300 - 400 g). Animals were individually housed in a climate-controlled vivarium. All animals received access to food and water *ad libitum*. All animals received a 12:12 h light-dark cycle (lights on at 7 a.m.). All experiments were conducted during the light phase cycle.

Handling

Each rat was handled daily for 3 minutes for 5 days prior to beginning of behavioral testing.

Apparatus

Water Plus-Maze

The water plus-maze apparatus used was identical to that described in Experiment 1a.

Conditioning Chamber

Conditioning chambers were identical to those used in Maren (2014). Conditioning chambers (30 x 24 x 21 cm) were constructed from aluminum (side walls) and Plexiglas (rear wall, ceiling, and hinged front door, and were located in sound-blocking cabinets in an isolated room. The floor of the chamber consisted of 19 stainless steel rods (4 mm diameter) spaced 1.5 cm apart (center to center). The floor rods were connected to a shock source for delivery of foot-shock US. A speaker was set up outside one wall of the chamber for delivery of auditory CS. Chambers were cleaned with 2% acetic acid before and after every conditioning and exposure trial. Stainless steel pans containing 2% acetic acid were also placed underneath the grid floor of each chamber to provide a distinct odor during conditioning and post-training exposure trials. Each chamber contained houselights that remained illuminated (15 W) over all trials.

Furthermore, the cabinets in which the chambers were contained were fitted with ventilation fans to supply background noise (65 dB).

Drugs and Injection Procedures

Propranolol hydrochloride (3 mg/kg) was dissolved in physiological saline and administered intra-peritoneal (i.p). This dose was chosen based on previous research that found that propranolol blocked corticosterone effects on memory (Roozendaal et al., 2006).

Behavioral Procedures

Fear Conditioning Procedure

24 hours prior to any water-maze training, all rats were exposed to the fear-conditioning chamber, identical to Experiment 5a. All groups of rats received three tone-shock pairings. Following fear conditioning rats were returned to their home cage.

Single-Solution Response Plus-Maze Task

The day following fear conditioning, rats were trained in the single-solution response plus-maze task identical to Experiment 5b. Following the sixth and final maze training trial, two groups of rats were immediately transported to the conditioning chamber in black plastic boxes. Of these two groups, one group (n = 8) received systemic injections of propranolol (3 mg/kg, i.p.) while the other group (n = 8) received vehicle injections. They were then exposed to three post-training tone (CS) presentations

identical to the procedures described during the fear conditioning phase. No shocks were presented at this phase for any group. Following tone presentations rats were returned to their home cages. Two other groups of rats that received tone-shock pairings during fear-conditioning did not receive exposure to fear-conditioned stimuli but instead were placed in a separate container in a different room for the same duration of time (i.e. 7 mins). Of these two groups, one group (n = 8) received propranolol injections (3 mg/kg, i.p.) and one group (n = 8) received vehicle injections. Post-training exposure to fear-conditioned stimuli and post-training drug injections only occurred following training on days 1-3.

Statistical Analysis

For analysis of the single-solution forced-response water plus-maze task a three-factor repeated measures ANOVA (Exposure x Drug x Day) from days 2-5 was carried out to examine if rats in all conditions learned the task and also to test for differences between groups at the end of training. A post-hoc Fisher's Least Significant Difference (LSD) test was run to determine the specific difference between groups, if any. A separate one-way ANOVA was carried out on data from Day 1 in order to determine that there were no differences in acquisition of the task between all groups prior to the first drug injection.

Results

Experiment 5a

Retrieval of Fear Memory through Exposure to Fear Cues Biases Rats Towards Response Strategy

A two-way one-repeated measures ANOVA (Group x Trial) revealed a main effect of trial ($F(1,28) = 8.87$, $p < 0.05$), suggesting that animals in both groups learned the task over six trials. There was no significant difference between groups during training ($F(1,28) = 0.10$, n.s.), indicating that there was no effect of fear conditioning on training 24 hours later.

The effect of post-training exposure to fear-conditioned cues on the use of "place" or "response" learning on a subsequent probe trial 24 hours following training is depicted in Figure 15. A χ^2 analysis was performed to assess the difference in the relative use of place or response learning on the probe trial. Fear-conditioned rats when exposed to post-training fear-conditioned cues displayed an absolute preference for the use of response learning on the probe trial (2 place rats, 9 response rats; $\chi^2 = 4.46$, $p < 0.05$). In contrast, rats that only received tone presentations without shock during fear conditioning showed no preference towards either place or response learning on the probe trial when exposed to post-training fear-conditioned cues ($\chi^2 = .82$, p = n.s.). Finally, a χ^2 analysis revealed that there was a significant difference between groups. Fear-conditioned rats were more likely to prefer a response strategy when presented with post-training fear-conditioned cues than rats that never received tone-shock pairings during conditioning trials ($\chi^2 = 4.70$, $p < 0.05$) (Figure 15).

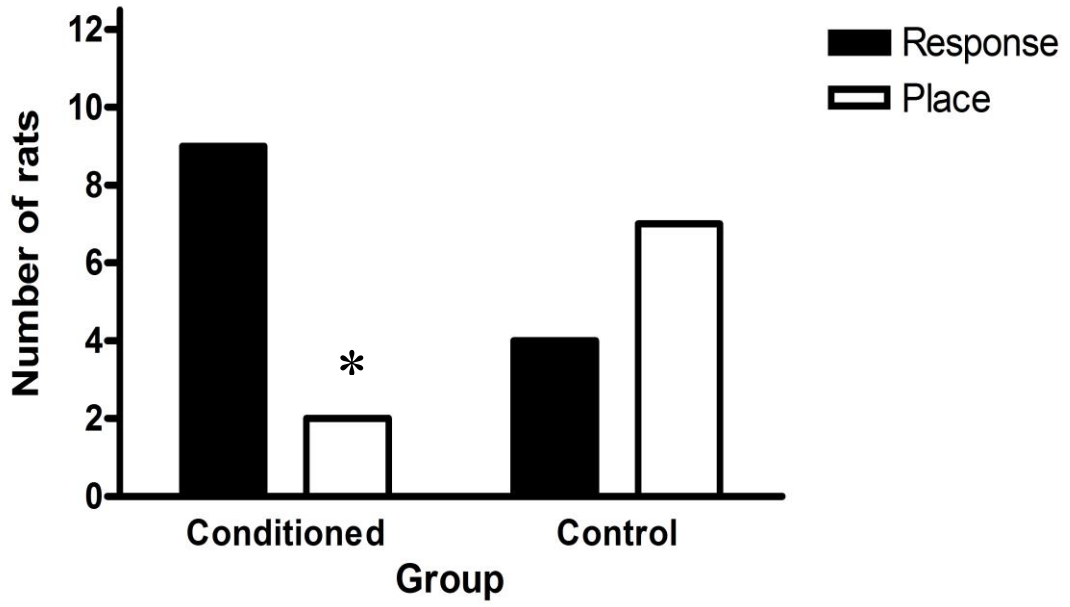

Figure 15. Effect of post-training exposure to fear-conditioned stimuli on learning strategy. Number of rats in each experimental group that exhibited place or response learning on the day 2 probe trial. Post-training peripheral exposure to fear-conditioned stimuli biased rats towards preferential use of a dorsolateral striatum-dependent response strategy in a dual-solution water maze task. Exposure to the same stimuli for rats that only received tone presentations without shock pairings during fear conditioning resulted in preferential use of the hippocampus-dependent strategy. Asterisks (*) denotes statistical significance at $p < 0.05$.

Experiment 5b

Retrieval of Fear Memory through Exposure to Fear-Conditioned Cues Enhances Response Consolidation

The effect of post-training exposure to fear-conditioned cues on the consolidation of the single-solution response learning task is depicted in Figure 16. A two-way repeated measures ANOVA (Group x Day) taking into account percentage of correct responses on days 2-5 revealed a significant effect of Day, ($F(1,18) = 38.16$, $p < 0.01$), suggesting that animals acquired this task over the period of 5 days. Furthermore, there was a significant difference between Group, ($F(2,18) = 6.368$, $p < 0.05$). There was no

significant Group x Day interaction ($F(2,18) = 1.20$, n.s.). A post-hoc Fisher's Least Significant Differences revealed significant differences on days 2-5 between fear-conditioned animals and animals that did not receive tone-shock pairings during conditioning ($p < 0.05$) and fear-conditioned animals that did not receive post-training exposure to fear-conditioned cues ($p < 0.05$). There was no significant difference between animals that did not receive tone-shock pairings and fear-conditioned animals that received no post-training exposure to fear-conditioned cues. A one-way ANOVA revealed no significant difference between groups on Day 1, ($F(2,18) = 1.74$, n.s.), indicating that there were no differences between all groups prior to the first post-training exposure to fear-conditioned cues. Taken together, these results indicate that fear-conditioned animals that received post-training exposure to fear-conditioned cues displayed enhanced consolidation of the single-solution response learning task relative to fear-conditioned animals that did not receive post-training exposure to fear-conditioned cues and animals that did not receive tone-shock pairings during conditioning (Figure 17).

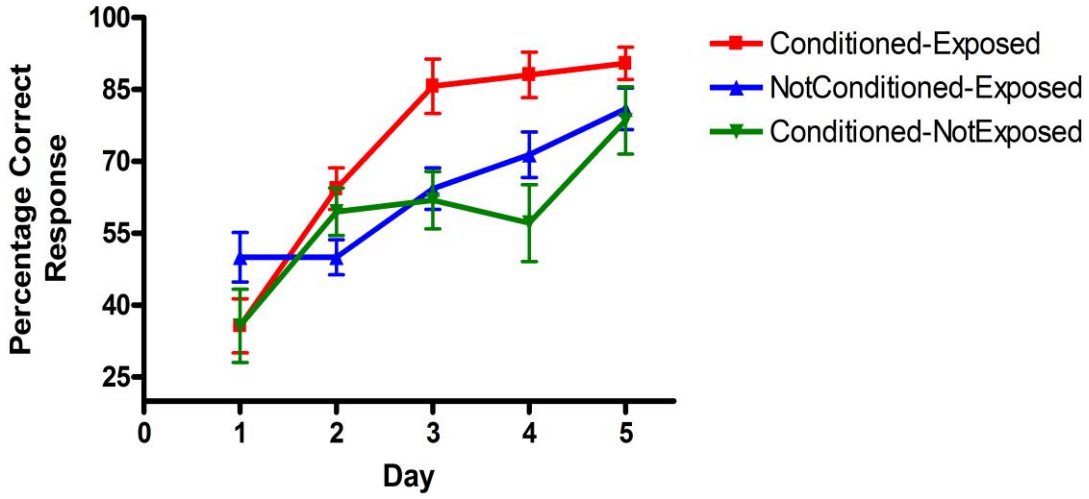

Figure 16. Effect of post-training exposure to fear-conditioned stimuli in a response task. Enhancing effects of post-training exposure to fear-conditioned stimuli on consolidation of a forced-response water plus-maze task relative to rats that were exposed to the same stimuli but did not receive tone-shock pairings or rats that received no exposure at all.

Experiment 6

Post-Training Peripheral Administration of Propranolol Prior to Exposure to Fear-Conditioned Stimuli Blocked the Enhancing Effect on Dorsolateral Striatum-Dependent Memory Consolidation

Freezing behavior on fear-conditioning days is depicted in Figure 17. A repeated measures ANOVA (Group x Trial) revealed a significant effect of Trial ($F(3,84) = 34.45$, $p < 0.01$), indicating that they displayed increased levels of freezing over the three tone-shock pairings. There was no effect of Group ($F(9, 28) = .68$, n.s.). This suggests that all rats, regardless of group, developed a fear response to tone-shock pairings during fear conditioning.

The effect of post-training peripheral administration of propranolol prior to exposure to fear-conditioned cues on the consolidation of the single-solution response

learning task is depicted in Figure 18. A one-way ANOVA revealed no significant difference between groups on Day 1, ($F(3,28) = 1.71$, n.s.), indicating that there were no differences between all groups prior to the first post-training exposure to fear-conditioned cues. A repeated measures ANOVA (Exposure x Drug x Day) taking into account percentage of correct responses on days 2-5 revealed a significant effect of Day, ($F(3,84) = 13.73$, $p < 0.01$), suggesting that animals acquired this task over the period of 5 days. There was no significant effect of Drug ($F(1,28) = 2.76$, n.s.) or of Exposure ($F(1,28) = .124$) alone. However, a significant Drug x Exposure interaction was revealed ($F(1,28) = 6.60$, $p < .05$). A post-hoc Fisher's Least Significant Differences test revealed significant differences in percentage of correct responses on days 2-5 between animals that received propranolol prior to exposure to fear-conditioned stimuli ($M = 58.33$) and animals that received vehicle prior to exposure ($M = 80.21$) ($p < 0.05$). Furthermore, significant differences were found between rats that received vehicle injections prior to exposure to fear-conditioned stimuli ($M = 80.21$) and rats that received vehicle injections but did not receive exposure to fear-conditioned stimuli ($M = 65.10$) ($p < 0.05$). The difference between rats that received vehicle prior to exposure ($M = 80.21$) and rats that received propranolol but no exposure to fear-conditioned stimuli ($M = 69.79$) showed a possible significant trend ($p = 0.16$). Finally, rats that received propranolol prior to exposure to fear-conditioned stimuli performed no different than either group that did not receive any exposure. Overall, these results suggest that the effect of propranolol in blocking the modulatory effect on consolidation of memory only occurs in rats that receive post-training exposure to fear conditioned stimuli.

