Out-of-Context Activation of Memory:

Limits of Stress-Induced Memory Enhancement

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PREFACE

Dedication

Acknowledgements

ABSTRACT

Posttraumatic stress disorder (PTSD) can be a sequela of a traumatic event that elicits an extremely fearful reaction, and is accompanied by re-experiencing of the trauma, numbing or avoidance behavior, and persistent hyperarousal. A signature feature of PTSD is the recurrent, involuntary, and intrusive recollection of a traumatic memory, colloquially referred to as flashbacks, which occur outside of the original experience. Whether an aberrant stress response is a contributing factor to eliciting these intrusive memories, our rodent studies suggest that the response to a stressful experience can activate previously acquired memories in a context that is unrelated to the original learning situation. This observation, which we call 'out-of-context activation of memory' (OCAM), may be a useful model with which to study how an extreme stress response can influence unrelated memories, and that the subsequent modification of these unrelated memories may interfere with normal functioning and contribute to the behavioral alterations and disturbances that characterize disorders such as PTSD.

Our previous work reported that after a swim-stress experience, the expression of an unrelated memory was enhanced. The learning environment and the swim environment had no physically identifiable common feature, and yet swim-stress, conducted one day after learning a left/right (L/R) discrimination task, enhanced the subsequent recall of the L/R discrimination task. In addition, swim-stress induced a stable memory to become susceptible to amnestic treatments such as propranolol and electro-convulsive shock. Taken together, this evidence suggests that the stress response to an adverse situation can modify stable, unrelated memories.

The goal of my work was to evaluate and characterize the limitations of a stress-induced modification of stable, unrelated memories. There four main findings: (1) the level of physiological stress was not a common feature between the learning and swim-stress experience, (2) corticosteroids play a necessary but not sufficient role in enhancing the expression of a stable, unrelated memory, (3) swim-stress can enhance the expression of a stable, unrelated memory within a time-limited window of up to at least one

week after learning, and (4) swim-stress does not enhance memories that are dependent on the hippocampus for its expression.

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Abbreviations

ACTH	Adrenocorticotropic Hormone
AFC	Auditory Fear Conditioning
AMPAR	α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptor
BDNF	Brain-Derived Neurotrophic Factor
BLA	Basolateral Amygdala
CBG	Corticosteroid-Binding-Globulin
CBT	Cognitive-Behavioral Treatments
CFC	Contextual Fear Conditioning
CS	Conditioned Stimulus
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
ECS	Electro-convulsive Shock
ELISA	Enzyme-linked Immunosorbant Assay
ERK	Extracellular Signal-Regulated Kinase
GR	Glucocorticoid Receptor
HPA	Hypothalamic-Pituitary-Adrenal
HPC	Hippocampus
IA	Inhibitory Avoidance
IHT	Interhemispheric Transfer
IP	Intraperitoneal
L/R	Left/Right
mEPSC	miniature Excitatory Postsynaptic Current
MR	Mineralocorticoid Receptor
OCAM	Out-of-Context Activation of Memory
PSA	Population Spike Amplitude
RIA	Radioactive Immunoassay
SEM	Standard Error of the Mean
SSRI	Selective Serotonin Reuptake Inhibitor
SWR	Sharp-Wave Ripples
TTX	Tetrodotoxin
US	Unconditioned Stimulus

Pharmacology

Aldosterone	MR agonist
Cortisol	Primary corticosteroid produced in humans
Corticosterone	Primary corticosteroid produced in rodents
Dexamethasone	GR agonist
Metyrapone	11beta-hydroxylase inhibitor (leads to inhibition of corticosterone synthesis)
RU 26988	GR agonist
RU 28318	MR antagonist
RU 28362	GR agonist
RU 38486 (mifepristone)	GR antagonist, progesterone receptor antagonist
Spironolactone	MR antagonist

INTRODUCTION

1. Posttraumatic Stress Disorder

1.1. DEFINITION OF POSTTRAUMATIC STRESS DISORDER

Classified as a trauma- and stressor-related disorder in the recently revised Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), posttraumatic stress disorder (PTSD) is a disorder with symptoms of altered emotions and behavioral disturbances that affect 7.7 million American adults, according to recent statistics from NIMH. Stress plays a pivotal role in causing some patients to re-experience a traumatic event, and although the precise mechanisms by which PTSD develops remain unknown, it is the memory of the event and the emotional reactions to that memory that underlie PTSD (Rubin et al, 2008). Subsequent, and continued, recall of the stressful memory causes some patients to respond and adapt to seemingly neutral circumstances in maladaptive ways. Selective serotonin reuptake inhibitors (SSRIs) (Stein et al, 2006) and cognitive-behavioral treatments (CBT) (Foa and Meadows, 1997) have shown some efficacy in blocking the emotional spiral and mitigating the symptoms. However, an Institute of Medicine report concluded that the only treatment for PTSD with sufficient empirical evidence demonstrating efficacy was exposure therapy, a type of behavior therapy in which the patient is exposed to the feared object and context in a controlled, safe environment to aid the patient to overcome his or her anxiety or fear (IOM, 2008), a paradigm similar to that of fear extinction in rodent research. Nonetheless, it is clear that PTSD remains a difficult disorder to treat as current medications and therapies are inadequate for many individuals with the disorder, and new treatments are currently being investigated (Cukor et al, 2009). Much of the current basic science research efforts on PTSD focus on the response to fear and its impact on the formation, storage, and extinction of fear memory. Popular animal models, including fear conditioning and inhibitory avoidance, require re-exposure to the initial learning conditions that act as a reminder to reactivate the acquired memory. However, a defining characteristic of PTSD is the recurrent, involuntary, and intrusive recollection of a traumatic memory, which is colloquially referred to as flashbacks. Currently, none of the animal models

that are used investigate an “out-of-context” trigger of an acquired memory. The proposed rodent studies are based on the surprising finding that stress hormones, in response to a stressful experience, can activate stable memories in a context that is unrelated to the learning situation, a phenomenon we call 'out-of-context activation of memory' (OCAM).

The major elements of posttraumatic stress disorder (PTSD) include: (1) re-experiencing of the trauma through recurrent and intrusive thoughts or dreams, (2) emotional numbing or feeling detached from others and significant activities, and (3) symptoms of elevated arousal such as hypervigilance, insomnia, increased irritability, and exaggerated startle response (Black and Andreasen, 2011; Vieweg et al, 2006). Although PTSD has recently been classified as a disorder in a new chapter titled “Trauma- and Stressor-Related Disorders” in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) (American Psychiatric Association, 2013), both DSM-V and DSM Fourth Edition, Text Revision (DSM-IV-TR) (American Psychiatric Association, 2000) still describe the main features of PTSD as a disorder with symptoms of altered emotions and behavioral disturbances. Importantly, the diagnosis of PTSD is still contingent upon an exposure to a traumatic event whose severity is beyond the range of normal human experience.

1.2. HPA AXIS DYSFUNCTION IN POSTTRAUMATIC STRESS DISORDER

Individuals with PTSD display particular neuroendocrinological alterations in the hypothalamic-pituitary-adrenal (HPA) axis in addition to dysfunction in noradrenergic, serotonergic, and glutamatergic systems. The biological alterations in patients with chronic PTSD include elevated levels of circulating corticotropin-releasing hormone, norepinephrine, and thyroid hormones in addition to an increased reactivity of alpha2-adrenergic receptors and an exaggerated suppression of cortisol in response to dexamethasone administration (Yehuda, 2002). There is accumulating evidence from psychological and biological studies that support the hypothesis that a failure to contain the biological stress response at the time of the stressor or trauma facilitates the development of PTSD. As a consequence of the altered trajectory, the lack of a normal adaptation and recovery of the stress response to the traumatic event

leads to intrusive recollections of the stressful event, avoidance of any reminders of the stressful event, and symptoms of hyperarousal (Nemeroff et al, 2006; Orr et al, 2002; Pitman et al, 2012; Vieweg et al, 2006; Yehuda and LeDoux, 2007).

Within the PTSD literature, some studies have shown that circulating cortisol levels are lower as compared their counterparts without PTSD (Glover and Poland, 2002; Kanter et al, 2001; Mason et al, 1986; Yehuda et al, 1990; Yehuda et al, 1993; Yehuda et al, 1995). In contrast, other studies have reported circulating cortisol levels that are higher as compared their counterparts without PTSD (De Bellis etl al, 1999; Liberzon et al, 1999; Pitman and Orr, 1990). It has been suggested that circulating cortisol levels do not reflect the severity or the progression in developing PTSD, and as a consequence, research has shifted its focus towards the effects that altered cortisol levels have on biological systems. Two prominent theories have been proposed. The glucocorticoid toxicity hypothesis suggests that the excess levels of cortisol lead to excitotoxic neuronal death, which provide an account for the smaller hippocampal volume seen in patients with PTSD (McEwen and Sapolsky, 1995; Reagan and McEwen, 1997; Sapolsky, 1996). In contrast, the enhanced glucocorticoid receptor responsiveness hypothesis suggests that the enhanced negative feedback inhibition induces lowered cortisol levels, which allows for an exaggerated catecholamine response that “over-consolidates” the traumatic memory (Yehuda, 2001; Yehuda, 2009).

1.3. CORTICOSTEROID TOXICITY HYPOTHESIS

Direct exposure of high levels of corticosteroids, as seen in stress, was associated with damage to the hippocampus (Uno et al, 1989; Sapolsky et al, 1990). Corticosteroids can exert some of their negative effects through the accumulation of extracellular glutamate (Stein-Behrens et al, 1994) while concurrently increasing the vulnerability of hippocampal neurons to endogenously released excitatory amino acids (Sapolsky and Pusinelli, 1985; Armanini et al, 1990; Virgin et al, 1991). These insults can lead to atrophy of dendritic branching (Woolley et al, 1990; Watanabe et al 1992), structural alterations of synaptic terminals (Magariños et al, 1997), and diminished neuronal regeneration (Gould et al, 1998).

The findings of stress leading to hippocampal damage extended into clinical studies in which magnetic resonance imaging (MRI)-based measurements of patients with PTSD showed smaller hippocampal volume compared to matched control subjects (Bremner et al, 1995; Bremner et al, 1997; Stein et al, 1997). In contrast to the decrease in hippocampal volume, there was no difference in the volume of either the amygdala or the caudate in patients with PTSD as compared to that of control subjects (Bremner et al, 1997).

1.4. ENHANCED GLUCOCORTICOID RECEPTOR RESPONSIVENESS HYPOTHESIS

Some early studies that showed decreased levels of cortisol in patients with previous trauma or chronic PTSD argue against the toxicity of excess corticosteroids due to stress (Mason et al, 1986; Yehuda et al, 1991; Resnick et al, 1995; Resnick et al, 1997). As an alternative, Yehuda proposes that the HPA alterations, which are a predominant feature of PTSD, stem from an enhanced negative feedback sensitivity of the HPA axis (Yehuda, 2009). The enhanced GR responsiveness (negative feedback inhibition) is consistent with an increased cortisol suppression in response to dexamethasone (Stein et al, 1997; Yehuda, 2001; Yehuda, 2009), and combined with elevated corticotropin-releasing hormone (CRH) levels, can result in patients with PTSD to have urinary and plasma cortisol levels that are comparable or lowered as compared to control subjects (Yehuda, 2009). In particular, the insufficient levels of cortisol allows for a prolonged and distress increased catecholamine-induced arousal and that this exaggerated catecholamine response initiates a process in which traumatic memories become “over-consolidated” or inappropriately remembered due to an exaggerated level of distress (Pitman, 1989).

2. Role of Corticosteroids

2.1. CORTICOSTEROID RECEPTORS AND AGONISTS

Receptors for adrenal steroids were first identified in brain tissue, in particular the hippocampal complex, by McEwen and colleagues (McEwen et al, 1968; McEwen et al, 1969), and were initially classified as type 1 and type 2. These intracellular receptors were renamed according to their preferential binding affinities to particular agonists in reference to the main physiological processes that they regulate. In a seminal paper, Reul and de Kloet investigated the distribution of the two corticosterone receptors in the rat brain. Type 1 receptors, or mineralocorticoid receptors (MRs), regulate mineral balance whereas type 2 receptors, or glucocorticoid receptors (GRs), regulate gluconeogenesis (Reul and de Kloet, 1985). The genes that encode these receptors in the brain are identical to those found in the rest of the body that mediate their respective physiological functions (Hollenberg et al, 1985; Arriza et al, 1987). Collectively, MRs and GRs will be referred to as “corticosteroid receptors” throughout this text.

In contrast to the receptors, the main steroidal hormones that are released to mediate the physiological response to a stressor are corticosterone (in rodents) and cortisol (in humans), both of which are classified as glucocorticoids. Systemically, mineralocorticoids, primarily aldosterone, bind to MRs regulate salt and water balance whereas glucocorticoids bind to GRs and act to raise blood glucose levels, but their actions in the forebrain have little relevance to glucose metabolism. Glucocorticoids have a 100- to 1000-fold higher circulating concentration than that of mineralocorticoids, and are able to bind to MRs. However, the enzyme 11-beta hydroxysteroid dehydrogenase deactivates glucocorticoids into 11-dehydro metabolites including cortisone, which then allows mineralocorticoids to bind to MRs. The enzyme 11-beta hydroxysteroid dehydrogenase is absent in the brain, which means that glucocorticoids can bind to both MRs and GRs. To better reflect the function as well as the binding affinity of corticosterone and cortisol to both MRs and GRs, the term “corticosteroids” instead of “glucocorticoids” will be used throughout this text (Joëls et al, 2012).

The anatomical distribution of GRs and MRs within the brain differs in that MRs are largely restricted to the septal-hippocampal complex whereas GRs have a widespread distribution (Reul and de Kloet, 1985; Reul and de Kloet, 1986). Specifically, a high concentration of GRs and MRs were found in limbic regions including CA1, CA3, dentate gyrus, dorsal subiculum, and ventral hippocampus, as well as the lateral septum (Agarwal et al, 1993; Reul and de Kloet, 1985; Reul and de Kloet, 1986). Moreover, the central and cortical amygdala, locus coeruleus, nucleus tractus solitarii, nucleus paraventricularis, and raphe area expressed high concentrations of GRs but low concentrations of MRs (Reul and de Kloet, 1985). Agarwal and colleagues found a rich density of both GRs and MRs in the dorsal and ventral hippocampus, but did not find MRs in the midbrain, septum, and striatum using radiolabeled antibodies targeted against MRs (Agarwal, 1993). In contrast to the autoradiographic data, a biochemical approach detected corticosterone binding proteins in adrenalectomized rats, and these binding proteins were functional GRs capable of nuclear translocation (Defiore and Turner 1983). Additional studies found GRs (Ahima and Harlan, 1990; Morimoto et al, 1996) and MRs (Ahima et al, 1991) in the rat striatum.

Intracellular MRs in the brain (Kd ~ 0.5 kM) have a six- to ten-fold higher affinity for corticosteroids than do intracellular GRs in the brain (Kd ~ 2.5-5 kM) (de Kloet et al, 1984; Reul and de Kloet, 1985; Veldhuis et al, 1982). In hippocampal slice studies, a high dose of corticosterone (e.g., 100 nM) is sufficient to fully activate GRs and MRs (de Kloet, 1991; Karst et al, 2000). Unlike GRs, low levels of corticosteroids can saturate MRs, which is associated with small amplitudes of voltage-dependent calcium currents (Joëls et al, 2003; Karst et al, 1994; Kerr et al, 1992), and is thought to maintain an inhibitory tone of the hippocampus on HPA axis activity (Reul et al, 2000). Responding to a stressor leads to additional release of corticosteroids that activate GRs to significantly increase calcium current amplitude even as voltage-dependent and kinetic properties remain unaffected (Chameau et al, 2007), and is thought to mediate the negative feedback activity arising from the elevated corticosteroid levels (Reul et al, 2000).

2.2. STRESS LEVEL AND PERFORMANCE RELATIONSHIP

Over a century ago, Robert Yerkes and John Dodson outlined a relationship between the shock intensity and trials to criterion as a measure of how quickly mice learned a task under different levels of stress. This relationship between the level arousal and the level of performance has been referred to as the Yerkes-Dodson Law (Yerkes and Dodson, 1908) (Figure 1).

The Hebbian version of the Yerkes-Dodson Law (Figure 1), which shows an inverted U-shaped relationship between arousal and performance, has commonly represented the Yerkes-Dodson Law for the last several decades. It is possible that the persistence of this model into behavioral studies stems from electrophysiological experiments of hippocampal slices in which synaptic potentiation follows an inverted-U shaped curve with increasing corticosterone concentration (Sandi, 1998). However, the dose-response relationships depend upon the investigated brain region as the hippocampal CA1 region shows a U-shaped relationship, the dentate gyrus shows a curvilinear relationship, and the hypothalamus and raphe regions shows an inverse relationship (Joëls et al, 2009).

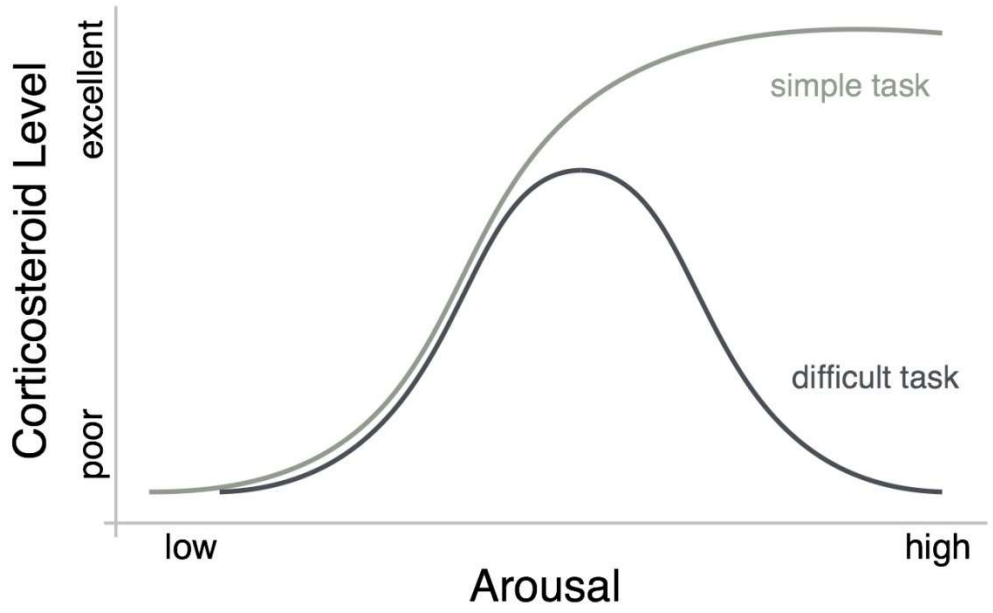


Figure 1. Yerkes-Dodson Law of arousal versus performance

Modified version of the Yerkes-Dodson Law, which is based on the findings and theorizing of Yerkes and Dodson (Yerkes and Dodson, 1908). The level of arousal correlates with the level of performance for simple tasks in a curvilinear relationship in that performance is poor at a low level of arousal, but performance improves as the level of arousal increases such that at a high level of arousal lead to a plateau of excellent performance (silver colored line for simple tasks). In contrast to simple tasks, difficult tasks show an inverted U-shaped curve in that either a low or a high level of arousal lead to poor performance, but at a moderate level of arousal lead to optimal performance (dark gray line for difficult tasks). In contrast to the original findings and theorizing of Yerkes and Dodson, the Hebbian version of the Yerkes-Dodson Law shows an inverted U-shaped curve between arousal and performance (dark gray line for difficult tasks), and it is this model that has commonly represented the Yerkes-Dodson Law for the last several decades, which seems to ignore or was unaware of the original findings and theorizing of Yerkes and Dodson (Yerkes and Dodson, 1908). Figure modified from Diamond et al, 2007.

According to the Yerkes-Dodson Law, performance on a simple task improves with increasing amounts of corticosterone until each additional increase in corticosterone results in a plateau in performance. A rise in corticosterone levels that exceed the corticosteroid binding capacity of corticosteroid-binding-globulin (CBG) enables corticosterone to cross the blood brain barrier (Breuner and Orchinik, 2002; Minni et al, 2012; Moisan et al, 2014; Qian et al, 2011), and if this elevated level of corticosterone was sustained for a sufficient duration of time (Droste et al, 2008), then corticosterone could modify behavioral performance. If a L/R discrimination task in a T-maze is comparable to a simple visual discrimination task such that there is an curvilinear relationship between corticosteroids and behavioral performance on a memory retrieval task (Diamond et al, 2007), then additional corticosterone produces one of two possibilities: (1) an outcome that does not show a trend towards an improved performance on a L/R discrimination task, which is what the findings presented in this thesis show, or (2) an outcome that shows an improved performance on a L/R discrimination task.

Conversely, also according to the Yerkes-Dodson Law, performance on a difficult task improves with increasing amounts of corticosterone until each additional increase in corticosterone results in a degradation in performance such that a high level of corticosterone produces an impairment. If a L/R discrimination task in a T-maze is comparable to a difficult visual discrimination task such that there is an inverted U-shaped relationship between corticosteroids and behavioral performance on a memory retrieval task (Diamond et al, 1992; Park et al, 2006), then additional corticosterone produces one of two possibilities: (1) an outcome that does not show a trend towards an improved performance on a L/R discrimination task, which is what the findings presented in this thesis show, or (2) an outcome that shows an impaired performance on a L/R discrimination task.

2.3. FAST AND SLOW EFFECTS OF CORTICOSTEROIDS

Classically, the effects of corticosteroids, like other steroidal hormones, involve DNA binding of receptor homodimers (Karst et al, 2000) to initiate gene-mediated pathways, a process that requires hours to days. In contrast to this classic view, early reports in the 1970s demonstrated rapid effects of corticosteroids (Edwardson and Bennett, 1974) and estrogens (Pietras and Szego, 1975). Orchinik and colleagues were one of the first groups to find evidence of membrane-bound corticosteroid receptors thereby providing a mechanism by which corticosteroids could exert their rapid, non-genomic effects (Moore and Orchinik, 1994; Orchinik et al, 1991; Orchinik et al, 1994). This membrane-bound receptor was highly specific for corticosterone and cortisol (Orchinik et al, 1991), and some considered this receptor protein to be distinct from that of intracellular GRs (Evans et al, 2000). However, these findings and others like them were largely ignored, presumably because they were conducted with brains from amphibians (Orchinik, 1998) or birds (Breuner et al, 1998). Nonetheless, evidence of non-genomic mechanisms of corticosteroid in rodents began to emerge (Sandi et al, 1996), and review articles of the non-genomic mechanisms of steroid effects followed shortly thereafter (Borski, 2000; Falkenstein et al, 2000; Makara and Haller, 2001).

Through a series of experiments conducted with rodent hippocampal slice cultures, Joëls and colleagues found that the fast, non-genomic response to rising corticosterone levels is mediated by a third type of corticosteroid receptor, a membrane-bound MR (Karst et al, 2005). Prior to this finding, it was presumed that the intracellular MRs, which have a high affinity for corticosterone, would be saturated under basal conditions, and thus the behavioral effects of corticosteroids would be mediated by GRs. These findings contributed to a model showing how the impact of stress on memory changes over time with both fast and slow effects of corticosteroids (Joëls et al, 2006).

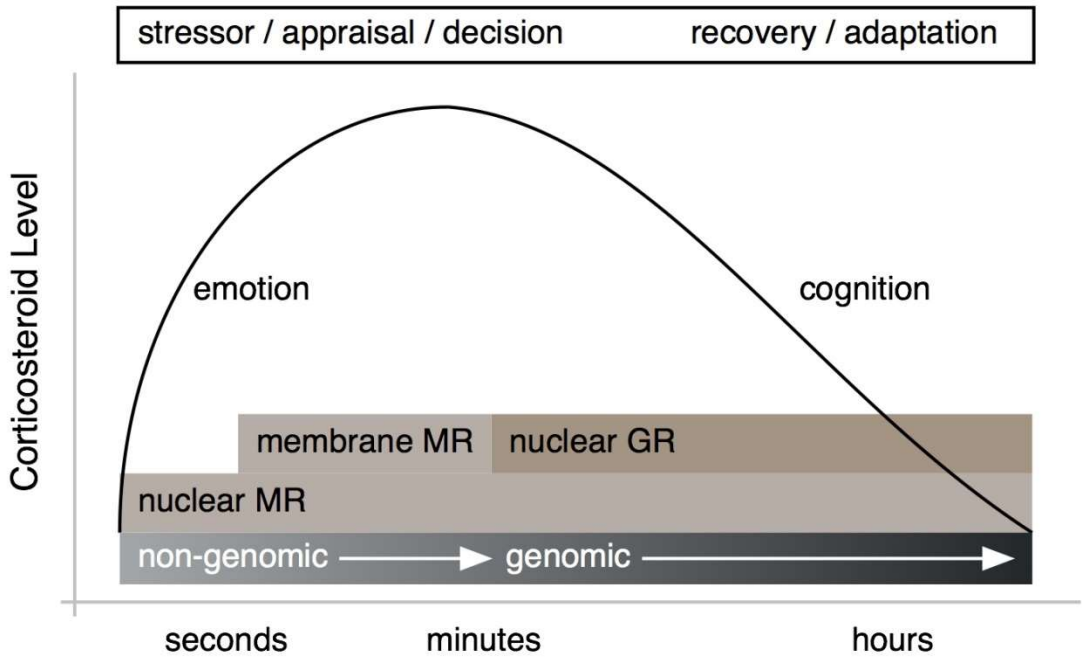


Figure 2. Fast and slow effects of corticosteroids.