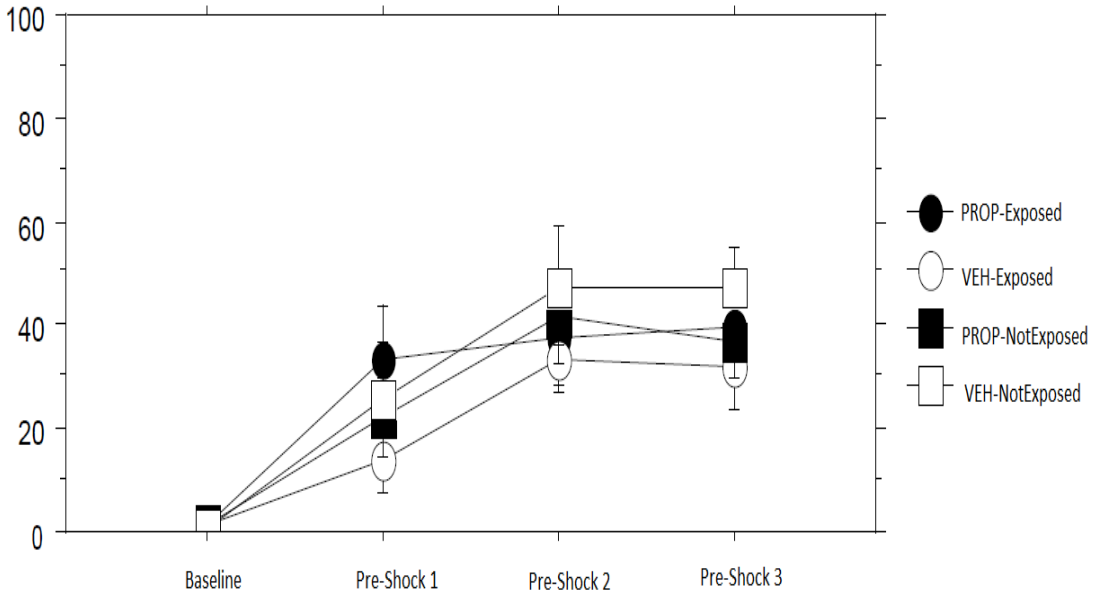

Figure 17. Effect of fear-conditioning on freezing behavior in rats. All groups displayed increased freezing behavior over three tone-shock pairings. Figure presents freezing over presentation of 20 second tone.

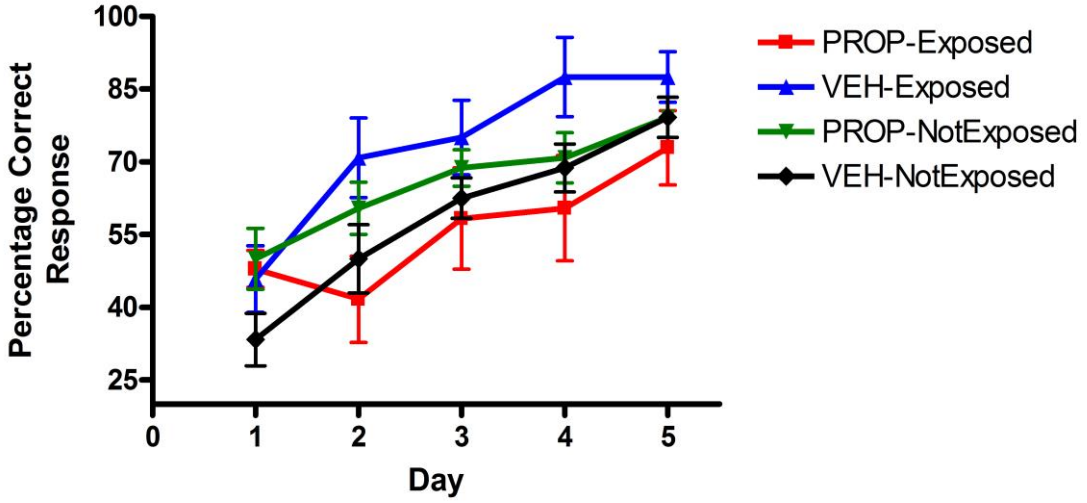

Figure 18. Effect of propranolol administration and exposure to fear-conditioned stimuli in a response task. Enhancing effects of post-training exposure to fear-conditioned stimuli (VEH-Exposed) on consolidation of a forced-response water plus-maze task is blocked by post-training peripheral administration of propranolol prior to exposure (PROP-Exposed). Rats that received no exposure (VEH-NotExposed) showed no enhancement in this task regardless of propranolol administration (PROP-NotExposed).

Summary

The current study revealed that post-training exposure to fear-conditioned cues biased rats towards preferential use of response learning over place learning in a dual-solution plus-maze task that could be acquired using either learning strategy. Furthermore, in a single-solution response plus-maze task, post-training exposure to the same fear-conditioned cues enhanced consolidation. Previous work from our lab has provided extensive evidence that emotional arousal can produce a bias towards response learning in a dual-solution task as well as enhance response learning in a single-solution response task. We have previously shown that post-training peripheral injections of the anxiogenic drug RS 79948-197 can bias animals towards the use of response learning in a dual-solution water plus-maze task (Packard & Wingard, 2004) and enhance consolidation of a single-solution response water plus-maze task (Wingard & Packard, 2008). Here, we extend these findings to support the role of emotional conditioned stimuli in modulating memory in a similar manner. It is unlikely that the aversive nature of the footshock itself during fear-conditioning trials modulated this memory effect as animals that received footshock but were not exposed to fear-conditioned stimuli post-training showed no enhancement in consolidation of the single-solution response task. Furthermore, the procedure of being placed in a novel chamber and experiencing a novel tone itself during fear-conditioning was not sufficient to produce memory modulation as animals did not show any memory modulation if the tone was never paired with the shock during conditioning. Memory modulation of the striatum-dependent memory system occurred only if animals were exposed to stimuli that have previously been

paired with a stressor (i.e. footshock). Overall, the findings support the hypothesis that exposure to a fear-conditioned stimulus can facilitate the consolidation and preferential use of habit memory in a manner similar to stress-related unconditioned stimuli.

Experiment 6 found that peripheral administration of propranolol blocked the memory enhancing effect of post-training exposure to fear-conditioned stimuli. This suggests that the enhancement of dorsolateral striatum-dependent memory consolidation through post-training exposure to fear-conditioned stimuli may be blocked through the attenuation of noradrenergic activity. These results are important because this implicates the noradrenergic system in mediating the memory consolidation of response memory through exposure to fear-conditioned stimuli. These findings extend previous research indicating that noradrenergic activity is important for the modulation of memory through emotional arousal (Roozendaal et al., 2006). It is important to consider that propranolol, at this dose, did not produce any effects on memory consolidation of this task by itself, therefore indicating that the effect of this drug on blocking the enhancing effect of fear-conditioned stimuli exposure is due to an interaction between the two factors. This suggests that noradrenergic activity is required for dorsolateral striatum-dependent memory consolidation during the presence of an emotionally arousing experience, but that the attenuation of activity is not sufficient to influence memory consolidation during the absence of post-training emotional arousal.

CHAPTER V

GENERAL DISCUSSION

Summary of Results

Overall, these experiments extend previous research investigating the role of emotional arousal on the relative use of multiple memory systems, focusing particularly on the dorsolateral striatum-dependent habit memory system. The results show that emotional arousal through exposure to an ethologically relevant stressor (TMT), administration of a physiologically relevant stressor (corticosterone), and exposure to fear-conditioned stimuli all facilitate dorsolateral striatum-dependent learning.

Specifically, results from experiment 1a found that pre-training exposure to predator odor can bias rats towards preferential use of a response strategy over a place strategy in a task that can be acquired using either strategy. Experiment 1b found that pre-training exposure to TMT facilitated response learning while having no influence on place learning in a single-solution water plus maze task. The possible role of the BLA in mediating this effect was uncovered in experiment 2. The pre-training effect of TMT on biasing rats towards use of a response strategy was blocked when the BLA was inactivated through infusions of the sodium-channel blocker, bupivacaine. This potentially suggests that the functional integrity of the BLA is important in modulating the effect on TMT on the relative use of memory systems.

Experiment 3 and 4 investigated the role of post-training corticosterone, the primary stress hormone in rats, on the dorsolateral striatum-dependent memory system. Both sets of results from experiment 3a and 3b indicated that the glucocorticoid system

plays an important role in the consolidation of dorsolateral striatum-dependent memory. This enhancement of dorsolateral striatum-dependent memory consolidation through administration of corticosterone was blocked with concurrent administration of the β-adrenoceptor antagonist propranolol. This extends previous findings indicating that the glucocorticoid-mediated modulation of memory requires activity of the noradrenergic system (for review see Roozendaal et al., 2009).

Finally, experiments 5 and 6 introduced the idea of employing a fear-conditioned stimulus as a stressor to modulate the relative use of memory systems, As results from experiment 5a showed, rats that received post-training exposure to previously fear-conditioned stimuli chose a predominantly response learning strategy in a dual-solution task. Similarly, as was found in experiment 5b, rats also displayed enhanced learning of a response task when exposed to fear-conditioned stimuli following training. This suggests that exposure to fear-conditioned stimuli produces emotional arousal similar to an unconditioned stressor and therefore produces similar behavioral effects on the dorsolateral striatum-dependent habit memory system. Furthermore, as indicated in experiment 6, this effect is mediated by noradrenergic activity. When the β-adrenoceptor antagonist, propranolol, was peripherally administered following training to coincide with exposure to fear-conditioned stimuli, this blocked the memory enhancing effect in a single-solution response task.

Overall, these results extends earlier research (e.g. Kim et al., 2001; Packard & Wingard, 2004; Wingard & Packard, 2008) that has found evidence for the role of emotional arousal in influencing the relative use of multiple memory systems. While

exposure to a predator odor or administration of corticosterone has been employed in previous studies to investigate other memory systems (Williams et al., 2005; Roozendaal et al., 2006), these experiments extended these findings by investigating the dorsolateral striatum-dependent habit memory system. Furthermore, these experiments also introduced a fairly recent idea of employing fear-conditioned stimuli as capable stressors in modulating memory systems in a manner similar to exposure to stressful unconditioned stimuli, while also providing evidence that this modulation also relies on similar neurocircuitry as that of unconditioned stressors.

TMT and the Modulation of Memory

This set of experiments suggest that pre-training exposure to the predator odor, TMT, biases rats towards preferential use of a dorsolateral striatum-dependent response strategy and that this effect may be mediated by the BLA. Furthermore, these studies also suggest that pre-training exposure to TMT facilitates acquisition of a dorsolateral striatum-dependent single-solution task. Previous studies have suggested that exposure to TMT produces anxiety-like behaviors in rodents (Vernet-Maury et al., 1984). However, the possibility remains that the results found in this set of experiments may be attributed to pre-training exposure to a novel odor, rather than a result of the stress-inducing properties of TMT. While this remains a possibility, evidence suggests that rats exposed to butyric acid, a noxious and rancid odor, failed to produce freezing compared to rats exposed to TMT (Wallace & Rosen, 2001). Therefore, the behavioral effects of pre-training exposure may be inferred to be a result of the stress-inducing properties of

TMT. Regardless, exposing rats to another novel odor could serve as a valuable control group in future studies.

Previous work from our lab found that emotional arousal facilitates response learning while impairing place learning in their respective single-solution tasks (Wingard & Packard, 2008). This led to the hypothesis that emotional arousal may facilitate dorsolateral striatum-dependent memory indirectly through the impairment of hippocampus-dependent memory, thus freeing the striatum-dependent habit memory system from online competition with the hippocampus (for review on competition see Poldrack & Packard, 2003). However, results from these experiments found that pre-training exposure to TMT did not influence place learning in a single-solution place task. There may be several reasons for this discrepancy. It is likely that exposure to TMT may not be as emotionally arousing as administration of an anxiogenic drug such as RS 79948-197. There is substantial evidence that suggests that the effect of emotional arousal on hippocampus-dependent learning and memory processes adheres to an inverted-U shape curve (Sandi et al., 1997; Williams et al., 2005). This suggests that stress at lower intensity levels may not be sufficient to affect hippocampus-dependent processes. However, this inverted-U shape relationship between emotional arousal and memory modulation has yet to have been established in dorsolateral striatum-dependent learning and memory processes, therefore the lower intensity levels of stress experienced by predator odor exposure may still be sufficient to modulate this type of learning. Another possibility for the lack of TMT-mediated effect on the single-solution place task might be a function of the task itself. The possibility remains that the single-solution

place task in its current incarnation may have been a "simpler" task than the single-solution response task, thus allowing for rapid acquisition for the task. The rapid acquisition of the single-solution place task may have prevented any impairing effect of pre-training exposure to TMT, especially if exposure to this predator odor did not produce highly arousing stress effects as discussed earlier. Finally, we cannot rule out the possibility that exposure to a predator odor may have produced emotional arousal in rats in a manner that directly enhanced dorsolateral striatum-dependent learning while leaving hippocampus-dependent learning relatively unaffected. Previous studies indicate that stress hormones, when administered directly into the dorsal striatum, enhances memory of a striatum-dependent task (Quirarte et al., 2009). Therefore, it is possible that stress effects may modulate dorsolateral striatum-dependent learning processes directly through potential activation of these GR receptors located within the dorsal striatum. Despite the discrepancy of these results with previous work from our lab, closer observation of the graph (Figure 7) suggests that pre-training exposure to TMT may have had a slight attenuating effect on place learning in earlier trials. Perhaps future studies could observe a significant effect of pre-training TMT on place learning through use of a larger sample size or through an experimental paradigm that produced a more difficult place task.

Experiment 2 provided evidence for the role of pre-training exposure to predator odor in biasing rats towards preferential use of a striatum-dependent response strategy in a task that could be acquired using either a hippocampus-dependent place strategy or dorsolateral striatum-dependent response strategy. Furthermore, this experiment also

implicates the BLA as a critical neuroanatomical region mediating this effect. It is important to note that although the BLA mediates the memory modulatory influence of TMT exposure on plus-maze behavior, the functional integrity of this brain region is not *necessary* for the acquisition of either place or response learning (Packard & Gabriele, 2009). Rather, the memory modulatory role of the BLA appears to involve activation of efferent projections that modulate memory processes occurring in other brain structures (for review see McGaugh, 2004). As discussed earlier, evidence indicates that both place and response learning are mediated by the hippocampus and dorsolateral striatum, respectively (e.g. Packard & McGaugh, 1996; for review see Packard, 2009). Similar to results from experiment 1, there is a possibility that TMT exposure activates BLA efferents and biases rats towards the use of dorsolateral striatal-dependent response learning by *directly* influencing synaptic plasticity within the striatum. Consistent with this hypothesis, pre-training predator exposure increases *c-fos* mRNA expression in the dorsolateral striatum in rats that display a suggested preference for a procedural learning strategy in a water radial-arm maze task (VanElzakker et al., 2011). Alternatively, the possibility remains that TMT exposure may *indirectly* favor the use of dorsolateral striatal-dependent response learning by impairing hippocampus-dependent place learning. Consistent with the latter hypothesis, both anxiogenic drug administration (Wingard & Packard, 2008; Packard & Gabriele, 2009) and exposure to a predator (presence of a cat), can impair hippocampus-dependent spatial learning (Park, Campbell & Diamond, 2002; Park et al., 2008; but see also Galliot, Levaillant, Beard, Millot & Pourie, 2010; Diamond et al., 2007) and retrieval (Diamond et al., 2006). Moreover, this

stress-induced impairment of hippocampus-dependent memory appears to involve a modulatory influence of the amygdala. Thus, pre-training exposure to a stress regimen (restraint and tail-shock) impairs hippocampus-dependent learning in a spatial water maze task, and this effect is blocked by lesions of the amygdala (Kim et al., 2001). The present findings using TMT exposure builds off previous work in which a pre-training stress regimen also enhanced the relative use of dorsolateral striatal-dependent memory in a dual-solution task (Kim et al., 2001).

As previously discussed, it is possible that bupivacaine administration may have had an effect on memory modulation through possible spread to adjacent regions to the BLA. However, it must be reiterated that the BLA, but not CeA, has been implicated in modulation of memory processes in other systems (Roozendaal & McGaugh 1996a; Roozendaal & McGaugh, 1997). Furthermore, the dose of bupivacaine administered in this study was used in previous research that implicated the role of the BLA in memory modulation (Packard & Gabriele, 2009). However, further research should still consider additional groups to control for this.

In sum, these findings indicate that pre-training exposure to the predator odor TMT biases animals towards the use of response learning/habit memory in a dual-solution plus-maze task, as well as facilitates acquisition of response learning in a single-solution plus-maze task. This effect of TMT appears to be mediated, at least in part, by the BLA, providing further evidence of a role for this brain region in emotional modulation of the relative use of multiple memory systems. An anxiety/stress induced bias towards the use of habit memory has also been recently observed in human studies

(e.g. Schwabe et al., 2007; Schwabe & Wolf, 2009), and it has been suggested that this modulatory influence of emotional arousal on the relative use of multiple memory systems may have implications for understanding the role of learning and memory processes in several human psychopathologies, as will be discussed later (for reviews see Goodman, Leong & Packard, 2012; Schwabe, Dickinson & Wolf, 2011).

Corticosterone and the Modulation of Memory

While most studies investigating the role of corticosterone on memory modulating have focused on other memory systems (e.g. Roozendaal et al., 2004; Sandi et al., 1997), the present set of experiments focused on extending these findings to the dorsolateral striatum-dependent habit memory system. There have been several studies that have observed a role of corticosterone, administered directly into the dorsal striatum in influencing memory consolidation. For example, Medina et al., (2007) found that administration of corticosterone directly into the dorsal striatum facilitated inhibitory avoidance memory. Further investigation suggested corticosterone administered directly into the dorsal striatum enhanced the procedural memory of this particular task. Similarly, corticosterone administered directly into the dorsal striatum immediately following training enhanced retention in a cued platform water maze task while having no effect on the spatial water maze task, suggesting that the corticosterone effect on memory consolidation was mediated by actions within the dorsal striatum (Quirarte et al., 2009). The present set of experiments did not determine the specific neural substrate that mediated this corticosterone effect on habit memory consolidation. Given the work

described above (Medina et al., 2007; Quirarte et al., 2009), it is possible that corticosterone may act directly on receptors within the dorsolateral striatum to alter synaptic plasticity and facilitate response learning. There is also a possibility that corticosterone may also be binding to GRs within the BLA to modulate consolidation of response memory. To support this, in the present studies concurrent administration of the β-adrenoceptor antagonist, propranolol, blocked the habit memory-enhancing effect of corticosterone. Previous work investigating this glucocorticoid-noradrenergic interaction in modulating memory suggests that the BLA is an important structure in mediating this effect (for review see Roozendaal et al., 2009). For example, intra-BLA administration of the β-adrenoceptor antagonist, propranolol, may block the modulatory effect of corticosterone in separate memory tasks (Roozendaal et al., 2006). Therefore, it is possible that in these present sets of experiments, corticosterone modulates habit memory consolidation through its action within the BLA and that this corticosterone-mediated effect also required noradrenergic activity within this brain region. Regardless, the neuroanatomical region mediating the effect found here has yet to be determined and it is possible that corticosterone may affect synaptic plasticity both in the dorsolateral striatum and BLA. As previously discussed, stress hormones may also bind to other brain regions that directly modulate independent memory systems. For example, corticosterone administered directly into the dorsal striatum facilitated memory consolidation in a dorsolateral striatum-dependent cued water maze task (Quirarte et al., 2009). However, this does not necessarily rule out the neuromodulatory role of the BLA. In another study, the memory enhancing effect of intra-hippocampal administration of

the GR agonist RU 28362 on an inhibitory avoidance task was blocked by intra-BLA infusions of lidocaine (Roozendaal & McGaugh, 1997). Similarly, the memory enhancing effect of intra-hippocampal administration of RU 28362 was also blocked through intra-BLA administration of the β-adrenoceptor antagonist atenolol (Roozendaal et al., 1999). Therefore, memory modulation through GR activation within the hippocampus is still dependent on noradrenergic activity within the BLA. Similarly, glucocorticoid action on modulation of dorsolateral striatum-dependent memory may involve GRs both within the striatum and BLA.

The molecular mechanisms underlying this phenomenon, though undetermined in this study, most likely adheres to the model proposed by Roozendaal (2000), which implicates the BLA as a critical region in which the glucocorticoid-noradrenergic interaction occurs (Figure 19). From this model, epinephrine activates afferents on the vagus nerve that project to the nucleus of the solitary tract. From there, noradrenergic neurons project to the BLA and to the LC (which also then projects to the BLA). Norepinephrine released from these projections bind to post-synaptic β-adrenoceptors and α1-adrenoceptors. Similarly, evidence suggests that glucocorticoid hormones freely enter through the blood-brain barrier and bind onto membrane GRs in the BLA (Roozendaal et al., 2002). These GRs are located on cells that are involved in norepinephrine signaling and therefore GR activation leads to potentiation of the norepinephrine signal through G-protein mediated effects. Glucocorticoids also binds to GRs located in the nucleus of the solitary tract that potentiates norepinephrine release from that region to the BLA and LC (Roozendaal et al., 1999). Here, researchers found

that the GR agonist RU 28362 infused directly into the nucleus of the solitary tract enhanced consolidation of an inhibitory avoidance task. This enhancement was blocked by intra-BLA infusions of a β-adrenoceptor antagonist. Furthermore, a study employing in vivo micro-dialysis suggested that glucocorticoid administration may facilitate training-induced release of norepinephrine within the amygdala (McIntyre et al., 2004).

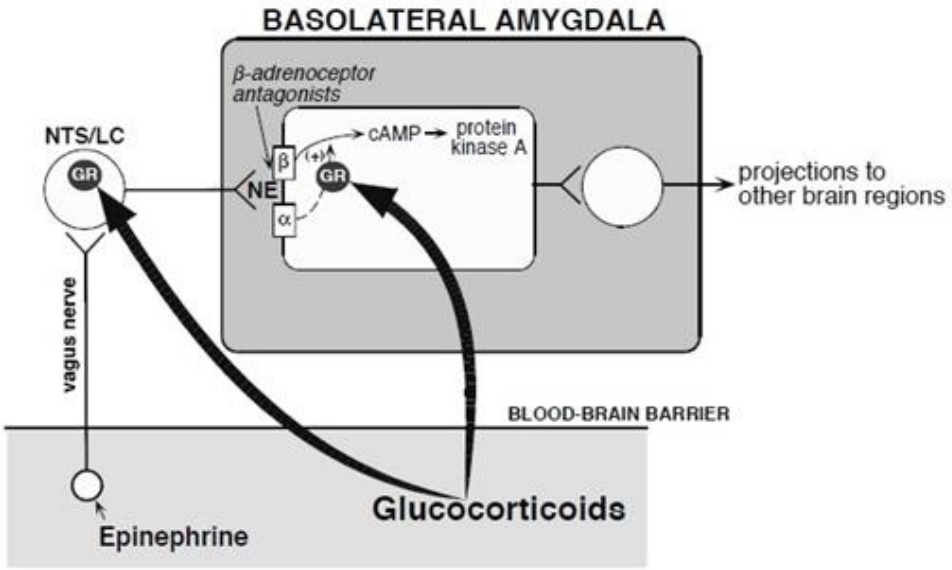

Figure 19. Summary schematic of the interaction between glucocorticoids and the noradrenergic system within the BLA. Norepinephrine (NE; noradrenaline) is released from projections from the nucleus of the solitary tract (NTS) and locus coeruleus (LC). NE binds to post-synaptic β-adrenoceptors and α1-adrenoceptors within the BLA. β-adrenoceptor activation stimulates cAMP formation. Glucocorticoids may bind to glucocorticoid receptors (GRs) within the NTS to facilitate downstream NE release and may influence the β-adrenoceptor-cAMP system through binding with intra-cellular GRs within the BLA. Adapted from Roozendaal, Okuda, de Quervain & McGaugh, 2004.

The subsequent increased activity within the BLA may influence memory processes in other brain regions through afferent projections from the BLA to brain regions associated with independent memory systems (Figure 20). The BLA projects to a

number of brain regions, such as the hippocampus and dorsal striatum, often through the stria terminalis (ST) (Petrovich et al., 2001; Pitkanen et al., 2000). Therefore it is highly likely that the glucocorticoid-noradrenergic interaction within the BLA following emotional arousal may modulate memory at other brain regions through projections from the BLA. Indeed, there have been a number of studies suggesting that the BLA plays an important neuromodulatory role in memory consolidation. For example, amphetamine administered post-training into the BLA modulates both hippocampus-dependent and dorsolateral striatum-dependent memory, depending on the type of task employed (Packard, Cahill, & McGaugh, 1994). Similarly, post-training intra-BLA administration of the glucocorticoid agonist RU 28362 enhanced memory in an inhibitory avoidance task (Roozendaal et al., 1999). Studies from our lab have also indicated that the BLA plays an important role in mediating the effect of systemic anxiogenic drug administration on the modulation of hippocampus-dependent and dorsolateral striatum-dependent memory (Packard & Gabriele, 2009).

These sets of experiments have provided evidence for the modulatory role of the glucocorticoid-noradrenergic interaction in dorsolateral striatum-dependent memory processes. Furthermore, the next set of experiments (experiment 6) presents evidence for the role of the amygdala in modulating this effect.