During the initial phase of a stress response, corticosteroids exert their short-term effects within seconds to minutes via non-genomic pathways that are mediated by membrane-bound MRs (Karst et al, 2005). During the recovery and adaptation phase of a stress response, corticosteroids exert their delayed effects several hours after the onset of stress via genomic pathways that are mediated by intracellular GRs (de Kloet et al, 1998; de Kloet et al, 2008). Figure modified from de Kloet, 2014.

According to the fast and slow model of the effects of corticosteroids on memory (Joëls et al, 2006), the short-term effects of corticosteroids, acting via non-genomic pathways that are mediated by membrane-bound MRs (Karst et al, 2005), play a role during the initial phase of a stress response (de Kloet et al, 2008) to facilitate learning and memory formation. Shortly thereafter, the delayed effects of corticosteroids, acting via genomic pathways that are mediated by intracellular GRs, play a role in the recovery and adaptation from that stress response (de Kloet et al, 1998; de Kloet et al, 2008) to facilitate consolidation of a recently acquired memory. A temporal dynamics model describes the impact that stress has on the processing of memory-related neuroplasticity in several structures including the hippocampus, amygdala, and prefrontal cortex (Diamond et al, 2007).

It is possible that the interval of time between the administration of stress or corticosteroids and its subsequent effects on behavior may reflect the fast and slow effects of corticosteroids. The study by de Quervain and colleagues showed that stress induced by foot-shock given 30 minutes before a retention test in a Morris water maze impaired retrieval; however, they also showed that foot-shocks given 2 minutes or 4h before a retention test did not impair retrieval (de Quervain et al, 1998). In view of the fast and slow effects of corticosteroids, the 2 min interval was too short for corticosteroids to exert any effects, particularly since peak corticosteroids levels in the brain require about 20 minutes after significant increase in systemic levels (Droste et al, 2008). At the other end of the spectrum, the 4h interval likely allowed sufficient time to elapse for corticosteroid levels to return back to baseline levels (Connor et al, 1997; Linthorst et al, 2008; Shors et al, 1999). The 30 min interval is consistent with the fast effects of corticosteroids, and during which can impair retrieval up to 90 minutes after stressor onset (Schwabe and Wolf, 2014).

Although the fast and slow model of the effects of corticosteroids predicts that the fast effects of corticosteroids facilitate learning (Diamond et al, 2007; de Kloet et al, 2008; Joëls et al, 2006), the results from the study by de Quervain and colleagues showed that stress induced by foot-shock given 30 minutes before a retention test impaired retrieval (de Quervain et al, 1998). An important point is that de

Quervain and colleagues did not study the effects of corticosteroids on learning, but rather studied the effects of corticosteroids on retrieval. MRs play a role in impaired retrieval (Khaksari et al, 2007), and it is possible that a process that facilitates learning may impair concurrent retrieval. The possible additional effects that corticosteroids exert are discussed further in the next section.

3. Memory Processes

3.1. ACTIVE AND INACTIVE MEMORIES

The findings presented in this thesis show that a forced-swim induced a significant rise in corticosterone levels, a physiological indicator of stress (study 1: Physiological Stress During Training And Forced-Swim). This finding combined with previous results that show dexamethasone blocked the memory enhancement effect on a L/R discrimination memory (Ježek, Lee et al, 2010) suggest two possibilities. One possibility is that corticosteroids induced a previously acquired, and inactive (stable) memory to become activated (labile). Dexamethasone blocked this process, and as such precluded the possibility that the expression of this memory would be modified towards enhancement. The other possibility is that corticosteroids do not have an appreciable impact as to whether a memory becomes activated (made labile). Nonetheless, subsequent to memory activation, an important step for a memory to be modified (Piñeyro et al, 2014), corticosteroids influence the modification of the expression that memory, and dexamethasone blocked this modification process. The role that corticosteroids play in memory activation and memory modification is discussed further in the upcoming sections.

Moving away from the classification of short-term memories and long-term memories (Ebbinghaus, 1913), the susceptibility of a memory to interference can be understood by distinguishing memories into active or inactive (Lewis, 1979). Active memories were considered to be a changing subset of all of the permanent memories that an organism possessed, which can either be formed anew from external stimulation or reinstated at a later time when the learning stimuli were presented again (Lewis, 1979). Unlike inactive memories, active memories are particularly open to disruption (Lewis, 1979)., and this susceptible to amnesic treatments is crucial for the effects of the intervention (Misanin et al, 1968).

The concept of active memories and inactive memories (Lewis, 1979) fits well with the notion that when a memory is reactivated during memory retrieval, the memory trace can re-enter an unstable (labile) state such that a reconsolidation process is needed to stabilize it anew (Dudai, 2006). An early study using an

inhibitory avoidance task showed that shortly after reactivation of the memory, systemic injection of propranolol, a non-specific beta-receptor antagonist, induced amnesia for the task when tested 1 or 2 days later (Przybyslawski et al, 1999).

3.2. STANDARD MODEL OF MEMORY CONSOLIDATION

Proposed more than a century ago, the memory consolidation hypothesis posits that new learning is followed by a time-dependent process of storage referred to as consolidation (Müller and Pilzecker, 1900; Lechner et al, 1999; McGaugh, 2000). This hypothesis offered an explanation as to how cerebral trauma could induce a loss of recent memory, but this view of retrograde amnesia was largely ignored by human memory research until 1949. The consolidation hypothesis gained renewed interest with papers reporting retrograde amnesia in rodents induced by electro-convulsive shock (ECS) (Duncan, 1949; Gerard, 1949) along with Hebb's seminal work *Organization of Behavior* (Hebb, 1949), which speculated that the stabilization of reverberating neural circuits that underlie short-term memory provides a mechanism to establish long-term memory.

Reviewing the literature on the time-dependent modulation of memory, a classic article by James McGaugh put forth a concept of consolidation that was widely accepted and would become the standard model of memory (McGaugh, 1966). This model of memory consolidation postulated that a time-limited process would occur after a learning episode to store long-term information, and once consolidated, a memory should not need to repeat the same process (McGaugh, 1966; McGaugh, 2000). This was further supported by Squire and Alvarez who described consolidation as "molecular cascades and morphological changes whereby synaptic modifications gradually become stable after learning." (Squire and Alvarez, 1995).

Contradicting this model of memory consolidation, Misanin and colleagues showed that a brief re-exposure to the fear cue one day after learning a simple Pavlovian fear conditioning task (Pavlov, 1927) made the memory vulnerable to electro-convulsive shock (ECS) treatment, whereas non-exposed

counterparts showed no impairment (Misanin et al, 1968). Further support of this fragile nature of a recently retrieved memory (Lewis, 1979) were shown by Sara and colleagues who showed that propranolol, a non-selective beta-adrenergic receptor antagonist, injected immediately after re-exposure to the training context impaired the recall of an inhibitory avoidance task (Przybyslawski et al, 1999). This initial finding was supported by a series of papers that followed (Land et al, 2000; Sara, 2000), notably a seminal report by Nader and colleagues (Nader et al, 2000).

3.3. MOLECULAR CONSOLIDATION HYPOTHESIS

Nader and colleagues used auditory fear conditioning to demonstrate that a stable memory could be made labile through reactivation, and that an infusion of the protein synthesis inhibitor anisomycin shortly after memory reactivation produced amnesia. The susceptibility of a memory trace to a protein synthesis inhibitor shortly after memory reactivation suggested that the stable memory became labile, and that restabilization of this reactivated memory required new protein synthesis (Nader et al, 2000). Since protein synthesis plays a key role in consolidation (Bailey et al, 1996), Nader and colleagues view consolidation and reconsolidation as intracellular molecular events ("molecular consolidation theory") in which new memories and reactivated memories exist in similar, but not identical, states (Nader et al, 2000). A theory proposed by Lewis posits that memories exist in a dynamic between two states: an active state in which memories are labile and susceptible to disruption, and an inactive state in which memories stabilize over time (Lewis, 1979). Using this dichotomy of active and inactive states as a framework, memories do not undergo a single process of consolidation or stabilization, but rather can be returned to a labile state upon reactivation from which it must reconsolidate or restabilize in order to persist (Nader and Hardt, 2009). Nader and colleagues further argue that if reconsolidation effects are not observed, one cannot conclude that a limit or boundary has been found. Debiec and colleagues used a contextual fear conditioning paradigm to show that memories up to 45-days-old could still be made labile upon reactivation (Debiec et al, 2002). As a result, any temporal limit or boundary with respect to the age of the memory is merely viewed as experimental limitations (Nader and Einarsson, 2010).

3.4. LINGERING CONSOLIDATION HYPOTHESIS

In contrast to the views proposed by Nader and colleagues, the lingering consolidation hypothesis (Dudai and Eisenberg, 2004) proposes that consolidation has two temporal domains. The fast domain, typically referred to as “synaptic,” or “cellular consolidation,” is completed within minutes to hours after training. The slow domain, typically referred to as “systems consolidation,” (Frankland and Bontempi, 2005) is a gradual process that lingers for days or weeks before completion. An acquired memory progressively undergoes consolidation such that the stability of the memory gradually increases and becomes more resilient to disruption in a kind of temporal gradient (Milekic and Alberini, 2002). Within this hypothesis, reconsolidation is viewed as a manifestation of a consolidation process that proceeds slowly rather than being a recapitulation of a finished process. The role of reconsolidation, then, is to strengthen a memory trace and prevent forgetting (Inda et al, 2011).

If reconsolidation is one contributing phase of a lingering consolidation process (Alberini, 2005; Dudai, 2004; Riccio et al, 2006), then is there a time point at which the consolidation process finalizes? And what would be the purpose of allowing a memory to undergo consolidation? Moreover, if a memory becomes labile, does it necessarily follow that the memory undergoes reconsolidation?

4. Out-of-Context Model

4.1. MNEMONIC MODEL OF POSTTRAUMATIC STRESS DISORDER

While a stressor criterion serves as a gatekeeper to receiving the diagnosis of PTSD, the underlying mechanism of PTSD may not require a traumatic experience (Rosen et al, 2008), and may be facilitated by other means. In contrast to the DSM perspective of PTSD, Rubin and colleagues have argued that PTSD is a memory-based problem, rather than an event-based problem. They argue that a mnemonic model of PTSD, a "pathologically strengthened memory" of the traumatic event rather than experiencing the aforementioned event, would allow a better connection to be made between clinical science research on PTSD and basic science research on memory and emotion (Rubin et al, 2008). Moreover, the claims made by the mnemonic model provide testable hypotheses, unlike the description provided by DSM-V or DSM-IV-TR. I will adopt the mnemonic model as a framework for the proposed work.

4.2. STRESS-INDUCED OUT-OF-CONTEXT

One defining characteristic of PTSD is the recurrent, involuntary, and intrusive recollections of the traumatic memory, which is colloquially referred to as flashbacks. Importantly, these flashbacks need not be associated with the location or environment in which the original event occurred. For example, a soldier who has returned home from serving a tour of duty may experience these intrusive recollections (e.g., flashbacks) at home, many miles away and a very different environment from that of the war zone. It is thought that the intrusive recollections are triggered by some external stimuli that act as a reminder of the trauma, and that this recollection lead to the emotional response (American Psychiatric Association, 2013). It is currently unknown what triggers these intrusive recollections and whether some unrelated event can trigger an emotional response that in turn triggers the intrusive recollections of the traumatic memory thereby enhancing the expression of that traumatic memory.

Behavioral paradigms using an animal model to study PTSD include auditory fear conditioning (also referred to as classical fear conditioning), contextual fear conditioning, and inhibitory avoidance. While

these animal models have been instrumental in elucidating many of the mechanisms of memory, reactivation of the acquired memory in all of these models requires re-exposure to the conditioned stimulus (CS) (e.g., auditory tone or shock chamber) but without the unconditioned stimulus (US) (e.g., shock), or the administration of a US to act as a reminder but delivered with a CS. Moreover, there is an element of the re-exposure or reminder that is also present in the training context, which implies that there is no “out-of-context” trigger of the initial acquired memory. And if the stimulus used to elicit recall is sufficiently similar to that of the initial stimulus used to train the animal, then either reconsolidation or extinction mechanisms can be induced depending upon the duration of the stimulus (Suzuki et al, 2004; Monfils et al, 2009).