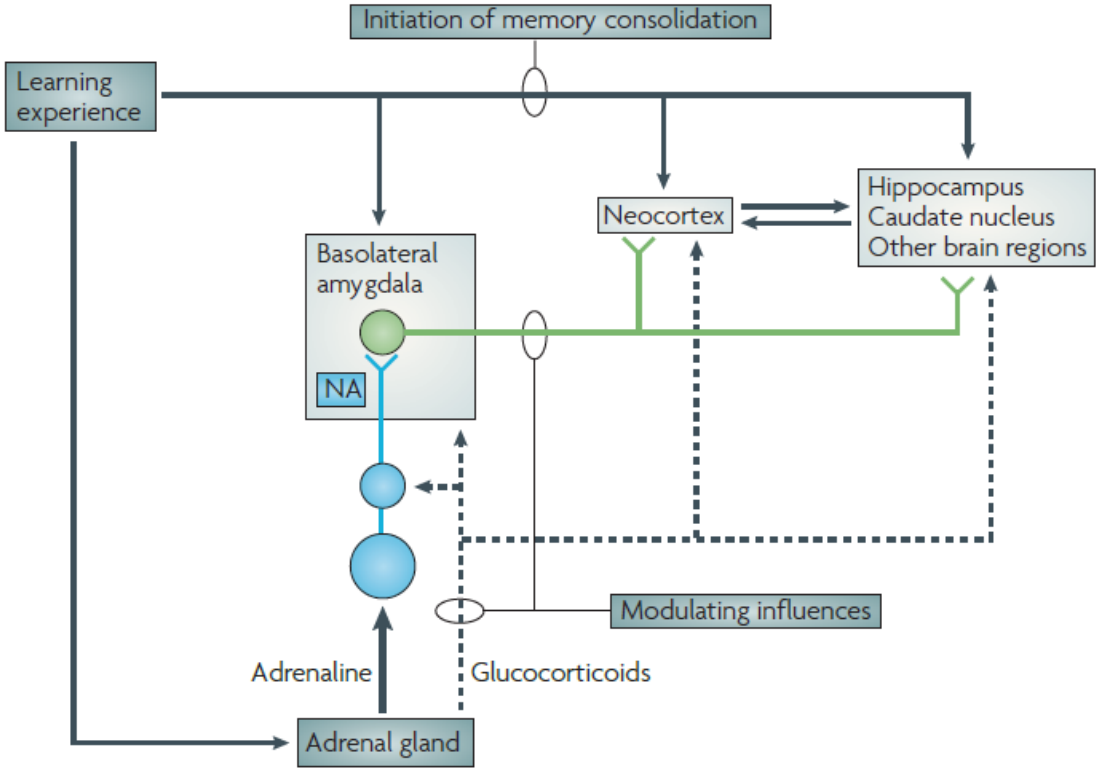

Figure 20. Diagram depicting the emotional arousal-induced modulation of memory. Information from learning experiences recruit different brain structures. For example, spatial information recruits the hippocampus while stimulus-response associations recruit the caudate nucleus (or dorsolateral striatum). Both glucocorticoids and noradrenaline (NA; norepinephrine) are released following emotionally arousing events. The glucocorticoid-noradrenergic interaction within the BLA modulates memory consolidation at other brain regions through projections from the BLA. Additionally, glucocorticoids directly modulate memory at other brain regions. Adapted from Roozendaal, McEwen & Chattarji, 2009.

Exposure to Fear-Conditioned Stimuli and the Modulation of Memory

As previously discussed, a number of studies have determined that emotional arousal modulates striatum-dependent habit memory (Wingard & Packard, 2008; Leong & Packard, 2014) and facilitates preferential use of response learning over place learning (Kim et al., 2001; Packard & Wingard, 2004). However, very few studies have observed the effect of "fear-provoking experience" or "fear-conditioned stimuli" on memory modulation. That is, few studies have observed the ability of a conditioned stimulus,

when paired with an unconditioned stressor, to modulate memory in a similar manner. There have been a handful of studies that have shown that reactivation of an emotional memory through exposure to inhibitory avoidance apparatus prior to retention or probe trials impaired spatial memory and biased learning strategy preference towards response learning (Zoladz et al., 2010; Hawley et al., 2013). While Hawley et al. (2013) found that reminders of an acute stressor can influence the relative use of learning strategy prior to retention; our study displays a similar effect of post-training exposure to fear-conditioned stimuli in shifting bias towards preferential use of response learning at consolidation. Additionally, the work of Holahan and White (2002) demonstrated that exposure to a discrete conditioned stimulus previously paired with a foot-shock can also modulate the consolidation of memory in a Y-maze task. Here, the researchers exposed rats to a chamber in which they had received tone-shock pairings a day prior to Y-maze training and found that post-training exposure to fear-conditioned cues modulated memory in an appetitive Y-maze task. Interestingly, if conditioned stimuli were paired with stimuli of positive valence (i.e. sucrose), post-training presentation of these positively-associated cues facilitated memory in a conditioned place preference (CPP) task (Holahan & White, 2013). The present study suggests that post-training exposure to fear-conditioned stimuli can modulate dorsolateral striatum-dependent memory consolidation and shift rats towards preferential use of a striatum-dependent learning strategy over a hippocampus-dependent place strategy. Furthermore, the present study found that post-training administration of propranolol, prior to exposure to fear-

conditioned stimuli, can block this enhancing effect on consolidation in a single-solution response task, suggesting that this effect is mediated by noradrenergic processes.

While these experiments provide evidence suggesting that post-training exposure to fear-conditioned stimuli modulates dorsal striatum-dependent memory, the dorsal striatum may also be directly involved in the behavioral response to fear-conditioned stimuli. Indeed, the dorsal striatum has been implicated in the behavioral response following fear-conditioning. Studies have found that lesions to the dorsal striatum impairs conditioned freezing to a discrete cue following fear-conditioning (Ferreira et al., 2003; 2008), while post-training infusions of amphetamine directly into the dorsal striatum enhances freezing to both a discrete cue and context following fear conditioning (White & Salinas, 2003).

Although future work must be carried out to determine the specific neurobiological mechanisms involved here, it is highly likely that these current results were, at least in part, modulated by elevated levels of stress hormones. Acute stress has been associated with elevated levels of corticosterone (Woodson et al., 2003; Park et al., 2008). Additionally, the administration of corticosterone directly into the dorsal striatum enhances consolidation of S-R memory in a cued water-maze task (Quirarte et al., 2009) and inhibitory avoidance training (Medina et al., 2007), as described earlier, suggesting a role of glucocorticoids in enhancing the consolidation of striatum-dependent memory. It is possible that post-training exposure to fear-conditioned stimuli may have caused stress-induced elevations corticosterone levels in a manner that modulated striatum-dependent memory consolidation and bias towards preferential use of response learning.

Indeed, exposure to fear-conditioned stimuli leads to a rise in corticosterone levels (Hagewoud et al., 2011) such that the amount of fear-like behaviors displayed during exposure is positively correlated to elevation of corticosterone in rats (Cordero, Merino, & Sandi, 1998). Therefore, it is plausible that post-training exposure to fear-conditioned stimuli produced enhancement in striatum-dependent memory consolidation through a mechanism that largely involves glucocorticoid function. That is, exposure to fear conditioned stimuli immediately following training may modulate memory through elevated glucocorticoid levels. These glucocorticoids may enhance dorsolateral striatum-dependent memory consolidation directly through binding to GRs within the dorsal striatum or through its action within the BLA (Figure 19). Future studies should be conducted to determine the specific neurotransmitter systems involved in mediating the effect of exposure to fear conditioned stimuli on the consolidation of dorsolateral striatum-dependent habit memory.

In sum, this study has found that exposure to fear-conditioned stimuli facilitates the preferential use of a striatum-dependent response strategy in a dual-solution task and enhances consolidation of striatum-dependent habit memory in a single-solution response task. Furthermore, this enhancement of dorsolateral striatum-dependent memory consolidation is mediated by noradrenergic activation. This study determined that fear-conditioned stimuli influences memory in a manner similar to its associated unconditioned stimulus, perhaps through the emotionally arousing or stressful nature of the re-experiencing of fear-conditioned stimuli. However, admittedly there are a number of future directions that may be undertaken in order to fully understand this

phenomenon. First and foremost, it would be important to dissociate the role of exposure to a discrete fear-conditioned cue, and exposure to a fear-conditioned context. It remains possible that exposure to a cue or context may modulate different memory systems in different ways. Furthermore, it is important to elucidate the specific neurobiological mechanisms of this phenomenon. The idea that stress hormones may play an influential role has been discussed but there are a number of possibilities regarding the neurotransmitters and anatomical structures involved. Regardless, it is important to show that conditioned stimuli, when paired with an emotionally arousing stimulus can striatum-dependent memory as this may provide significant implications for the understanding of stress influences on habit memory, particularly as it related to several stress-related psychopathologies.

Neuroanatomical Substrates Modulating Stress-Induced Competition between Memory Systems

The present set of experiments implicated the role of a glucocorticoid-noradrenergic interaction in mediating the effect of emotional arousal on modulation of memory, particularly dorsolateral striatum-dependent memory. Additionally, the amygdala is implicated as a possible neuroanatomical substrate mediating this effect (Roozendaal et al., 2006). However, the precise neuroanatomical mechanisms mediating the relative use of learning strategy following emotional arousal has yet to be fully identified. One possible neural substrate mediating the glucocorticoid-noradrenergic interaction is the BLA, as discussed earlier (Figure 19). From the model presented in

Roozendaal et al. (2004), epinephrine released following stressful experience modulates noradrenergic activity in the BLA via the NTS. Additionally, concurrent glucocorticoid release may facilitate noradrenergic activity through binding with intracellulular GRs within the BLA, this highlighting the importance of the glucocorticoid-noradrenergic interaction and implicating the BLA as an important structure in mediating this effect. Research from our lab, including the studies presented here, has suggested that emotional arousal produces enhancement in the dorsolateral striatum-dependent memory system, and shifts preferential use of learning strategy away from the hippocampus-dependent memory system.

As previously discussed, research suggests that the hippocampus and dorsolateral striatum-dependent may compete directly in certain tasks (for review see Poldrack & Packard, 2003). One potential mechanism that might mediate this effect of emotional arousal on enhancement of dorsolateral striatum-dependent memory is through releasing it from competition with the hippocampus-dependent memory system. Specifically, the BLA may modulate the dorsolateral striatum-dependent memory system *indirectly* by impairing hippocampus-dependent learning and memory. Previous studies have provided consistent evidence with this hypothesis. For example, intra-BLA administration of the anxiogenic drug RS 79948-197 impaired hippocampus-dependent learning and enhanced dorsolateral striatum-dependent learning, depending on the type of task employed (Wingard & Packard, 2008). Similar effects were seen with peripheral administration of RS 79948-197, and these effects were blocked with intra-BLA administration of the sodium channel bupivacaine (Packard & Gabriele, 2009). Finally, amygdala lesions

blocked the effect of pre-training exposure to a stress regimen on hippocampus-dependent learning impairments (Kim et al., 2001). Taken together, these studies suggest that the BLA may impair the hippocampus-dependent memory system thus releasing the dorsolateral striatum-dependent system from competition.

While the studies presented here have not directly investigated the role of the locus coeruleus in mediating the effect of emotional arousal on memory modulation, evidence suggests that this structure may still play a critical role. The locus coeruleus is an important structure in mediating noradrenergic activity following stressful exposure. Additionally the locus coeruleus interacts with the hippocampus and cortex (for review see Valentino & Van Bockstaele, 2008), and therefore is an important structure in noradrenergic regulation within the brain following stress. Patients suffering from stress-related disorders have shown hypersensitivity to noradrenergic drugs such as yohimbine (Sullivan, Coplan, Kent, & Gorman, 1999). Similar results have been found in non-human primates following exposure to early stress (Rosenblum et al., 1994). Therefore, it can be postulated that people suffering from anxiety disorders and animals that experienced developmental stress may develop dysregulation of the noradrenergic system, thus implicated the locus coeruleus in this dysregulation.

Studies have found that noradrenergic activity within the locus coeruleus plays an important role in hippocampus-dependent learning (Lemon, Aydin-Abidin, Funke, & Manahan-Vaughan, 2009; Gibbs, Hutchinson, & Summers, 2010). Furthermore, the locus coeruelus sends (Williams & Clayton, 2001) and receives (Van Bockstaele, Colago, & Valentino, 1998) projections from the amygdala. It is possible that the locus

coeruleus influences hippocampus-dependent learning and memory through its interaction with the amygdala. In line with the previously discussed model (Figure 19), epinephrine release potentiates noradrenergic activity within the locus coeruleus that indirectly facilitates noradrenergic activity within the BLA. Furthermore, the locus coeruleus may also modulate hippocampus-dependent memory following afferent input from the amygdala (Van Bockstaele, Colago, & Valentino, 1998). Previous studies from our lab have shown that administration of yohimbine can produce preferential use of a striatum-dependent response strategy over a hippocampus-dependent place strategy in rats (Packard & Wingard, 2004). This may have mimicked the dysregulation of noradrenergic activity following stress thus implicating the role of the locus coeruleus in this effect. The dysregulation of noradrenergic activity mediated by the locus coeruleus may impair hippocampus-dependent learning and memory, thus *indirectly* facilitating dorsolateral striatum-dependent learning and memory. Indeed, future studies should further investigate the role of noradrenergic activity within the locus coeruleus in mediating the effect of emotional arousal on the modulation of multiple memory systems.

Emotional Arousal and the Relative Use of Memory Systems: An Evolutionary Perspective

Some researchers have postulated that the development of multiple memory systems has been adaptive for animals (Sherry & Schacter, 1987) and humans (Klein, Cosmides, Tooby, & Chance, 2002). For example, accessing hippocampus-dependent

cognitive memories from typically produce accurate memories, but this comes at the cost of speed. This type of memory also takes into account situational variables, allowing for flexibility. The dorsolateral striatum-dependent habit memory system typically results in rigid behaviors that are "immune" to variance across environments, resulting in responses that remain consistent across episodes. The overall suggestion is that these types of memories are not reliant on details regarding time, space, or context, which allows for automatic execution of behavior. Due to the seemingly "incompatible" nature of the types of information processed by these memory systems (e.g. flexible vs. inflexible), it seemed adaptive to compartmentalize the processing of these two separate types of information. The development of these two independent memory systems allowed for an animal to access various types of information to respond to a variety of situations. For example, the hippocampus-dependent cognitive memory system may be beneficial in certain situations, such as remembering locations of stored foods, while the dorsal striatum-dependent memory system may be useful in instances where behavioral processes need to be rigid and automatic, such as when engaged in a procedural task.

As previously discussed, experiences of anxiety and stress are typically adaptive in that they allow an animal to respond to perceived threats in their surrounding environment (Gutierrez-Garcia & Contreras, 2013). The general theme of this dissertation proposes that emotional arousal enhances the dorsolateral striatum-dependent memory system and produces a bias towards use of a striatum-dependent strategy over a hippocampus-dependent strategy. Therefore, the shift from hippocampus-dependent strategy to striatum-dependent strategy following emotional arousal may

serve an adaptive purpose, such as avoiding hesitation and delays with coping with the stressor (Schwabe & Wolf, 2013). For example, if an animal has been reinforced to approach a particular location, the introduction of an immediate threat may lead the animal to automatically approach that same particular location. It would hypothetically be beneficial for the animal to disengage from any processing of information regarding time and context during a potentially fatal encounter and channel all resources towards engaging a response that might lead to safety, thus leading to behaviors that rely on a response memory rather than a cognitive memory. This is consistent with the results presented here in which presentation of an acute stressor, such as a predator odor, led rats to preferential use of a striatum-dependent strategy which typically favors rigid, automatic behaviors.

The idea that multiple memory systems developed as a result of a naturally adaptive process suggests a possibility that it may have been adaptively beneficial to develop a memory system that could be engaged *despite* the experience of stress. Results from studies presented here suggests that stressors, both unconditioned and conditioned, modulates dorsolateral striatum-dependent memory. It may have been evolutionarily beneficial for animals to develop a memory system that could still acquire, consolidate, and retrieve information in states of heightened emotional arousal, when cognitive functioning is typically impaired. Granted, the dorsolateral striatum-dependent memory system mediates information containing fewer *details* (i.e. temporal or spatial) about the episode, but may also require fewer resources to engage. However, while the mechanisms involved in modulating striatum-dependent memory following emotional

arousal may have evolved with an adaptive purpose, the engagement of the habit memory system following stress and anxiety may also lead to maladaptive behaviors.

Emotional Arousal, Habit Memories, and Psychopathologies

The finding that an ethologically-valid stressor or a conditioned stimulus, when paired with an aversive stimulus, modulates memory provides an exciting avenue of research in the field of emotion and memory. Specifically, evidence suggesting that exposure to TMT or exposure to fear-conditioned stimuli may modulate striatum-dependent habit memory provides implications into various stress-related psychopathologies such as post-traumatic stress disorder (PTSD) or drug addiction. Indeed, it can be argued that these specific psychopathologies may result from dysfunction of the dorsolateral striatum-dependent habit memory system (for review see Goodman, Leong, & Packard, 2012). For example, in patients suffering from PTSD, it can be argued that the strong manifestation of stimulus-response (S-R) avoidance behaviors following exposure to traumatic cues may be, in part, due to the maladaptation of the dorsal striatum in guiding these automatic-like avoidance behaviors. It is plausible that this over-consolidation of S-R learning might be exacerbated by extreme emotional arousal. Here, we show that exposure to fear-conditioned stimuli, which parallels exposure to traumatic cues, can produce robust use of habit-like response strategies during learning as well as enhance the consolidation of habit memory. This notion that a fear-conditioned stimulus can produce robust effects on the habit memory system similar to a fearful unconditioned stimulus has strong implications for PTSD. Similarly, the

initial acquisition of drug-seeking and drug-taking behaviors may depend on the hippocampus-dependent memory system, while subsequent drug-taking behaviors following addiction might result in a shift of control in favor of the dorsal striatum-dependent memory system, as evidenced by the rigid and habit-like nature of these behaviors (White, 1996). This is consistent with the hypothesis that emotional arousal produces a bias in use of the habit memory system (Packard & Wingard, 2004; Schwabe et al., 2011), as stressful life experience has often been attributed to increased drug abuse (Newcomb & Bentler, 1988) and relapse (Wallace, 1989). Again, our study suggests that stress presented in the form of exposure to emotionally-associated stimuli can produce a robust enhancement of the striatum-dependent habit memory system, which holds strong implications in the role of stress and drug addiction.

Finally, while the present set of experiments do not cover the topic of extinction; an important question remains whether emotional arousal can modulate extinction learning, specifically with regards to S-R or response extinction. A prominent theory in extinction learning suggests that extinction is a form of "new learning", in that the original memory association does not degrade, but rather during extinction training (e.g. action without outcome, or CS without US) a new association is formed that exists alongside the original stimulus association (Bouton 2002). Furthermore, evidence suggests that extinction learning is mediated by multiple systems (Gabriele & Packard, 2006). Specifically, evidence suggests that extinction paradigms involving S-R associations or responses may be mediated by the dorsolateral striatum. Therefore, under the assumption that extinction training is a separate form of learning that is also

mediated by multiple, individual systems, it is plausible to expect that emotional arousal may also play a role in modulating extinction. Several studies have proposed that stress impairs fear extinction (for review see Akirav & Maroun, 2007), while other studies have also suggested that glucocorticoid administration facilitates extinction (de Quervain et al., 2011). Regardless, the ability for emotional arousal to modulate extinction processes would be an extremely important direction with regards to extinction of various habit-based psychopathologies.

Future Directions

While the present experiments have extended research regarding the role of emotional arousal in modulating multiple memory systems, these experiments have also laid the groundwork for future studies to build from. As alluded to earlier, the present studies suggest an important role for the glucocorticoid-noradrenergic interaction in modulating dorsolateral striatum-dependent memory. Previous work from other labs (Roozendaal et al., 2006) have suggested that noradrenergic activity within the BLA is critical in this interaction. However, few studies have determined the specific site of action for glucocorticoids following emotional arousal-driven modulation of habit memory. As discussed earlier, there is a possibility that glucocorticoids may modulate striatum-dependent memory through binding with GRs within the striatum or within the BLA. However, the question remains whether glucocorticoid binding to either of these structures plays an important role in this glucocorticoid-noradrenergic interaction that is critical to the modulation of memory through emotional arousal. Future studies could

replicate the current design of Experiment 4 but instead administer corticosterone directly into the BLA or dorsolateral striatum in order to determine the specific neuroanatomical structure mediating this effect. While previous studies have shown that corticosterone, when administered directly into the dorsal striatum, modulates habit memory (Quirarte et al., 2009), few studies have observed examined this effect with concurrent intra-BLA administration of propranolol. It would be worthwhile to determine if corticosterone-mediated enhancement of striatum-dependent memory consolidation is blocked by intra-BLA propranolol depending on whether corticosterone was administered directly into the dorsal striatum or BLA.

The present set of experiments also examined the ability for exposure to fear-conditioned stimuli to modulate dorsolateral striatum-dependent memory. Under the current experimental paradigm, exposure to fear-conditioned stimuli was achieved through exposing rats to the same context and auditory cue as during fear-conditioning trials. While this experimental paradigm was sufficient for the purposes of our hypothesis, future studies should attempt to differentiate the role of exposure to the context and discrete auditory cue in modulating striatum-dependent memory consolidation. Previous studies have found modest results when separating exposure to context and cue in modulating memory (Holahan & White, 2002), in that the both exposure to just the context and just the cue modulated memory but in a less robust manner. The question remains whether these findings would translate to the dorsolateral striatum-dependent habit memory system. From a purely speculative point of view, it may be possible that exposure to the fear-related context may modulate striatum-

dependent memory differently when compared to exposure to the fear-related cue, due to evidence suggesting that processing of contextual and spatial information is largely dependent on the hippocampus. Similarly, future studies should also examine the role of exposure to fear-conditioned stimuli on its ability to modulate hippocampus-dependent memory. Previous work from our lab has suggested that hippocampus-dependent memory is impaired following emotional arousal (Wingard & Packard, 2008; Packard & Gabriele, 2009). Therefore, the current effect of exposure to fear-conditioned stimuli should also be examined within the context of a hippocampus-dependent task.

General Summary

To summarize, the experiments described in this dissertation have extended research regarding the role of emotional arousal on the relative use of multiple memory systems, with particular focus on its role in modulating dorsolateral striatum-dependent memory. These experiments built on previous research using stressors, such as TMT and corticosterone, to examine their impact on the dorsolateral striatum-dependent memory system. Additionally, one set of studies also put forth a relatively novel idea of using fear-conditioned stimuli to modulate the relative use of memory systems, again focusing on the dorsolateral striatum-dependent memory system. Furthermore, the combined experiments also suggest that emotional modulation of the habit memory system is mediated by similar neurobiological mechanisms as other memory systems (i.e. glucocorticoid-noradrenergic interaction) and highlights a critical role of the BLA in this process. The multiple memory systems theory provides an important fabric in which to

study the effects of emotional arousal on memory and to continue research on the neurobiological mechanisms underlying the ability for emotional arousal to modulate memory.

REFERENCES

Akirav, I., Kozenicky, M., Tal, D., & Sandi, C. (2004). A facilitative role for corticosterone in the acquisition of a spatial task under moderate stress. *Learning and Memory, 11,* 188-195.

Akirav, I., & Maroun, M. (2007). The role of the medial prefrontal cortex-amygdala circuit in stress effects of extinction of fear. *Neural Plasticity, 2007,* 1-11.

Akirav, I. & Richter-Levin, G. (2002). Mechanisms of amygdala modulation of hippocampal plasticity. *Journal of Neuroscience, 22,* 9912-9921.

Balleine, B. W. & O'Doherty, J. P. (2010). Human and rodent homologies in action control: Corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology, 35,* 48-69.

Barden, N., Reul, J. M. H. M., & Holsboer, F. (1995). Do antidepressants stabilize mood through actions on the hypothalamic-pituitary-adrenocortical system? *Trends in Neurosciences, 18,* 6-11.

Barrett, D., & Gonzalez-Lima, F. (2004). Behavioral effects of metyrapone on Pavlovian extinction. *Neuroscience Letters, 371,* 91-96.

Blanchard, R. J., Blanchard, D. C., Wiess S. M., & Meyers S. (1990). The effects of ethanol and diazepam on reactions to predatory odors. *Pharmacology Biochemistry and Behavior, 35,* 775-780.

Bohus, B., & Lissak, K. (1968). Adrenocortical hormones and avoidance behavior of rats. *International Journal of Neuropharmacology, 7,* 301-306.

Bohus, B., & de Wied, D. D. (1981). Actions of ACTH-and MSH-like peptides on learning, performance and retention. In Martinez Jr., J. L. (Eds.). *Endogenous Peptides and Learning and Memory Processes* (pp. 59-78). New York: Academic Press.

Borrell, J., de Kloet, E. R., Versteeg, D. H., & Bohus, B. (1983). Inhibitory avoidance deficit following short-term adrenalectomy in the rat: The role of adrenal catecholamines. *Behavioral Neural Biology, 39,* 241-258.

Bouton, M.E. (2002). Context, ambiguity, and unlearning: Sources of relapse after Brooks, D. N., & Baddeley, A. D. (1976). What can amnesic patients learn? *Neuropsychologia, 14,* 111-122.

Burwash, M. D., Tobin, M. E., Woolhouse, A. D., & Sullivan, T. P. (1998). Laboratory evaluation of predator odors for eliciting an avoidance response in roof rats (Rattus rattus). *Journal of Chemical Ecology, 24,* 49-66.

Buwalda, B., Kole. M. H. P., Veenema, A. H., Huininga, M., de Boer, S. F., Korte, S. M., & Koolhaas, J. M. (2005). Long-term effects of social stress on brain and behavior: A focus on hippocampal functioning. *Neuroscience & Biobehavioral Reviews, 29,* 83-97.

Cai, W. H., Blundell, J., Han, J., Greene, R. W., & Powell, C. M. (2006). Postreactivation glucocorticoids impair recall of established fear memory. *Journal of Neuroscience, 26,* 9560-9566.

Campeau, S. & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *Journal of Neuroscience, 15,* 2301-2311.

Carr, G. D., & White, N. M. (1984). The relationship between stereotypy and memory improvement produced by amphetamine. *Psychopharmacology, 82,* 203-209.