In contrast to the behavioral paradigms currently used to study PTSD, our rodent studies have focused on the observation that a previously acquired memory can be modified by a stressful experience that occurs at a different time and with no identifiable feature in common with the original learning context. Importantly, this characteristic in our “out-of-context” rodent studies begins to resemble an important biological and behavioral sequelae of PTSD, namely that of the intrusive re-experiencing or recollection of a previously acquired memory. Questions arise as to the mechanism by which this phenomenon occurs, and the impact that it potentially has on acquired memories, but we know that the release of stress hormones in response to the stressful situation plays a pivotal role in inducing this observed “out-of-context” phenomenon (Ježek, Lee et al, 2010). Further studies of this observed phenomenon in which an “out-of-context” stressful experience can modify a previously acquired, stable memory may lead to a useful tool in understanding how memories can be triggered, how memories can be modified subsequent to activation, and how memory processes can be disrupted.

SPECIFIC AIMS

The general aim is to study the conditions by which a stressful experience, mediated by corticosteroids, act on the hippocampus to modify stable, unrelated memories so that the expression of that memory appears enhanced within a time-limited window.

Aim 1. Are corticosteroids the key mediator of the stress response during forced-swim to induce a stable memory to become modified?

The state-dependency hypothesis, also referred to as the mood congruency effect (McGaugh, 1973; Tulving and Thomson, 1973; Mactutus et al, 1980b; Judge and Quartermain, 1982; Izquierdo et al, 1989) suggests that when stress hormones are given prior to testing, it reproduces the internal hormonal state of the animal at the time of training, and that the retrieval of the acquired memory depends upon the influence of that hormonal state. In other words, the hormones act as retrieval cues at the time of testing by reproducing a component of the motivational aspect of the task (Vianna et al, 2001). The working hypothesis is that the forced-swim experience reproduced the stress of the learning, which acted as a reminder cue such that the expression of the left/right discrimination memory appeared enhanced. I tested the central hypothesis by measuring serum corticosterone levels, as a marker of physiological stress, to determine whether the stress level is a common factor during both the learning and swim experiences.

Aim 2. Is there a time-limited window within which swim-stress can modify an unrelated memory?

The lingering consolidation hypothesis (Dudai and Eisenberg, 2004) proposes that an acquired memory progressively undergoes consolidation with time such that the stability of the memory gradually increases and becomes more resilient to disruption in a kind of temporal gradient (Milekic and Alberini, 2002). The working hypothesis is that the stability of a memory increases such that swim-stress can modify memories only within a time-limited window. I tested the central hypothesis by increasing the time

interval between learning and swim-stress to determine whether there is a time-limited window within which swim-stress can enhance the expression of an unrelated memory.

Aim 3. Does swim-stress modify a spatial memory?

Cognitive map theory predicts that animals in a T-maze will approach the arm in the same spatial location that was baited during training whereas stimulus-response theory predicts that animals will approach the arm with the same body turn that was baited during training (Restle, 1957). This indicates that a L/R discrimination task can be solved with two different strategies (a place strategy or response strategy). The active place avoidance task is a very sensitive spatial memory paradigm in which successful avoidance requires the use of a place strategy. The working hypothesis is that swim-stress enhances the expression of both place strategy and response strategy memories. I tested the central hypothesis by measuring the behavior in a spatial memory task to determine whether corticosteroids that are released during swim-stress can enhance the expression of spatial memories.

MATERIALS AND METHODS

Subjects

7-12 week-old male Long-Evans rats weighing at least 300 grams were housed in pairs in a vivarium with a light / dark cycle at 07:00 / 19:00. All rats were allowed at least 5 days to acclimate to the vivarium upon arrival. Afterwards, each rat was handled for several days prior to any experimental procedure. Experiments for all of the studies were conducted during the light period, primarily between the hours of 10:00 and 15:00.

Pharmacological Stress

ORAL DOSING OF EXOGENOUS CORTICOSTERONE

A homogenized suspension of corticosterone in oil from commercially available peanut butter was made at varying doses (Pung et al, 2003). To make a concentration of 5 mg/mL, 5 mg of corticosterone was measured and placed into a 10-mL beaker with 0.5 mL of 100% ethanol on mild heat to dissolve the corticosterone. Once the corticosterone dissolved, 1.0 mL of peanut butter oil was then added to the solution. The vehicle was prepared in the same manner except that no corticosterone was added to the solution. The corticosterone suspension and vehicle solution were made the previous night (approximately 1800h), and placed on a magnetic stirrer with mild heat overnight to evaporate the ethanol.

The oral dosing procedure was modified from the gavage procedure as an alternate means of drug delivery to minimize stress for the animal (Küster et al, 2012). The procedure involved placing a 1-mL tuberculin syringe (without needle) into the mouth of a rat, and then slowly injecting a volume of 1 mL/kg of peanut butter oil. Rats were habituated to the procedure for 2-4 days during which the animal was

held in the experimenter's hands with the posterior aspect of the animal's jaw was held in place by the experimenter's fingers to stabilize the animal's head while the tuberculin syringe was placed into the rat's mouth upon which peanut butter oil was delivered (Figures 5, 6, 7, and 8).

CORTICOSTERONE ASSAY

Rats were anesthetized with isofluorane for 20 min after the administration of vehicle or corticosterone (an interval of time that mimics the duration as that of the forced-swim). Trunk blood was collected into 15-mL conical or centrifuge tube(s) (the tubes were not coated with EDTA or other anti-coagulant) that were previously chilled with wet ice. The centrifuge tubes that contained the blood samples were then put on wet ice until they were placed into a 4°C refrigerator on the date of collection for overnight storage. The day after trunk blood collection, each of the 15-mL conical or centrifuge tube(s) that contained the coagulated blood samples was centrifuged for 10 minutes at 4000 rpm. Using a serological pipette, the supernatant was withdrawn and placed into 1.5-mL eppendorf tubes. The frozen supernatant (i.e., serum) was stored in a –80°C freezer until assayed for corticosterone levels. Serum corticosterone levels were measured with a corticosterone ELISA kit (AssayPro, St. Charles, MO).

Left/Right (L/R) Discrimination Paradigm

Left/Right (L/R) discrimination training was administered using a short training and an intensive training protocol. Both protocols were followed by the identical forced-swim experimental and non-swim control treatments, but the retention test was training-specific, as described below.

SHORT TRAINING APPETITIVELY-CONDITIONED

The T-maze had transparent walls (50cm x 15cm x 43cm (L x W x H) arms) (Figures 3 and 4). During 5-6 days, the rats were habituated to the T-maze and to eat 3 cocoa puffs during 2 min at the choice point. All rats then received five acquisition trials (Figures 3 and 4).

Acquisition Training (Day 1): Rats were food-deprived to 85% of their weight. Each animal was habituated to the T-maze for 5 min after which they were trained to perform a left/right (L/R) discrimination task for food reward on a T-maze starting from a fixed start arm to one of two choice arms. Each trial starts when the rat was placed in the start arm, and ends when the rat entered a choice arm by at least half of its body length or 120 s elapsed. The rewarded and incorrect arm designation was based on the response of the rat on its first trial: the first choice was always considered an error, and the other arm was then designated the rewarded arm (Acquisition Arm). Classifying the first choice as an error enables the rat to experience one arm as incorrect and the other arm as rewarded. In addition, this controlled for any turn preferences that the rat may have had. If the rat did not enter a choice arm within 120 s, it was placed in the goal arm, given 3 cocoa puffs, and an error was scored. Each rat received 5 training trials, with each trial separated by 2 min. Rats with three or more errors were excluded from the study.

Stressor (Day 2): One day after learning the L/R discrimination task, one group of rats was forced to swim for 20 min in a tall plastic bucket (diameter 32 cm) filled to 28 cm with 27±1°C water. Afterwards, these rats were dried with a towel and returned to their home cage. A group of control rats was treated exactly like the experimental animals except that these rats were placed in a plastic bucket containing 1 cm of water for 20 min.

Retention (Day 3): One day after the Stressor treatment, rats were tested for retention of the L/R discrimination memory acquired during Acquisition Training (on Day 1) by a single non-reward trial (Retention). If the rat did not enter a choice arm within 120 s, an error was scored.

INTENSIVE TRAINING AVERSIVELY-CONDITIONED

The T-maze had opaque walls (50cm x 15cm x 43cm (L x W x H) arms) and an electrifiable floor that was a metal sheet (Figures 9, 10, 11, and 12).

Acquisition Training (Day 1): Each animal was habituated to the T-maze for 5 min before starting the left/right (L/R) discrimination task in which the rat escapes from a fixed start arm to one of two choice arms. Each trial starts when the rat was placed in the middle arm, and 5 s later foot-shocks (50 Hz, 0.5 mA, 0.5 s) were given every 3 s. Escaping directly into the safe arm was scored correct and foot-shocks ceased, but entering the incorrect arm by at least half of its body length was scored an error and foot-shocks continued. Failure to escape to the safe arm within 60 s was scored an error, at which point the rat was manually put in the safe arm. Each rat then spent 30 s in the safe arm before starting a new trial. The safe and incorrect arm designation was based on the response of the rat on its first trial: the first choice was always considered an error, and the other arm was then designated the safe arm (Acquisition Arm). Rats exhibit spontaneous alternation, but stress decreases this natural tendency (Bats et al, 2001; Lalonde, 2002). Classifying the first choice as an error enables the rat to experience one arm as shock and the other arm as safe. In addition, this controlled for any turn preferences that the rat may have had. After 9 of 10 consecutive correct responses, 30 additional trials were given.

Stressor (Day 2, Day 8, Day 15, or Day 31, respectively): After an interval of one, 7, 14, or 30 days after learning the L/R discrimination task in the T-maze, one group of rats was forced to swim for 20 min in a tall plastic bucket (diameter 32 cm) filled to 28 cm with 27±1°C water. Afterwards, these rats were dried with a towel and returned to their home cage. A group of control rats was treated exactly like the experimental animals in the same experimental room except that these rats were placed in the plastic bucket containing 1 cm of water for 20 min.

Reversal Training (Day 3, Day 9, Day 16, Day 32, respectively): One day after the Stressor treatment, rats were tested for retention of the L/R discrimination memory by Reversal Training in which the rat must escape to the opposite arm as that on Day 1 (Reversal Arm). The intensive training regimen on Day 1 creates a robust memory that persists for at least a week such that both the control and experimental groups perform perfectly on retention testing. Administering a forced-swim to naïve rats one day before L/R discrimination training did not impair learning (Ježek, Lee et al, 2010). As such, more errors (rats entering Acquisition Arm) during Reversal Training indicated better retention of Day 1 memory. The number of errors to the criterion of four consecutive correct responses was used to compare Acquisition Training and Reversal Training.

Active Place Avoidance Paradigm

The active place avoidance apparatus consisted of a circular metal arena measuring 82 cm in diameter that rotated clockwise at 1 rotation per minute (rpm) (Figures 13 and 14).

SHORT TRAINING AVERSIVELY-CONDITIONED

Acquisition (Day 1): Each animal was habituated to the rotating arena without foot-shock for a single 10-min trial before starting the active place avoidance acquisition (Habituation). After a 10-min habituation session, each rat received three 10-min acquisition trials with 10-min inter-trial intervals. During the Acquisition trials, the rats have to learn how to avoid an invisible and stationary 60° sector shock area on the rotating arena that was oriented according to distal cues on the walls of the room (Wesierska et al, 2005). The position of the rat was tracked by PC-based software that analyzed the images from an overhead camera at 30 Hz. Avoidance was reinforced by foot-shock (60 Hz, 500 ms, 0.3 – 0.5 mA). The foot-shock was delivered when the rat entered the shock area for a duration longer than 500 ms with additional shocks administered every 1.5 s thereafter until the rat exited the shock area.

Stressor (Day 2): One day after learning the active place avoidance task, one group of rats was forced to swim for 20 min in a tall plastic bucket (diameter 32 cm) filled to 28 cm with 27±1°C water. Afterwards, these rats were dried with a towel and returned to their home cage. A group of control rats was treated exactly like the experimental animals in the same experimental room except that these rats were placed in a plastic bucket containing 1 cm of water for 20 min.

Retention (Day 3): One day after the Stressor treatment, rats were tested for retention of the active place avoidance memory by a single 10-min trial without foot-shock.

INTENSIVE TRAINING AVERSIVELY-CONDITIONED

Acquisition (Day 1): Each animal was habituated to the rotating arena without foot-shock for a single 10-min trial before starting the active place avoidance acquisition (habituation). After a 10-min habituation session, each rat received eight 10-min acquisition trials with 10-min inter-trial intervals. During the Acquisition trials, the rats have to learn how to avoid an invisible and stationary 60° arc shock area on the rotating arena that was oriented according to distal cues on the walls of the room (Wesierska et al, 2005). The position of the rat was tracked by a PC-based software that analyzed the images from an overhead camera at 30 Hz. Avoidance was reinforced by foot-shock (60 Hz, 500 ms, 0.3 – 0.5 mA). The foot-shock was delivered when the rat entered the shock area for a duration longer than 500 ms with additional shocks administered every 1.5 s thereafter until the rat exited the shock area.

Stressor (Day 2): One day after learning the active place avoidance task, one group of rats was forced to swim for 20 min in a tall plastic bucket (diameter 32 cm) filled to 28 cm with 27±1°C water. Afterwards, the rats were dried with a towel and returned to their home cage. A group of control rats were treated exactly like the experimental animals in the same experimental room except that the rats were placed in a plastic bucket containing 1 cm of water for 20 min.

Retention (Day 3): One day after the Stressor treatment, rats were tested for retention for the active place avoidance memory by a single 10-min trial without foot-shock.

RESULTS

5. Physiological Stress During Training And Forced-Swim

I investigated whether stress was a common factor during both the acquisition training and forced-swim experiences by measuring serum corticosterone levels as a marker of physiological stress. A forced-swim experience can modify seemingly unrelated memories such that its subsequent expression was enhanced (Ježek, Lee et al, 2010). Previously, a short training aversively-conditioned left/right (L/R) discrimination task was used to create a memory of a L/R discrimination task. The next day, animals were subjected to either a forced-swim or non-swim control conditions, and then tested for retention on the third day. It was found that the animals in the forced-swim group scored more correct response than those in the non-swim group (Ježek, Lee et al, 2010). The learning environment and the swim environment had no physically identifiable feature in common with each other, but both experiences may have been stressful (Ježek, Lee et al, 2010).

Typically, a return to the learning environment (Misanin et al, 1968; Lewis et al, 1972) or exposure to contextual cues (Lewis et al, 1973) have long been used to trigger the retrieval of a previously acquired memory. Besides using contextual cues to reactivate a memory, Riccio and Cancannon proposed that a pretest administration of stress-related hormones could facilitate retrieval of an avoidance task by acting as a reminder treatment (Riccio and Concannon, 1981). A pretest injection of ACTH attenuated a hypothermia-induced retrograde amnesia (Mactutus et al, 1980a) and increased the latency of a passive avoidance task (Izquierdo et al, 1988; Mactutus et al, 1980a).

According to the state-dependency hypothesis, also referred to as the mood congruency effect (McGaugh, 1973; Tulving and Thomson, 1973; Mactutus et al, 1980b; Judge and Quartermain, 1982; Izquierdo et al, 1989), the circulating stress hormones during training and swim reproduces the internal hormonal state of the animal at the time of training, and that the retrieval of the acquired memory depends upon the influence of that hormonal state. Hormones released during the aversively-

conditioned left/right (L/R) discrimination training may have also been released at the time of the forced-swim thereby acting as retrieval cues by reproducing a component of the task context (Vianna et al, 2001). This could facilitate the enhanced recall of the L/R discrimination memory during retention testing.

5.1. FORCED-SWIM ENHANCES APPETITIVELY-CONDITIONED MEMORIES

The forced-swim experience is presumed to be aversive for the animal, and hence an experience that induces a stress response. If the forced-swim experience reproduced the stress of the learning, and acted as a reminder cue for the L/R discrimination memory, then there are two possible approaches to dissociate this confound by making the learning and forced-swim conditions have different levels of stress hormones. One is to block the stress response during the forced-swim such that the hormonal aspects are less likely to act as retrieval cues during the forced-swim. This approach has already been investigated (Ježek, Lee et al, 2010), and will be discussed further in the next chapter. Alternatively, a non-aversive training protocol can be used, and it is this method that is discussed in this chapter.

The short training appetitively-conditioned L/R discrimination task was used. Details of the protocol are described in the Materials and Methods section. Briefly, each animal was habituated to a T-maze for 5 min after which they were trained to perform a L/R discrimination task for food reward. Each rat received 5 training trials (Day 1, Acquisition Training). One day after learning the L/R discrimination task, one group of rats was forced to swim for 20 min, while one group of control rats was placed in 1 cm of water for 20 min (Day 2, Stressor). One day after the Stressor treatment, rats were tested for retention by a single non-reward trial (Day 3, Retention).

All animals that received the forced-swim chose the rewarded arm whereas those animals that received the non-swim experience chose either arm at the level of chance. This suggests that the forced-swim enhanced the expression of an appetitively-conditioned L/R discrimination task (Figures 3 and 4).

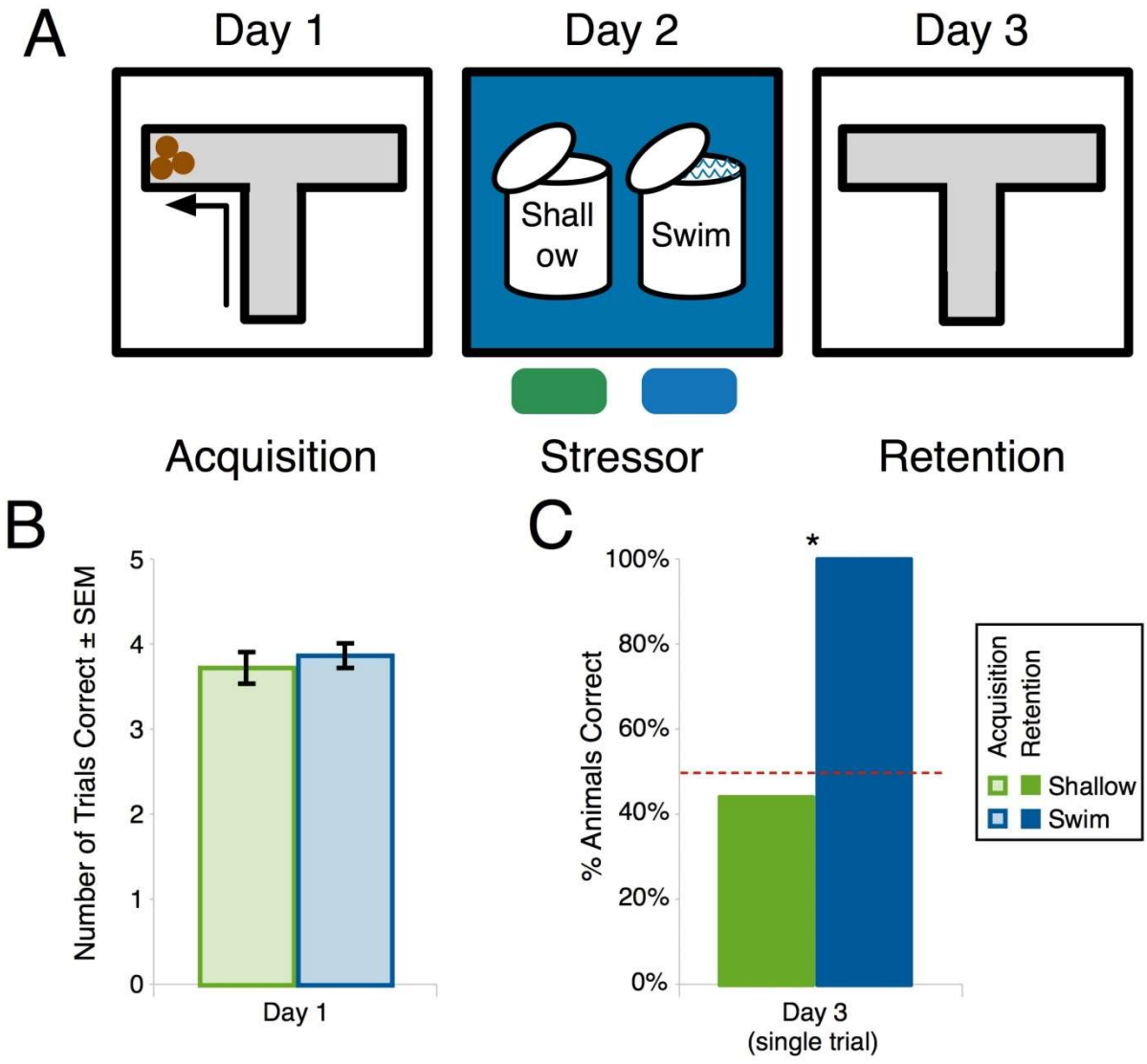


Figure 3. Forced-swim enhanced memory expression of a short training appetitively-conditioned left/right discrimination task.

(A) *To determine whether the forced-swim could induce the observed memory enhancement effect for a non-aversive behavioral task similar to that for aversive behavioral task, a short-training appetitively-conditioned left/right (L/R) discrimination task was used. On Day 1, animals received 5 Acquisition trials for food reward. On Day 2, one group of animals was placed in a bucket with 1 cm of water (Shallow, n = 7) whereas the other group of animals was placed in a bucket and forced to swim (Swim, n = 7). On Day 3, animals were tested for retention with a single trial without food reward.*

(B) *There was no difference in the number of correct trials between the Shallow group (3.71 ± 0.18) and the Swim group (3.86 ± 0.14) during Acquisition Training on Day 1 (two-tailed $t_{12} = 0.6124$; $p = 0.5517$). The Acquisition trials in each group are listed as correct trials ± SEM.*

(C) *On Day 3, animals in the Swim group chose the same arm during Retention as the arm that had been rewarded during the Acquisition Training whereas animals in the Shallow group chose either arm at the level of chance during Retention ($X^2_1 = 4.9524$; $p = 0.0261$). One animal in the Swim group failed to leave the start arm, and was not included in the X^2 calculation. This finding suggests that the forced-swim enhanced the expression of a previously acquired appetitively-conditioned left/right (L/R) discrimination memory. A red dashed line is set at the level of chance.*

5.2. TRAINING AND SWIM CONTEXTS ARE DIFFERENT

In a parallel series of experiments, a separate cohort of animals underwent the same experimental paradigm but were sacrificed at different stages of the protocol to measure their serum corticosterone levels as an estimate of physiological stress (Figure 4). In addition, a non-handled control group was added as an additional control group to assess physiological stress levels of the handling procedure itself.

Physiological stress was estimated by measuring the serum corticosterone levels in each group. The corticosterone levels in the acquisition group was not significantly different from that of animals in either the pre-training (handling only) group or non-handled group. In contrast, the corticosterone levels in the forced-swim group were significantly greater than both the shallow water group and the acquisition group. Moreover, the shallow water group had significantly higher corticosterone levels than that of the acquisition group (Figure 4).

The results of the study reveal three important observations regarding the short training appetitively-conditioned L/R discrimination task. The first observation is that the acquisition and the recall of the L/R

discrimination memory is not stressful as compared to both a pre-training (handling only) control group as well as to a non-handled control group.

The second observation is that the forced-swim is stressful as compared to either acquisition or recall of the L/R discrimination memory as indicated by a significant increase in serum corticosterone levels as compared to that of the Acquisition Training or Retention levels, respectively.

These two observations taken together demonstrate that the internal hormonal state of the animal at the time of training and the internal hormonal state of the animal at the time of the forced-swim are significantly different from each other. Since the physical environment of the training and forced-swim experiences are intentionally constructed to be different from each other, and combined with the demonstration that the internal hormonal state of the animal during training and forced-swim are different from each other, these results strongly suggest that the modification of a previously acquired memory occurred in an “out-of-context” situation.

The third observation is that the increase in serum corticosterone levels of the shallow water group is significantly higher than that of the acquisition group, but significantly lower than that of the forced-swim group. This finding suggests the possibility that the absence of a behavioral effect subsequent to the shallow water experience may be due to systemic corticosterone levels in the shallow water group failing to reach a threshold level to sufficiently activate corticosteroid receptors in the brain. Increases in hippocampal corticosterone levels have a 20 min delay from any increase in systemic corticosterone levels, but a drop in hippocampal corticosterone levels coincides with that of systemic levels (Droste et al, 2008). As such, systemic corticosterone levels may not have increased quickly enough or failed to reach a sufficient level before returning to baseline to have an appreciable impact on the circulating corticosterone levels around the hippocampus in the shallow water group. In addition, corticosteroid-binding-globulin (CBG) may attenuate any change in systemic corticosterone levels (Qian et al, 2011; Minni et al, 2012; Moisan et al, 2014), which would reduce the amount of corticosterone that could reach

the brain during the shallow water experience. In contrast, the increase in systemic corticosterone levels in the forced-swim group may have rapidly and sufficiently exceeded the binding capacity of CBG, thereby allowing a threshold corticosterone concentration to reach the brain.