Chekley, S. (1996). The neuroendocrinology of depression and chronic stress. *British Medical Bulletin, 52,* 597-617.

Clayton, E. C., & Williams, C. L. (2000). Noradrenergic receptor blockade of the NTS attenuates the mnemonic effects of epinephrine in an appetitive light-dark discrimination learning task. *Neurobiology of Learning and Memory, 74,* 135-145.

Cohen, N. J. & Squire, L. R. (1980). Preserved learning and retention of pattern-analyzing skill in amnesia: Dissociation of knowing how and knowing that. *Science, 210,* 207-209.

Conrad, C. D., Galea, A. M., Kuroda, Y., & McEwen, B. S. (1996). Chronic stress impairs rat spatial memory on the Y maze, and this effect is blocked by tianeptine treatment. *Behavioral Neuroscience, 110,* 1321-1334.

Conrad, C. D., Jackson, J. L., Wieczorek, L., Baran, S. E., Harman, J. S., Wright R. L., & Korol, D. L. (2004). Acute stress impairs spatial memory in male but not female rats: Influence of estrous cycle. *Pharmacol Biochem Behav, 78,* 569-579.

Cordero, M. I., Merino, J. J., & Sandi, C. (1998). Correlational relationship between shock intensity and corticosterone secretion on the establishment and subsequent expression of contextual fear conditioning. *Behavioral Neuroscience, 112,* 885-891.

Cordero, M. I., & Sandi, C. (1998). A role for brain glucocorticoid receptors in contextual fear conditioning: Dependence upon training intensity. *Brain Research, 786,* 11-17.

Critchley, H. (2003). Emotion and its disorders: Imaging in clinical neuroscience. *British Medical Bulletin, 65,* 35-47.

Dachir, S., Kadar, T., Robinzon, B., & Levy, A. (1993). Cognitive deficits induced in young rats by long-term corticosterone administration. *Behavioral and Neural Biology, 60,* 103-109.

Davis, M. (1992). The role of the amygdala in fear and anxiety. *Annual Review of Neuroscience, 15,* 353-375.

Day, H. E., Masini, C.V., Campeau, S. (2004). The pattern of brain *c-fos* mRNA induced by a component of fox odor, 2,5-dihydro-2,4,5-trimethylthiazoline (TMT), in rats, suggests both systemic and processive stress characteristics. *Brain Research, 1025,* 139-151.

de Boer, S. F., Koopmans, S. J., Slangen, J. L., & Van der Gugten, J. (1990). Plasma catecholamine, corticosterone and glucose response to repeated stress in rats: Effect of interstressor interval length. *Physiological Behavior, 47,* 1117-1124.

de Kloet, E. R. (1991). Brain corticosteroid receptor balance and homeostatic control. *Frontiers in Neuroendocrinology, 12,* 95-164.

de Kloet, E. R., Harst, M., & Joels, M. (2008). Corticosteroid hormones in the central stress response: Quick-and-slow. *Frontiers in Neuroendocrinology, 29,* 268-272.

de Quervain, D. J. F., Bentz, D., Michael, T., Bolt, O. C., Wierderhold, B. K., Margraf, J., & Wilhelm, F. H. (2010). Glucocorticoids enhance extinction-based psychotherapy. *Proceedings of the National Academy of Sciences, 108,* 6621-6625.

de Quervain, D. J. F., Roozendaal, B., & McGaugh, J. L. (1998). Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature, 394,* 787-790.

de Quervain, D. J. F., Roozendaal., B., Nitsch, R. M., McGaugh, J. L., & Hock, C. (2000). Acute cortisone administration impairs retrieval of long-term declarative memory in humans. *Nature Neuroscience, 3,* 313-314.

de Wied, D. D., & Bohus, B. (1966). Long term and short term effects on retention of a conditioned avoidance response in rats by treatment with long acting pitressin and α-MSH. *Nature, 212,* 1484-1486.

Diamond, D. M., Campbell, A. M., Park C. R., Halonen, J., & Zoladz, P. R. (2007). The temporal dynamics model of emotional memory processing: A synthesis on the neurobiological basis of stress-induces amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson Law. *Neural Plasticity,* 1-33.

Diamond, D. M., Campbell, A. M., Park, C. R., Woodson, J. C., Conrad, C. D., Bachstetter, A. D., & Mervis, R. F. (2006). Influence of predator stress on the consolidation versus retrieval of long-term spatial memory and hippocampal spinogenesis. *Hippocampus, 16,* 571-576.

Diamond, D. M., Park, C. R., Heman, K. L., & Rose, G. M. (1999). Exposing rats to a predator impairs spatial working memory in the radial arm water maze. *Hippocampus, 9,* 542-552.

Dielenberg, R. A. & McGregor, I. S. (2001). Defensive behavior in rats towards predatory odors: A review. *Neuroscience & Biobehavioral Reviews, 25,* 597-609.

Divac, I., Rosvold, H. E., & Szwarcbart, M. K. (1967). Behavioral effects of selective ablation of the caudate nucleus. *Journal of Comparative and Physiological Psychology, 63,* 184-190.

Dolan, R. J. (2002). Emotion, Cognition, Behavior. *Science, 298,* 1191-1194.

Donley, M. P., Schulkin, J., & Rosen, J. B. (2005). Glucocorticoid receptor antagonism in the basolateral amygdala and ventral hippocampus interferes with long-term memory of contextual fear. *Behavioural Brain Research, 164,* 197-205.

Eichenbaum, H., Stewart, C., Morris, R. G. M. (1990). Hippocampal representation in spatial learning. *Journal of Neuroscience, 10,* 331-339.

Elliott, A. E. & Packard, M. G. (2008). Intra-amygdala anxiogenic drug infusion prior to retrieval biases rats towards the use of habit memory. *Neurobiology of Learning and Memory* 90: 616-623.

Elsinger, P. J. & Damasio, A. R. (1985). Severe disturbance of higher cognition after bilateral frontal lobe ablation Patient EVR. *Neurology, 35,* 1731-1741.

Endres, T., & Fendt, M. (2007). Conditioned behavioral responses to a context paired with the predator odor trimethythiazoline. *Behavioral Neuroscience, 121,* 594-601.

Endres, T. & Fendt, M. (2008). Inactivation of the lateral septum blocks fox odor-induced fear behavior. *NeuroReport, 19,* 667-670.

Fanselow, M. S., & Kim, J. J. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphono-valeric acid in the basolateral amygdala. *Behavioral Neuroscience, 108,* 210-212.

Fendt, M., Endres, T., & Apfelbach, R. (2003). Temporary inactivation of the bed nucleus of the stria terminalis but not of the amygdala blocks freezing induced by trimethylthiazoline, a component of fox feces. *Journal of Neuroscience, 23,* 23-28.

Fendt, M., Endres, T., Lowry, C. A., Apfelbach, R., & McGregor, I. S. (2005). TMT-induced autonomic and behavioral changes and the neural basis of its processing. *Neuroscience & Biobehavioral Reviews, 29,* 1145-1156.

Ferreira, T. L., Moreira, K. M., Ikeda, D. C., Bueno, O. F. A., & Oliveira, M. G. M. (2003). Effects of dorsal striatum lesions in tone fear conditioning and contextual fear conditioning. *Brain Research, 987,* 17-24.

Ferreira, T. L., Shammah-Lagnado, S. J., Bueno, O. F. A., Moreira, K. M., Fornani, R. V. & Oliveira, M. G. M. (2008). The indirect amygdala-dorsal striatum pathway mediates conditioned freezing: Insights on emotional memory networks. *Neuroscience, 153,* 84-94.

Flavell, C. R. & Lee, J. L. C. (2012). Post-training unilateral amygdala lesions selectively impair contextual fear memories. *Learning and Memory, 19,* 256-263.

Flood, J. F., Bennett, E. L., Orme, A. E., Rosenzweig, M. R., & Jarvik, M. E. (1978). Memory: Modification of anisomycin-induced amnesia by stimulants and depressants. *Science, 199,* 324-326.

Gabriele, A. and Packard, M.G. (2006). Evidence of a role for multiple memory systems in behavioral extinction. *Neurobiology of Learning and. Memory, 85,* 289-299.

Galliot, E., Levaillant, M., Beard, E., Millot, J-L., & Pourie, G. (2010). Enhancement of spatial learning by predator odor in mice: Involvement of amygdala and hippocampus. *Neurobiology of Learning and Memory, 93,* 196-202.

Gibbs, M. E., Hutchinson, D. S., & Summers, R. J. (2010). Noradrenaline release in the locus coeruleus modulates memory formation and consolidation; roles for α-and β-adrenergic receptors. *Neuroscience, 170,* 1209-1222.

Gold, P. E., & McGaugh, J. L. (1975). A single-trace, two-process view of memory storage processes. In *Short Term Memory* (pp. 355-378). New York: Academic.

Gold, P. E., & van Buskirk, R. (1975). Facilitation of time-dependent memory processes with posttrial epinephrine injections. *Behavioral Biology, 13,* 145-153.

Goldstein, L. E., Rasmusson, A. M., Bunney, B. S., & Roth, R. H. (1996). Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *Journal of Neuroscience, 16,* 4787-4798.

Goodman, J., Leong, K. C., & Packard, M. G. (2012). Emotional modulation of multiple memory systems: Implications for the neurobiology of post-traumatic stress disorder. *Reviews in Neurosciences, 23,* 627-643.

Gorzalka, B. B., Hanson, L. A., & Brotto, L. A. (1998). Chronic stress effects on sexual behavior in male and female rats: Mediation by 5-HT_{2A} receptors. *Pharmacology Biochemistry and Behavior, 61,* 405-412.

Griffith, C. R. (1919). A possible case of instinctive behavior in the white rat. *Science, 50,* 166-167.

Griffith, C.R .(1920). The behavior of white rats in the presence of cats. *Psychobiology, 2,* 19-28.

Gutiérrez-García, A. G., & Contreras, C. M. (2013). Anxiety: An adaptive emotion. In Dr. Federico Durbano (Ed.), *New Insights into Anxiety Disorders*. InTech.

Hacquemand, R., Jacquot, L., & Brand, G. (2012). Postnatal exposure to predator odor (TMT) enhances spatial learning in mice adulthood. *Behavioural Brain Research, 229,* 113-117.

Hagewoud, R., Bultsma, L. J., Barf, R. P., Koolhaas, J. P., & Meerlo, P. (2011). Sleep deprivation impairs contextual fear conditioning and attenuates subsequent behavioural, endocrine, and neuronal responses. *Journal of Sleep Research, 20,* 259-266.

Handley, S. L., & Mithani, S. (1984). Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn Schmiedebergs Archives of Pharmacology, 327,* 1-5.

Hatfield, T., & McGaugh, J. L. (1999) Norepinephrine infused into the basolateral amygdala posttraining enhances retention in a spatial water maze task. *Neurobiology of Learning and Memory, 91,* 232-239.

Hawley, W. R., Grissom, E. M., & Dohanich, G. P. (2011). The relationships between trait anxiety, place recognition memory, and learning strategy. *Behavioural Brain Research, 216,* 525-530.

Hawley, W. R., Grissom, E. M., Patel, J. M., Hodges, K. S., & Dohanich, G. P. (2013). Reactivation of an aversive memory modulates learning preference in male rats. *Stress, 16,* 73-86.

Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: Central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in Neurosciences, 20,* 78-84.

Holahan, M. R. & White, N. M. (2002). Conditioned memory modulation, freezing, and avoidance as measures of amygdala-mediated conditioned fear. *Neurobiology of Learning and Memory, 77,* 250-275.

Holahan, M R., & White, N. M. (2013). Memory enhancement produced by post-training exposure to sucrose-conditioned cues. *F1000Research* 2013, 2:22.

Hotsenpillar, G. & Williams, J. L. (1997). A synthetic predator odour (TMT) enhances conditioned analgesia and fear when paired with a benzodiazepine receptor inverse agonist (FG-7142). *Psychobiology, 25,* 83-88.

Hull, C. L. (1943). Principles of Behavior. New York: Appleton-Century-Crofts.

Iwata, J., Chida, K., & LeDoux, J. E. (1987). Cardiovascular responses elicited by stimulation of neurons in the central amygdaloid nucleus in awake but not anesthetized rats resemble conditioned emotional responses. *Brain Research, 418,* 183-188.

Joels, M., Pu, Z., Wiegert, O., Oitzl, M. S., & Krugers, H. J. (2006). Learning under stress: How does it work? *Trends in Cognitive Sciences, 10,* 152-158.

Karten, Y. J. G., Nair, S. M., van Essen, L., Sibug, S., & Joels, M. (1999). Long-term exposure to high corticosterone levels attenuates serotonin responses in rat hippocampus CA1 neurons. *Proceedings of the National Academy of Sciences, 96,* 13456-13461.

Kessner, R. P., & Wilburn, M. W. (1974), A review of electrical stimulation of the brain in context of learning and retention. *Behavioral Biology, 10,* 259-293.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256,* 675-677.

Kim, J. J., Koo, J. W., Lee, H. J., & Han, J. S. (2005). Amygdalar inactivation blocks stress-induced impairments in hippocampal long-term potentiation and spatial memory. *The Journal of Neuroscience, 25,* 1532-1539.