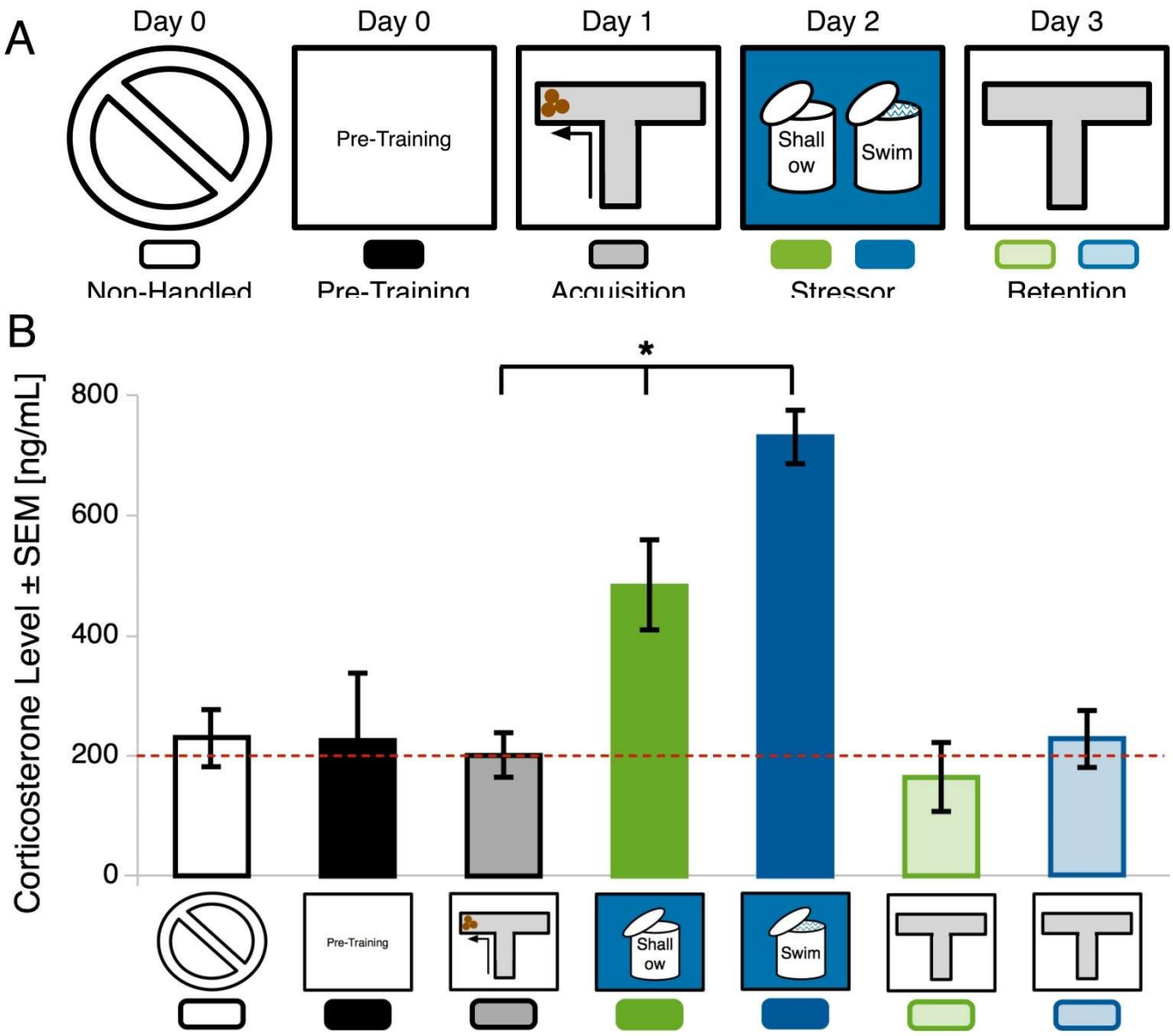


Figure 4. Forced-swim induced a significant increase in serum corticosterone levels.

(A) *Another cohort of animals were trained in a short-training appetitively-conditioned L/R discrimination task, as was previously described, to determine circulating corticosterone levels. To measure circulating corticosterone levels, animals were removed from the study and sacrificed to collect trunk blood at different phases of the experiment: Non-Handled (n = 4), Pre-Training (n = 4), Acquisition (n = 5), Shallow (n = 5), Swim (n = 4), Retention Shallow (n = 6), Retention Swim (n = 7).*

(B) *There was no difference in the serum corticosterone levels among the Acquisition group (200.49 ± 35.89) as compared to that of the Pre-Training (225.31 ± 111.54) and Non-Handled (229.83 ± 47.49) groups ($F_{2,10}$ = 0.0568; p = 0.9451). This finding suggests that the Acquisition trials of the short-training appetitively-conditioned L/R discrimination task is not significantly stressful. In contrast, the forced-swim induced a significant increase in serum corticosterone levels in the Swim group (730.46 ± 62.34) as compared to that of both the Acquisition and Shallow groups (482.01 ± 74.71) ($F_{2,11}$ = 18.8583; p = 0.0003). A post hoc Tukey HSD test showed that serum corticosterone levels were significantly increased in the Shallow group as compared to that of the Acquisition group (p < 0.05), that serum corticosterone levels were significantly increased in the Swim group as compared to that of the Shallow group (p < 0.05), and that serum corticosterone levels were significantly increased in the Swim group as compared to that of the Acquisition group (p < 0.01). This finding suggests that the forced-swim is a stressful experience as compared to that of the Acquisition trials. The elevated serum corticosterone levels in the Swim and Shallow groups returned to baseline by the next day as there was no difference in serum corticosterone levels in the Acquisition group as compared to that of the Retention Shallow group (163.56 ± 57.05) and Retention Swim group (226.55 ± 47.01) ($F_{2,15}$ = 0.4458; p = 0.6485). A red dashed line is set to the Acquisition group (gray bar), which acted as the reference group to which all other groups were compared. The serum corticosterone levels in each group are listed as ng/mL ± SEM.*

5.3. SUMMARY

The forced-swim experience modifies a previously acquired memory in which the expression of a L/R discrimination memory is enhanced. The recall was enhanced regardless of whether the L/R discrimination task is aversively-conditioned (Ježek, Lee et al, 2010) or appetitively-conditioned. This suggests that stress can modify a previously acquired memory even though that memory is not associated with stress. Moreover, this memory enhancement is unlikely to be due to any reminder cues present in the environment because the training and stressor experiences were conducted in different rooms with different physical appearances. Specifically, the high corticosteroid levels during the forced-

swim do not act as contextual reminder cues, supporting the assertion that the memory activation was truly an out-of-context phenomenon.

6. Corticosteroid Actions During Swim-Stress

I investigated whether corticosterone, the levels of which significantly increase during the forced-swim, is the key mediator by which swim-stress acts to enhance the expression of unrelated memories. Although an increasing body of knowledge suggests that sufficient stress and corticosteroids impair the retrieval of previously acquired information (McGaugh and Roozendaal, 2002), there have been mixed results and no conclusions are definitive. These mixed results could be due to behavioral manipulations used in the experiments that acted as a stressor to induce an increase in corticosterone levels as well as influence catecholamine levels, particularly epinephrine and norepinephrine. Additionally, the interval of time between the administration of stress or corticosteroids and the subsequent retrieval of a memory may have an influence as to whether corticosteroids act to impair or enhance the retrieval of a particular memory. A model proposed by Joëls and colleagues (Joëls et al, 2006) suggests that the short-term effect of corticosterone, acting via non-genomic pathways, facilitate learning and memory formation.

In addition, Riccio and Cancannon proposed that a pretest administration of stress-related hormones, particularly ACTH, could facilitate retrieval of an avoidance task by acting as a reminder treatment (Riccio and Concannon, 1981). The working hypothesis is that corticosterone is sufficient to induce the memory enhancement effect as observed subsequent to a forced-swim if the retrieval of a previously acquired memory occurs after the gene-mediated pathway has normalized the earlier raised activity. I tested the central hypothesis by administering corticosterone itself to determine whether corticosteroids are the key mediator by which swim-stress acts to enhance the expression of unrelated memories.

Dexamethasone, a synthetic glucocorticoid with potent glucocorticoid effects and negligible mineralocorticoid effects, has a higher binding affinity to GRs than does that of either corticosterone or cortisol (de Kloet et al, 1975; Meikle and Tyler, 1977). Dexamethasone binds to GRs in the pituitary very well (Cole et al, 2000; de Kloet et al, 1974; de Kloet et al, 1975), but poorly penetrates the blood-brain barrier (de Kloet et al, 1974; de Kloet, 1997). As a consequence, dexamethasone that is administered

systemically suppresses the production of corticosterone via negative feedback (Carroll et al, 1976) by suppressing both adrenocorticotropic hormone (ACTH) release (Cole et al, 2000; de Kloet et al, 1974; de Kloet et al, 1975) and corticotropin-releasing hormone (CRH) release (Cole et al, 2000; Warembourg, 1975). Thus, systemically administered dexamethasone acts to suppress the activity of the hypothalamic-pituitary-adrenal (HPA) system that results in a significant decrease of plasma corticosterone levels within 2h after injection (Lurie et al, 1989).

Previously, an intensive training aversively-conditioned left/right (L/R) discrimination task was used to create a memory of a L/R discrimination task. The next day, animals received either dexamethasone or saline, and were subjected to either a forced-swim or non-swim control conditions. Retention was then tested on the third day. Blocking the endogenous release of corticosterone at the time of the forced-swim also blocked the swim-induced enhancement of the left/right (L/R) discrimination memory. This suggests that corticosterone is necessary for the swim-induced enhancement of memory (Ježek, Lee et al, 2010). If corticosterone is necessary for the swim-induced enhancement of memory, is it also sufficient to induce the memory enhancement effect observed subsequent to a forced-swim?

6.1. ORAL DOSING IS AN EFFECTIVE, NON-STRESSFUL METHOD OF DELIVERING EXOGENOUS CORTICOSTERONE

The forced-swim experience induces a complex stress response that elicits movement and exercise related activity (Linthorst et al, 2008), behavioral struggling (Armario et al, 1995), and a drop in body temperature (Drugan et al, 2005; Linthorst et al, 2008). Moreover, a forced-swim also induces a whole cascade of biochemical changes such as the release of corticosterone (Abel, 1993), adrenocorticotropic hormone (ACTH) (Armario et al, 1995), corticotropin-releasing hormone (CRH) (Jiang et al, 2004), catecholamines (Jordan et al, 1994), vasopressin (Jiang et al, 2004), glucose (Abel, 1993; Armario et al, 1995; Kelliher et al, 2000), prolactin (Abel, 1993; Armario et al, 1995), 5-hydroxytryptamine (5-HT, serotonin) (Peñalva et al, 2002; Linthorst et al, 2002), and tryptophan hydroxylase (Azmitia and McEwen, 1974) among a host of other biochemical changes.

To focus on the role of corticosteroids alone, a method of administering exogenous corticosterone needs to deliver a dose of sufficient magnitude, demonstrate a physiological time course comparable to the physiological stress response induced by a forced-swim, and minimize the impact on circulating levels of endogenous catecholamines. This eliminates commonly used methods including corticosterone pellets implanted subcutaneously and the addition of corticosterone to the drinking water. Moreover, many methods of administering exogenous corticosterone are themselves aversive including subcutaneous injection, intraperitoneal (ip) injection, and gavage (Brown et al, 2000).

Several studies have demonstrated that an oral route of administration is effective in inducing a rapid rise in corticosterone levels without eliciting the endogenous stress response from the procedure itself in sparrows (Breuner et al, 1998), in mice (Dalm et al, 2008; Hoggatt et al, 2010), and rats (Pung et al, 2003). One study used a different method of voluntary oral administration of daily medication by using a store-bought cookie dough ball. Several strains of rats were used, and most strains of rats reliably ate the cookie dough with the medication mixture within a single session. Surprisingly, the female Long-Evans strain (males were not used in the study) were significantly different from the other strains in that they were much more likely to incompletely ingest the cookie dough ball (Corbett et al, 2012).

Since rats do not have a gag reflex, the contents delivered into the mouth of the rat will be swallowed. One study found that daily administration of orogastric gavage over the course of 28 days did not negatively affect the wellbeing of the animals; however, the procedure required the experimenter to firmly immobilize the animal while inserting the gavage needle to deliver the contents directly into the esophagus (Turner et al, 2012). To eliminate the concerns of inducing restraint-associated distress, two studies investigated methods of delivering drugs by placing the contents into the mouth and allowing the animal to consume vehicle and drug mixture. One study created a suspension of drug in honey (Küster et al, 2012) whereas another study used a drug-containing 10% sucrose solution (Atcha et al,

2010). Since corticosterone can be made into a suspension with oil (Pung et al, 2003), the delivery of exogenous corticosterone was modified from published reports (Atcha et al, 2010; Küster et al, 2012).

To determine whether an oral dosing method is (1) a non-stressful means of administering exogenous corticosterone, and (2) can induce an appreciable rise in serum corticosterone levels within 20 minutes of delivery, I conducted the following experiment.

Animals were separated into three groups to receive the following treatments (Figure 5):

(1) handling only,

(2) vehicle (peanut butter oil), and

(3) vehicle + 20 mg/kg corticosterone.

The results of the oral dosing study show three important points. First, serum corticosterone levels between the handling only group and the vehicle group were not different from each other. This suggests that the oral dosing procedure itself is a non-stressful method by which corticosterone can be administered. Second, oral delivery of corticosterone results in a significant and measurable rise in corticosterone levels in the systemic circulatory system within 20 minutes, which is the duration of the forced-swim.

Third, an exogenous dose of 20 mg/kg dose of corticosterone results in serum corticosterone levels that greatly exceed the levels found after a 20-min swim, thereby indicating an upper limit in the dose of corticosterone that needs to be administered (Figure 5). These findings suggest that oral dosing is a non-stressful, effective method of delivering exogenous corticosterone.

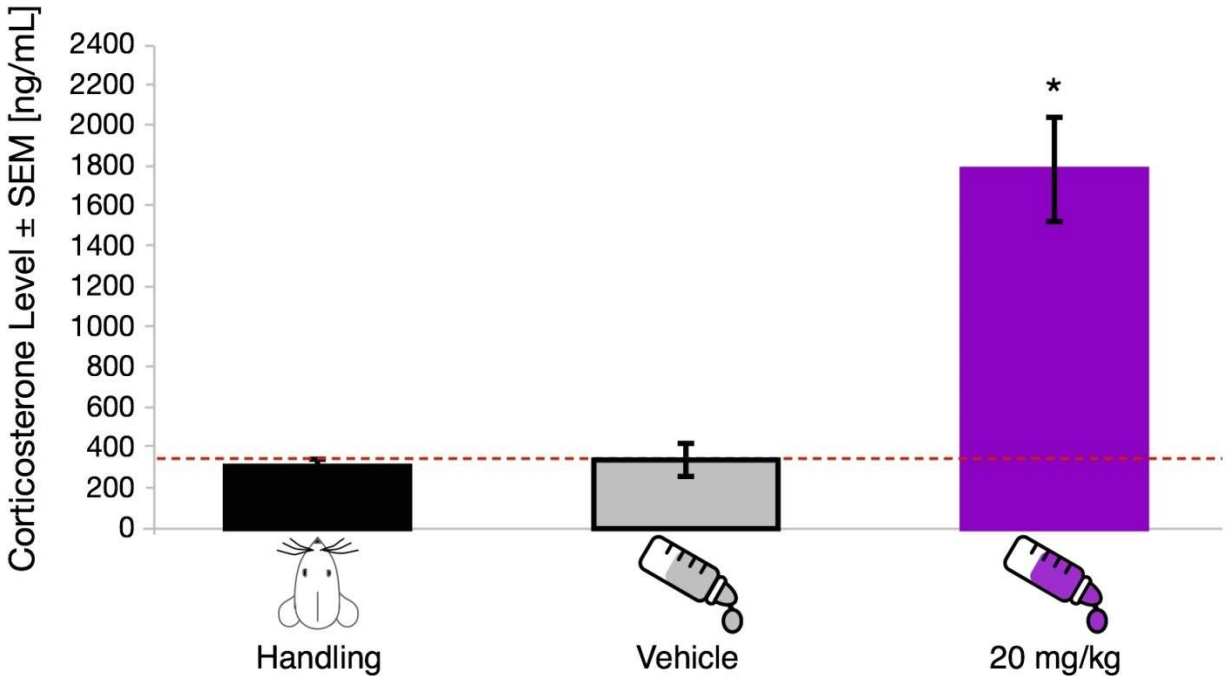


Figure 5. Oral dosing procedure is a non-aversive and effective method for delivering exogenous corticosterone.

An oral dosing procedure was devised to determine whether exogenous corticosterone could be given such that the method itself was effective at raising serum corticosterone levels, measured 20 min after administration, without inducing any appreciable increase in endogenous corticosterone levels. There was no difference in the serum corticosterone levels between the Handling group (n=4, 311.09 ± 35.13) and Vehicle group (n=4, 342.72 ± 82.28) (two-tailed $t_6 = 0.3536$; $p = 0.7357$). This finding suggests that the oral dosing procedure is not a stressful method of administering exogenous corticosterone. In contrast, a 20 mg/kg dose of exogenous corticosterone (n=4, 1792.35 ± 259.92) resulted in a significant increase in serum corticosterone levels as compared to that of the Vehicle group (two-tailed $t_6 = 5.3172$; $p = 0.0018$). This finding suggests that oral dosing procedure is an effective method of delivering exogenous corticosterone. A red dashed line is set to the vehicle group (gray bar), which acted as the reference group to which the other two groups were compared. The serum corticosterone levels in each group are listed as ng/mL ± SEM.

6.2. DOSE-RESPONSE CURVE OF EXOGENOUS CORTICOSTERONE

In study 2A, I showed that oral dosing is an effective, non-stressful method of administering exogenous corticosterone. It is unknown as to what dose of exogenous corticosterone is needed to raise serum corticosterone levels to that induced by a 20-min forced-swim. As such, a dose-response curve is needed.

To determine the dose needed to raise serum corticosterone levels to to that induced by a forced-swim, animals were divided into the following groups (Figure 6):

(1) handling only,

(2) vehicle (peanut butter oil) only,

(3) vehicle + 1 mg/kg corticosterone,

(4) vehicle + 3 mg/kg corticosterone,

(5) vehicle + 5 mg/kg corticosterone,

(6) vehicle + 10 mg/kg corticosterone.

Twenty minutes after the oral dosing procedure (the time duration of the forced-swim), trunk blood was collected under isoflurane anesthesia. After overnight storage, the blood was centrifuged for 10 minutes at 4000 rpm, the supernatant was withdrawn, and then stored frozen until assayed with a corticosterone ELISA kit.

Once the corticosterone dose-response curve has been determined, these doses were then compared to the increase in corticosterone levels induced by the forced-swim to find the optimal dose of exogenous corticosterone that needs to be delivered. The results are as follows.

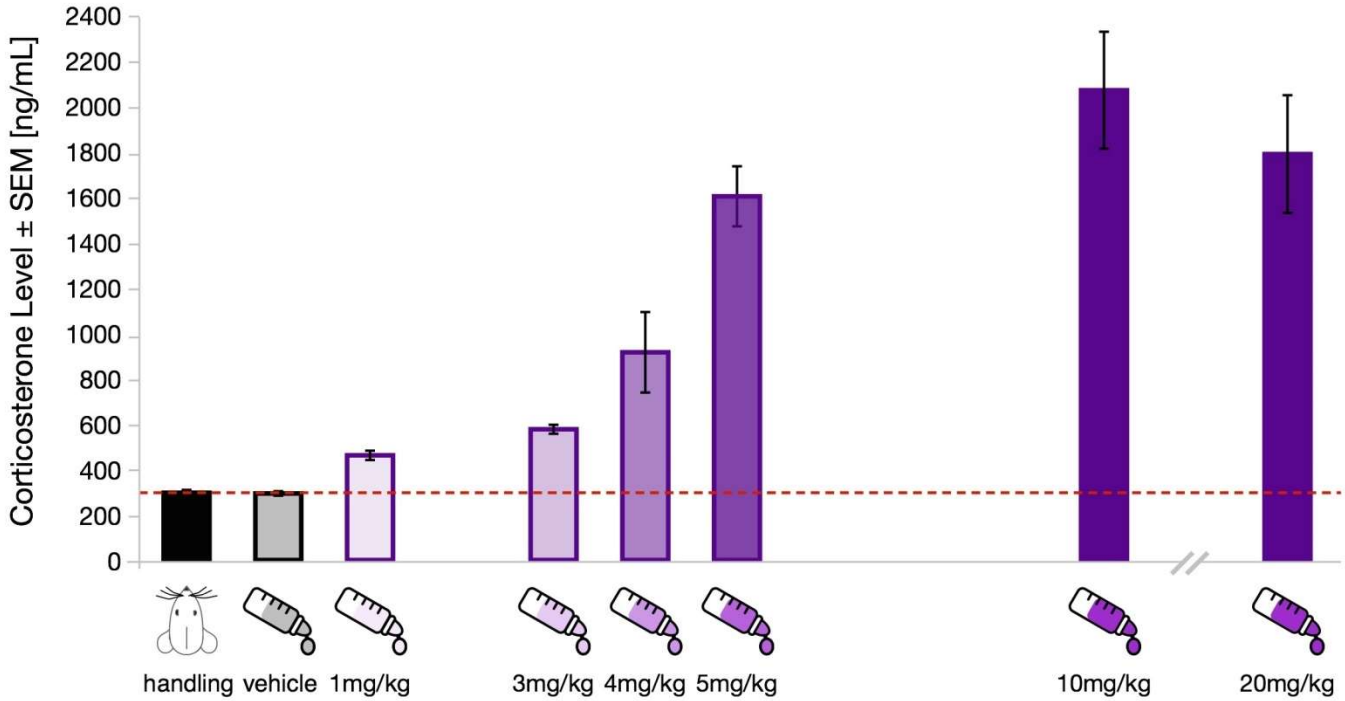


Figure 6. Dose-response curve of oral dosing exogenous corticosterone administration.

Various doses of exogenous corticosterone were administered to determine a dose-response curve of exogenous corticosterone and subsequent serum corticosterone levels, when measured 20 min after administration. The serum corticosterone levels of each group are as follows (n's, ng/mL ± SEM): Handling (n=7, 297.10 ± 12.74), Vehicle (n=6, 294.00 ± 9.96), 1 mg/kg corticosterone (n=5, 462.28 ± 20.50), 3 mg/kg corticosterone (n=9, 577.35 ± 19.81), 4 mg/kg corticosterone (n=4, 917.08 ± 177.70), 5 mg/kg corticosterone (n=6, 1605.82 ± 132.53), 10 mg/kg corticosterone (n=5, 2074.14 ± 258.16). The value for the 20 mg/kg dose (n=4, 1792.35 ± 259.92), which is from the data presented in Figure 5, is provided for reference. A red dashed line set to the vehicle group (gray bar).

6.3. DETERMINATION OF EXOGENOUS CORTICOSTERONE

Comparing the exogenous corticosterone dose response curve to that of the corticosterone levels induced by a forced-swim, the corticosterone increase from baseline levels to 600.53 ± 42.01 ng/mL (vehicle with forced-swim) and 726.62 ± 32.65 ng/mL (appetitive study with forced-swim).