Kim, J. J., Lee, H. J., Han, J. S., & Packard, M. G. (2001). Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. *The Journal of Neuroscience, 21,* 5222-5228.

Kim, M., & Davis, M. (1993). Lack of a temporal gradient of retrograde amnesia in rats with amygdala lesions assessed with the fear-potentiated startle paradigm. *Behavioral Neuroscience, 107,* 1088-1092.

Klein, S. B., Cosmides, L., Tooby, J., & Chance, S. (2002). Decisions and the evolution of memory: Multiple systems, multiple functions. *Psychological Review, 109,* 306-329.

Kluver, H. & Bucy, P. C. (1937). "Psychic blindess" and other symptoms following bilateral temporal lobectomies in rhesus monkeys. *American Journal of Physiology, 119,* 352-353.

Kluver, H. & Bucy, P. C. (1939). Preliminary analysis of the functions of the temporal lobes in monkeys. *Archives of Neurological Psychiatry, 42,* 979-1000.

Kopin, I. J. (1995). Definitions of stress and sympathetic neuronal responses. *Annals of the New York Academy of Sciences, 771,* 19-30.

Korte, S. M. (2001). Corticosteroids in relation to fear, anxiety, and psychopathology. *Neuroscience Biobehavioral Research, 25,* 117-142.

Kovacs, G. L., Telegdy, G., & Lissak, K. (1977). Dose-dependent action of corticosteroids on brain serotonin content and passive avoidance behavior. *Hormones and Behavior, 8,* 155-165.

LeDoux, J. E. (1993). Emotional memory systems in the brain. *Behavioural Brain Research, 58,* 69-79.

LeDoux, J. E. (1995). Emotion: Clues from the brain. *Annual Review of Psychology, 46,* 209-235.

Lee, A. S., Duman, R. S., & Pittenger, C. (2008). A double dissociation revealing bidirectional competition between striatum and hippocampus during learning. *Proceedings of the National Academy of Sciences, 105,* 17163-17168.

Lemon, N., Aydin-Abidin, S., Funke, K., & Manahan-Vaughan, D. (2009). Locus coruleus activation facilitates memory encoding and induces hippocampal LTD that depends on β-adrenergic receptor activation. *Cerebral Cortex,* bhp065.

Leong, K. C., Goodman, J., & Packard, M. G. (2012). Buspirone blocks the enhancing effect of the anxiogenic drug RS 79948-197 on consolidation of habit memory. *Behavioural Brain Research, 234,* 299-302.

Leong, K. C. & Packard, M. G. (2014). Exposure to predator odor influences the relative use of multiple memory systems: Role of basolateral amygdala. *Neurobiology of Learning and Memory, 109,* 56-61.

Liang, K. C., Juler, R. G., & McGaugh, J. L. (1996). Modulating effects of post-training epinephrine on memory: Involvement of the amygdala noradrenergic system. *Brain Research, 368,* 125-133.

Liang, K. C., McGaugh, J. L., & Yao, H.-Y. (1990). Involvement of amygdala pathways in the influence of post-training intra-amygdala norepinephrine and peripheral epinephrine on memory storage. *Brain Research, 508,* 255-233.

Lupien, S. J., Gillin, C. J., & Haugher, R. L. (1999). Working memory is more sensitive than declarative memory to the acute effects of corticosteroids: A dose-response study in humans. *Behavioral Neuroscience, 113,* 420-430.

Lupien, S. J., & McEwen, B. S. (1997). The acute effects of corticosteroids on cognition: Integration of animal and human model studies. *Brain Research Reviews, 24,* 1-27.

Maheu, F. S., Joober, R., Beaulieu, S., & Lupien, S. J. (2004). Differential effects of adrenergic and corticosteroid hormonal systems on human short- and long-term declarative memory for emotionally arousing material. *Behavioral Neuroscience, 118,* 420-428.

Mahut, H. (1985). Dissociations of two behavioral functions in the monkey after early hippocampal ablations. In B. E. Will, P. Schmitt, & J. C. Dalrymple-Alford (Eds.). *Brain plasticity, learning, and memory* (pp. 353-362). New York: Plenum Press

Maier, S. F., & Seligman, M. E. (1976). Learned helplessness: Theory and evidence. *Journal of Experimental Psychology, 105,* 3-46.

Malin, E. L. & McGaugh, J. L. (2006). Differential involvement of the hippocampus, anterior cingulate cortex, and basolateral amygdala in memory for context and footshock. *Proceedings of the National Academy of Sciences, 103,* 1959-1963.

Maren, S., Aharonov, G., & Fanselow, M. (1996). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: Absence of a temporal gradient. *Behavioral Neuroscience, 110,* 718-726.

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and Pavolovian fear conditioning in rats. *Behavioural Brain Research, 88,* 261-274.

Maren, S. & Fanselow, M. S. (1996). The amygdala and fear conditioning: Has the nut been cracked? *Neuron, 16,* 237-240.

Maren, S. (2001). Neurobiology of pavlovian fear conditioning. *Annual Review in Neurosciences, 24,* 897-931.

Maren, S. (2014). Fear of the unexpected: Hippocampus mediates novelty-induced return of extinguished fear in rats. *Neurobiology of Learning and Memory, 108,* 88-95.

McDonald, R. J., & White, N. M. (1993). A triple dissociation of memory systems: Hippocampus, amygdala, and dorsal striatum. *Behavioral Neuroscience, 107,* 3-22.

McDonald, R. J., & White, N. M. (1994). Parallel information processing in the water maze: Evidence for independent memory systems involving dorsal striatum and hippocampus. *Behavioral and Neural Relay, 61,* 260-270.

McEwen, B. S., & Sapolsky, R. M. (1995). Stress and cognitive function. *Current Opinion in Neurobiology, 5,* 205-216.

McGaugh, J. L. (2000). Memory – a century of consolidation. *Science, 287,* 248-251.

McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience, 27,* 1-28.

McIntyre, C. K., Roozendaal, B., & McGaugh, J. L. (2004). Glucocorticoid treatment enhances training-induced norepinephrine release in the amygdala. *Society for Neuroscience Abstract,* 772.12 online. Available at http://sfn.scholarone.com/itin2004/

Medina, A. C., Charles, J. R., Espinoza-Gonzalez, V., Sanchez-Resendis, O., Prado-Alcala, R. A., Roozendaal, B., & Quirarte, G. L. (2007). Glucocorticoid administration into the dorsal striatum facilitates memory consolidation of inhibitory avoidance training but not of the context or footshock components. *Learning and Memory, 14,* 673-677.

Messier, C. & White, N. M. (1984). Contingent and non-contingent actions of sucrose and saccharin reinforcers: Effects of taste preference and memory. *Physiology & Behavior, 32,* 195-203.

Milner, B., Corkin, S., & Teuber, H. L. (1968). Further analysis of the hippocampal amnesic syndrome: 14-year follow-up study of H.M. *Neuropsychologia, 6, 215-234*.

Mishkin, M. & Petri, H. L. (1984). Memories and habits: Some implications for the analysis of learning and retention. *In* Squire, L. R. & Butters, N. (Eds.), *Neuropsychology of memory (pp. 287-296*). New York: Guilford.

Mishkin, M. (1954). Visual discrimination performance following partial ablations of the temporal lobe: II. Ventral surface vs. hippocampus. *Journal of Comparative and Physiological Psychology, 47,* 187-193.

Mitchell, D., Osborne, E. W., & O'Boyle, M. W. (1985). Habituation under stress: Shocked mice show nonassociative learning in T-maze. *Behavioral Neural Biology, 43,* 212-217.

Mitchell, J. A., & Hall, G. (1988). Caudate-putamen lesions in the rat may impair or potentiate maze learning depending on the availability of stimulus cues and relevance of response cues. *The Quarterly Journal of Experimental Psychology, 40,* 243-258.

Morris, R. G. M., Anderson, E., Lynch, G. S. & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature, 319,* 774-776.

Morrow, B. A., Elsworth, J. D., & Roth, R. H. (2002). Fear-like biochemical and behavioral responses in rats to the predator odor, TMT, are dependent on the exposure environment. *Synapse, 46,* 11-18.

Morrow, B. A., Roth, R. H., & Elsworth, J. D. (2000). TMT, a predator odor, elevates mesoprefrontal dopamine metabolic activity and disrupts short-term working memory. *Brain Research Bulletin, 52,* 519-523.

Muller, M. & Fendt, M. (2006). Temporary inactivation on the medial and basolateral amygdala differentially affects TMT-induced fear behavior in rats. *Behavioural Brain Research, 167,* 57-62.

Newcomb, M. D. & Bentler, P. M. (1988). Impact of adolescent drug use and social support on problems of young adults: A longitudinal study. *Journal of Abnormal Psychology, 97,* 64-75.

O'Keefe, J., & Conway, D. H. (1978). Hippocampal place cells in the freely moving rat: Why they fire where they fire. *Experimental Brain Research, 31,* 573-590.

O'Keefe, J., & Nadel, L. (1978). *The hippocampus as a cognitive map.* Oxford, England: Clarendon Press.

Oitzl, M. S., & de Kloet, E. R. (1992). Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. *Behavioral Neuroscience, 108,* 62-71.

Oitzl, M. S., & de Kloet, E. R. (1992). Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. *Behavioral Neuroscience, 108,* 62-71.

Okuda, S., Roozendaal, B., & McGaugh, J. L. (2004). Glucocorticoid effects on object recognition memory require training-associated emotional arousal. *Proceedings of the National Academy of Sciences, 101,* 853-858.

Olton, D. S., Becker, J. T., & Handelmann, G. E. (1979). Hippocampus, space, and memory. *Behavioral and Brain Sciences, 2,* 313-365.

Orbach, J., Milner, B., & Rasmussen, T. (1960). Learning and retention in monkeys after amygdala-hippocampus resection. *Archives of Neurology, 3,* 230-251.

Packard, M. G., Cahill, L., & McGaugh, J.L. (1994). Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory systems. *Proceedings of the Natural Academy of Sciences, 91,* 8744-8481.

Packard, M. G. & Gabriele, A. (2009). Peripheral anxiogenic drug injections differentially affect cognitive and habit memory: Role of basolateral amygdala. *Neuroscience* 164: 457-462.

Packard, M. G. & Goodman, J. (2012). Emotional arousal and multiple memory systems in the mammalian brain. *Frontiers in Behavioral Neuroscience, 6,* 1-9.

Packard, M. G., Hirsh, R., White, N. M. (1989). Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: Evidence for multiple memory systems. *Journal of Neuroscience, 9,* 1465-1472.

Packard, M. G., McGaugh, J. L. (1996). Inactivation of hippocampus or caudate with lidocaine differentially affects expression of place and response learning. *Neurobiology of Learning and Memory, 65,* 65-72.

Packard, M. G. & Teather, L. A. (1998). Amygdala modulation of multiple memory systems: Hippocampus and caudate putamen. *Neurobiology of Learning and Memory, 69,* 163-203.

Packard, M. G. & Wingard, J. C. (2004). Amygdala and "emotional" modulation of the relative use of multiple memory systems. Neurobiology of Learning and Memory. *82*, 243-252.

Packard, M. G. (1999). Glutamate infused posttraining into the hippocampus or caudate-putamen differentially strengthens place and response learning. *Proceedings of the National Academy of Sciences, 96,* 12881-12886.

Packard, M. G. (2009). Exhumed from thought: Basal ganglia and response learning in the plus-maze. *Behavioural Brain Research, 199,* 24-31.

Pagani, J. H. & Rosen, J. B. (2009). The medial hypothalamic defensive circuit and 2,5-dihyro-2,4,5-trimathylthiazoline (TMT) induced fear: Comparison of electrolytic and neurotoxic lesions. *Brain Research, 1286,* 133-146.

Park, C. R., Campbell, A. M., & Diamond, D. M. (2002). Chronic psychosocial stress impairs learning and memory and increases sensitivity to yohimbine in adult rats. *Biological Psychiatry,15,* 994-1004.

Park, C. R, Zoladz, P. R., Conrad, C. D., Fleshner, M., & Diamond, D. M. (2008). Acute predator stress impairs the consolidation and retrieval of hippocampal-dependent memory in male and female rats. *Learning & Memory, 15,* 271-280.

Pavlov, I. P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex.* London: Oxford University Press.

Petrovich, G. D., Canteras, N. S. & Swanson, L. W. (2001). Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Research Reviews, 38,* 247-289.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behavioral Neuroscience, 106,* 274-285.

Pitkanen, A., Pikkarainen, M., Nurminen, N., & Ylinen, A. (2006). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat: A review. *Annals of the New York Academy of Sciences, 911,* 369-391.

Poldrack, R. A., & Packard, M. G. (2003). Competition among multiple memory systems: Converging evidence from animal and human brain studies. *Neuropsychologia, 41,* 245-251.

Pugh, C. R., Tremblay, D., Fleshner, M., & Rudy, J. W. (1997). A selective role for corticosterone in contextual-fear conditioning. *Behavioral Neuroscience, 111,* 503-511.

Quirarte, G. L., de la Teja, I. S. L., Casillas, M., Serafin, N., Prado-Alcala, R. A., & Roozendaal, B. (2009). Corticosterone infused into the dorsal striatum selectively enhances memory consolidation of cued water-maze training. *Learning and Memory, 16,* 586-589.

Quirarte, G. L., Roozendaal, B., & McGaugh, J. L. (1997). Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences, 94,* 14048-14053.

Reul, J. M. H. M. & de Kloet, E. R. (1985). Two receptor systems for corticosterone in rat brain: Microdistribution and differential occupation. *Endocrinology, 117,* 2505-2511.

Roozendaal, B., Carmi, O., & McGaugh, J. L. (1996). Adrenocortical suppression blocks the memory-enhancing effects of amphetamine and epinephrine. *Proceedings of the National Academy of Sciences, 93,* 1429-1433.

Roozendaal, B., Griffith, Q. K., Buranday, J., de Quervain, D. J. F., & McGaugh, J. L. (2003). The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: Dependence on basolateral amygdala. *Proceedings of the National Academy of Sciences, 100,* 1328-1333.

Roozendaal, B., Hahn, E. L., Nathan, S. V., de Quervain, D. J. F., & McGaugh, J. L. (2004). Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala. *Journal of Neuroscience, 24,* 8161-8169.

Roozendaal, B., Koolhaas, J. M., & Bohus, B. (1991). Central amygdala lesions affect behavioral and autonomic balance during stress in rats. *Physiological Behavior, 50,* 777-781.

Roozendaal, B., McEwen, B. S., & Chattarji, S. (2009). Stress, memory and the amygdala. *Nature Reviews Neuroscience, 10,* 423-433

Roozendaal, B., & McGaugh, J. L. (1996a). Amygdaloid nuclei lesions differentially affect glucocorticoid-induced memory enhancement in an inhibitory avoidance task. *Neurobiology of Learning and Memory, 65,* 1-8.

Roozendaal, B., & McGaugh, J. L. (1996b). The memory modulatory effects of glucocorticoids depend on an intact stria terminalis. *Brain Research, 709,* 243-250.

Roozendaal, B., & McGaugh, J. L. (1997a). Basolateral amygdala lesions block the memory-enhancing effects of glucocorticoid administration in the dorsal hippocampus of rats. *European Journal of Neuroscience, 9,* 76-83.

Roozendaal, B., & McGaugh, J. L. (1997b). Glucocorticoid receptor agonist and antagonist administration into the basolateral amygdala but not central amygdala modulates memory storage. *Neurobiology of Learning and Memory, 67,* 176-179.

Roozendaal, B., Okuda, S., de Quervain, D. J. F., & McGaugh, J. L. (2006). Glucocorticoids interaction with emotion-induced noradrenergic activation in influencing different memory functions. *Neuroscience, 138,* 901-910.

Roozendaal, B., Okuda, S., Van der Zee, E. A., & McGaugh, J. L. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences, 103,* 6741-6746.

Roozendaal, B., Quirarte, G. L., & McGaugh, J. L. (2002). Glucocorticoids interact with the basolateral amygdala β-adrenoceptor-cAMP/PKA system in influencing memory consolidation. *European Journal of Neuroscience, 15,* 553-560.

Roozendaal, B., Williams, C. L., & McGaugh, J. L. (1999). Glucocorticoid receptor activation in the rat nucleus of the solitary tract facilitates memory consolidation: Involvement of the basolateral amygdala. *European Journal of Neuroscience, 11,* 1317-1323.

Roozendaal, B. (2000). Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrinology, 25,* 213-238.

Rosen, J. B. (2004). The neurobiology of conditioned and unconditioned fear: A neurobehavioral system analysis of the amygdala. *Behavioral and Cognitive Neurosciences, 3,* 23-41.

Sage, J. R. & Knowlton, B. J. (2000). Effects of US-devaluation on win-stay and win-shift radial maze performance in rats. *Behavioral Neuroscience, 114,* 295-306.

Sanders, S. K., & Shekhar, A. (1991). Blockade of GABA-A receptors in the region of the anterior basolateral amygdala of rats elicits increases in heart rate and blood pressure. *Brain Research, 567,* 101-110.

Sandi, C., Loscertales, M., & Guaza, C. (1997). Experience-dependent facilitating effect of corticosterone on spatial memory formation in the water maze. *European Journal of Neuroscience, 9,* 637-642.

Sandi, C., & Rose, S. P. R. (1994). Corticosterone enhances long-term potentiation in one-day-old chicks trained in a weak passive avoidance learning paradigm. *Brain Research, 647,* 106-112.

Schwabe, L., Dalm, S., Schachinger, H., & Oitzl, M. (2008). Chronic stress modulates the use of spatial and stimulus-response learning strategies in mice and man. *Neurobiology of Learning and Memory, 90,* 495-503.

Schwabe, L., Dickinson, A., & Wolf, O. T. (2011). Stress, habits, and drug addiction: A psychoneuroendocrinological perspective. *Experimental and Clinical Psychopharmacology, 19,* 53-63.

Schwabe, L., Oitzl, M., Philippsen, C., Richter, S., Bohringer, A., Wippich, W., & Schachinger, H. (2007). Stress modulates the use of spatial and stimulus-response learning strategies in humans. *Learning and Memory, 14,* 109-116.

Schwabe, L., Tegenthoff, M., Hoffken, O., & Wolf, O. T. (2010). Concurrent glucocorticoid and noradrenergic activity shifts instrumental behavior from goal-directed to habitual control. *Journal of Neuroscience, 30,* 8190-8196.

Schwabe, L., & Wolf, O. T. (2013). Stress and multiple memory systems: From 'thinking' to 'doing'. *Trends in Cognitive Sciences, 17,* 60-68.

Scoville, W.B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery and Psychiatry, 20, 11-21.*

Sherry, D. F., & Schacter, D. L. (1987). The evolution of multiple memory systems. *Psychological Reviews, 94,* 439-454.

Shors, T. J. (2006). Stressful experience and learning across the lifespan. *Annual Review of Psychology, 57,* 55-85.

Smith, M. & McDougall, W. M. (2011). Some experiments in learning and retention. *British Journal of Psychology, 10,* 199-209

Squire, L. R. (2004). Memory systems of the brain: A brief history and current perspective. *Neurobiology of Learning and Memory, 82,* 171-177.

Stone, E. A., Egawa, M., & McEwen, B. S. (1988). Novel effects of chronic corticosterone treatment on escape behavior in rats. *Behavioral and Neural Biology, 50,* 120-125.

Sullivan, G. M., Coplan, J. D., Kent, J. M., & Gorman, J. M. (1999). The noradrenergic system in pathological anxiety: A focus on panic with relevance to generalized anxiety and phobias. *Biological Psychiatry, 46,* 1205-1218.

Takahashi, L. K., Chan, M.M., & Pilar, M. L (2008). Predator odor fear conditioning: Current perspectives and new directions. *Neuroscience and Biobehavioral Reviews, 32,* 1218-1227.

Takahashi, L. K., Nakashima, B. R., Hong, H., & Watanabe, K. (2005). The smell of danger: A behavioral and neural analysis of predator odor-induced fear. *Neuroscience and Biobehavioral Reviews, 29,* 1157-1167.

Thompson, M.E., & Thompson, J.P. (1949). Reactive inhibition as a factor in maze learning: II. The role of reactive inhibition in studies of place learning versus response learning. *Journal of Experimental Psychology* 39: 883-891.

Tricomi, E., Balleine, B. W., & O'Doherty, J. P. (2009). A specific role for posterior dorsolateral striatum in human habit learning. *European Journal of Neuroscience, 29,* 2225-2232.

Valentino, R. J. & Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as adaptive response to stress. *European Journal of Pharmacology, 583,* 194-203.

Van Bockstaele, E. J., Colago, E. E. O., & Valentino, R. J. (1998). Amygdaloid corticotropin-releasing factor targets locus coeruleus dendrites: Substrate for the co-ordination of emotional and cognitive limbs of the stress response. *Journal of Neuroendocrinology*, *10*, 743-758.

VanElzakker, M. B., Zoladz, P. R., Thompson, V. M., Park, C. R., Halonen, J. D., Spencer, R. L., & Diamond, D. M. (2011). Influence of pre-training predator stress on the expression of c-fos mRNA in the hippocampus, amygdala, and striatum following long-term spatial memory retrieval. *Frontiers in Behavioral Neuroscience, 5,* 1-13.

Vernet-Maury, E., Polak, E. H., & Demael, A. (1984). Structure-activity relationship of stress inducing odorants in the rat. *Journal of Chemical Ecology, 10,* 1007-1018.

Vernet-Maury, E. (1980). Trimethyl-thiazoline in fox feces: A natural alarming substance for the rat. In H. van der Starre (Ed.), *Olfaction and taste.* Washington DC: IRL Press.

Viaud, M. D. & White, N. M. (1987). Dissociation of visual and olfactory conditioning in the neostriatum of rats. *Society for Neuroscience Abstracts, 13,* 645.

Vyas, A., Mitra, R., Shankaranarayana Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *Journal of Neuroscience, 22,* 6810-6818.

Wallace, B. C. (1989). Psychological and environmental determinants of relapse in crack cocaine smokers. *Journal of Substance Abuse Treatment, 6,* 95-106.

Wallace, K. J. & Rosen, J. B. (2001). Neurotoxic lesions of the lateral nucleus of the amygdala decrease conditioned fear but not unconditioned fear of a predator odor: Comparison with electrolytic lesions. *Journal of Neuroscience, 21,* 3619-3627.

Wang, M. E., Fraize, N. P., Yin, L., Yuan, R. K., Petsagourakis, D., Wann, E. G., & Muzzio, I. A. (2013). Differential roles of the dorsal and ventral hippocampus in predator odor fear conditioning. *Hippocampus, 23,* 451-466.

Watson, J. B., & Rayner, R. (1920). Conditioned emotional reactions. *Journal of Experimental Psychology, 3,* 1-14.

Weiskratz, L. (1956). Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *Journal of Computational Physiological Psychology, 49,* 381-391.

White, D. A. & Birkle, D. L. (2001). The differential effects of prenatal stress in rats on the acoustic startle reflex under baseline conditions and in response to anxiogenic drugs. *Psychopharmacology, 154,* 169-176.

White, N. M., & McDonald, R. J. (2002). Multiple parallel memory systems in the brain of the rat. *Neurobiology of Learning and Memory, 77*, 125–184.

White, N. M., Packard, M. G., & McDonald, R. J. (2013). Dissociation of memory systems: The story unfolds. *Behavioral Neuroscience, 127,* 813-834.

White, N. M. & Salinas, J. A. (2003). Mnemonic functions of dorsal striatum and hippocampus in aversive conditioning. *Behavioural Brain Research, 142,* 99-107.

White, N. M. (1996). Addictive drugs as reinforcers: Multiple partial actions on memory systems. *Addiction, 91,* 921-950.

Williams, C. L., & Clayton, E. C. (2001). Contribution of brainstem structures in modulating memory storage processes. In: P.E. Gold, & W.T. Greenough (Ed.), *Memory consolidation: Essays in honor of James L. McGaugh* (pp. 141-163). Washington, D.C.: American Psychological Association.

Williams, J. L., Baez, C., Hladky, K. J., & Camacho, C. A. (2005). Effects of a synthetic predator odor (TMT) on freezing, analgesia, stereotypy, and spatial memory. *The Psychological Record, 55,* 3-38.

Williams, J. L., Baker, S. L., Gress, J. E., & Givens, B. (1998). Effects of predator-induced stress and age on working memory in rats (Rattus norvegicus). *The Psychological Record, 48,* 355-372.

Wingard, J. C & Packard, M. G. (2008). The amygdala and emotional modulation of competition between cognitive and habit memory. *Behavioral Brain Research, 193,* 126-131.

Wolf, O. T., Schommer, N. C., Hellhammer, D. H., McEwen, B. S., & Kirschbaum, C. (2001). The relationship between stress induced cortisol levels and memory differs between men and women. *Psychoneuroendocrinology, 26,* 711-720.

Woodson, J. C., Macintosh, D., Fleshner, M. & Diamond, D. M. (2003). Emotion-induced amnesia in rats: Working memory-specific impairment, corticosterone-memory correlation, and fear versus arousal effects on memory. *Learning and Memory, 10,* 326-336.

Young, S. L., & Fanselow, M. S. (1992). Associative regulation of Pavlovian fear conditioning: Unconditioned stimulus intensity, incentive shifts, and latent inhibition. *Journal of Experimental Psychology: Animal Behavior Processes, 18,* 400-413.

Zoladz, P. R., Woodson, J. C., Hayes, V. F. & Diamond, D. M. (2010). Activation of a remote (1-year old) emotional memory interferes with the retrieval of a newly formed hippocampus-dependent memory in rats. *Stress, 13,* 36-52.

Zola-Morgan, S. & Squire, L. R. (1985). Complementary approaches to the study of memory: Human amnesia and animal models. In N. W. Weinberger, J. L. McGaugh, & G. Lynch (Eds.). *Memory systems of the brain: Animal and human cognitive process* (pp. 463-477). New York: Guilford Press.