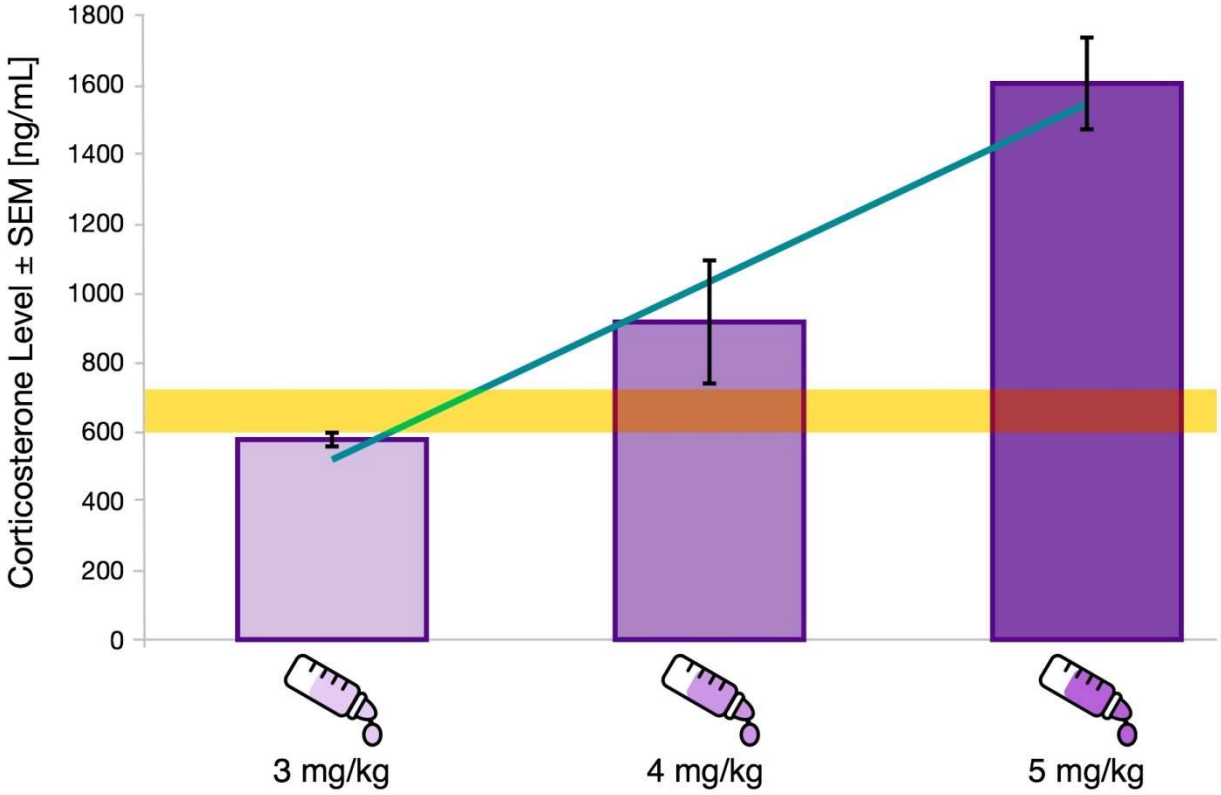


Figure 7. A 3.5 mg/kg dose of exogenous corticosterone raises serum corticosterone levels to that induced by a forced-swim.

Serum corticosterone levels induced by a forced-swim were compared to serum corticosterone levels after administration of exogenous corticosterone. A subset of the data from Figure 6 was selected to focus on the range of 3 to 5 mg/kg doses of exogenous corticosterone, and a trend line was calculated (green line). The range of serum corticosterone levels induced by a forced-swim is generated by combining the data from the appetitively-conditioned L/R discrimination study (Swim group) and a preliminary aversely-conditioned L/R discrimination study (Vehicle plus Swim group) (translucent yellow colored bar). The targeted dose of exogenous corticosterone lies at the intersection of the green line and translucent yellow bar, which corresponds to an approximate dose of 3.2 to 3.4 mg/kg of exogenous corticosterone. A 3.5 mg/kg dose of corticosterone in vehicle was chosen for two reasons: (1) corticosterone levels are highly variable and subject to both circadian and ultradian rhythms, and (2) an error towards a slightly higher dose of corticosterone ensures that the delivered dose exceeds a minimum threshold. Corticosteroid-binding globulin absorbs any increase in corticosterone and may

have prevented levels from exceeding a threshold concentration needed to cross the blood-brain barrier and bind to receptors in the brain in the Shallow group (appetitive study, Figures 3 and 4), and as such precluded the memory enhancement effect as observed subsequent to a forced-swim.

6.4. ACTIONS OF CORTICOSTEROIDS ALONE

To test whether corticosterone alone is sufficient to replicate the observed memory enhancement effect induced by a forced-swim, the L/R discrimination task was used. Although the corticosterone levels were measured using the short training appetitively-conditioned L/R discrimination task, the intensive training aversively-conditioned L/R discrimination task was used because: (1) the intensive training regimen is a more robust paradigm (see Aim 2), and (2) more trials are conducted during retention testing (Reversal Training) in the intensive training aversively-conditioned regimen as compared to the single retention trial of the short training appetitively-conditioned regimen, which provides a better assay to characterize any graded effects of exogenous corticosterone on the expression of a L/R discrimination memory.

The intensive training aversively-conditioned L/R discrimination task was used. Details of the protocol are described in the Materials and Methods section. Briefly, each animal was habituated to a T-maze for 5 min after which they were trained to perform a L/R discrimination task to avoid foot-shocks. After reaching a criterion of 9 of 10 consecutive correct responses, 30 additional trials were given (Day 1, Acquisition Training). One day after learning the L/R discrimination task, one group of rats received an oral dose of 3.5 mg/kg exogenous corticosterone in vehicle and returned to their home cage in a familiar room to avoid any endogenous effects due to novelty (experimental group). After 20 minutes, these rats were returned to the vivarium. One group of control rats received vehicle only (peanut butter oil) (negative control). A second group of control rats received vehicle and were then forced to swim for 20 min (positive control) (Day 2, Stressor). One day after the Stressor treatment, rats were tested for retention by Reversal Training in which the rat must escape to the opposite arm as that on Day 1 (Reversal Arm) to the criterion of four consecutive correct responses (Day 3, Reversal Training).

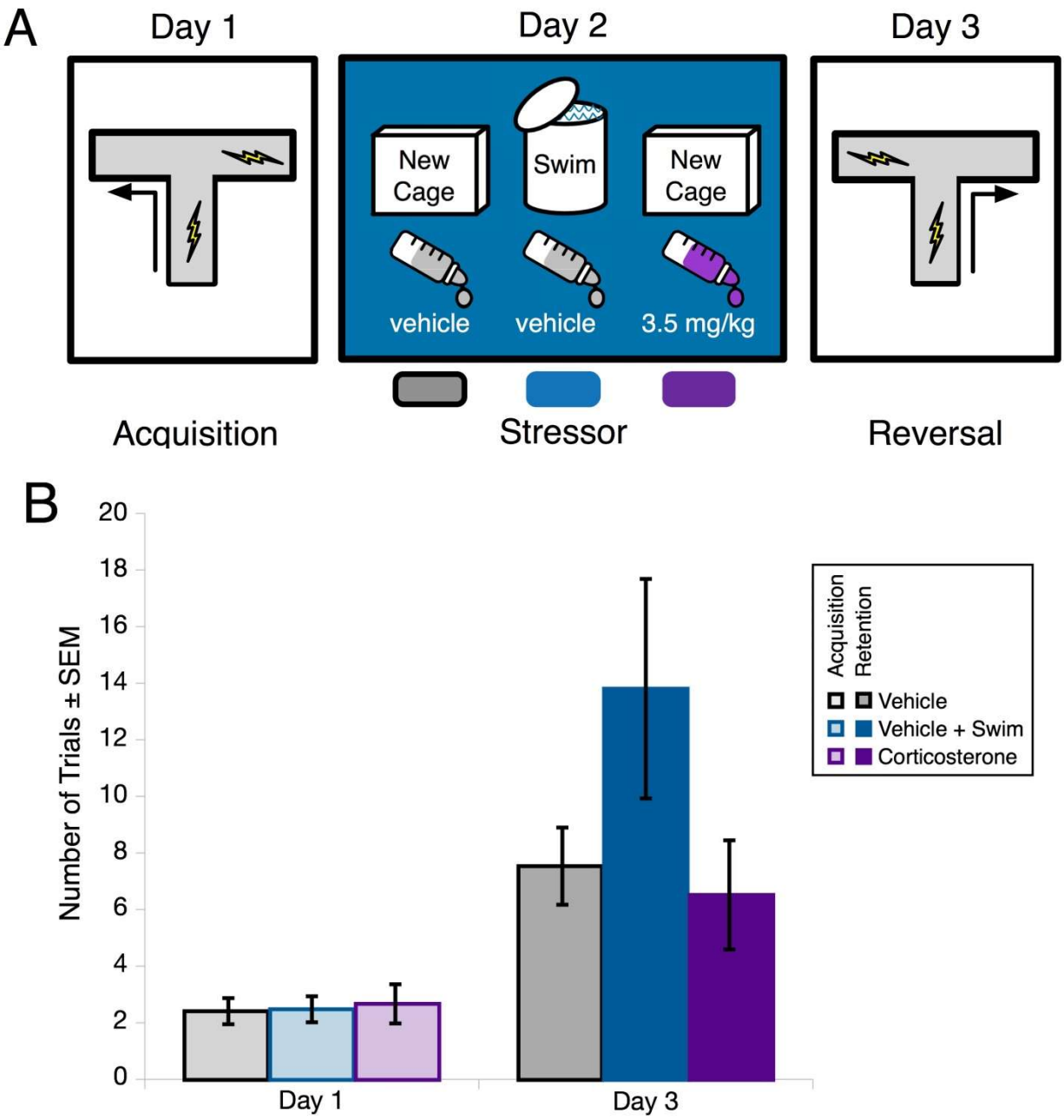


Figure 8. Exogenous corticosterone itself does not replicate the observed memory enhancement effect induced by a forced-swim.

(A) *To determine whether a dose exogenous corticosterone itself could replicate the observed memory enhancement effect induced by a forced-swim, an intensive training aversively-conditioned left/right (L/R) discrimination task with administration of exogenous corticosterone was used. On Day 1, animals were trained to avoid foot-shocks to a criterion of 9/10 consecutive correct trials followed by an additional 30 Acquisition trials. On Day 2, one group of animals received a 3.5 mg/kg dose of exogenous*

corticosterone via oral dosing and then returned to its home cage (experimental group). Another group of animals were treated like the experimental animals but instead received vehicle (negative control group). A third group of animals received vehicle and then subjected to a forced-swim for 20 min (positive control group). On Day 3, animals were tested for retention by Reversal Training in which the safe/shock arms were reversed as compared to that on Day 1.

(B) *There was no difference in the number of Acquisition trials among the Vehicle group (n = 14, 2.57 ± 0.49) as compared to that of the Vehicle + Swim (n = 14, 2.64 ± 0.49) and 3.5 mg/kg Corticosterone (n = 13, 2.85 ± 0.73) groups ($F_{2,38}$ = 0.0606; p = 0.9413). Also, there was no difference in the number of Reversal trials among the Vehicle group (8.00 ± 1.45) as compared to that of the Vehicle + Swim (14.43 ± 4.15) and 3.5 mg/kg Corticosterone (6.92 ± 2.04) groups ($F_{2,38}$ = 2.0638; p = 0.1410). This surprising finding suggests that the forced-swim did not induce a significant increase in the number of Reversal trials in the Vehicle + Swim group as compared to that of the Vehicle group. This finding also suggests that the 3.5 mg/kg dose of exogenous corticosterone did not induce a significant increase in the number of Reversal trials in the 3.5 mg/kg Corticosterone group as compared to that of the Vehicle group. Moreover, there was no difference in the number of Reversal trials in a direct comparison between the Vehicle group to the 3.5 mg/kg Corticosterone group (two-tailed t_{25} = 0.4354; p = 0.6670). The Acquisition and Reversal trials in each group are listed as the number of trials ± SEM.*

Nonetheless, it is possible that adding additional animals may unmask any graded effect of a 3.5 mg/kg dose of corticosterone on the expression of a L/R discrimination memory. A post hoc power analysis showed an achieved power of 0.0703 (protocol of power analysis shown in Table 4 below). This very low power suggests that it is very unlikely that a meaningful difference can be found between the vehicle group and the corticosterone group. An a priori analysis to compute the appropriate sample size confirms this by calculating that over 1000 animals in total are needed to reach significance (protocol of sample size analysis shown in Table 4 below). As such, this analysis supports the assertion that sufficiently elevated corticosterone levels alone do not replicate the observed memory enhancement effect induced by a forced-swim.

It is possible that exogenous corticosterone did not replicate the observed memory enhancement effect induced by a forced-swim because the vehicle + swim group (positive control) did not have the memory enhancement effect as compared to that of the vehicle only group (negative control) as confirmed statistically ($F_{2,38} = 2.0638$; $p = 0.1410$). With an effect size of 0.6138, a post hoc power analysis showed an achieved large power of 0.9325 (protocol of the power analysis is provided in Table 5 below). This high power suggests that it is likely that a meaningful difference could be found, and should have been found, between the vehicle group and the corticosterone group. An a priori analysis to compute required sample size confirms this by calculating that an additional 4 animals in total are needed reach a power of 0.95 (protocol of sample size analysis shown in Table 5 below).

It is surprising that such results that reach a large power fail to reach statistical significance. One possibility is that the vehicle group (negative control) was placed into a new home cage, and this experience may have produce a small, but measurable effect as compared to that of the shallow water group. Animals in this vehicle group required 8.00 ± 1.44 (average ± SEM) trials to reach criterion in Reversal Training (this study). In comparison, animals in the shallow water group required 5.75 ± 3.77 (average ± SEM) trials to reach criterion in Reversal Training (see Aim 2). The averages comparing the vehicle group to the shallow water group do not appear to be different from each other, and statistical analysis supports this observation (two-tailed $t_{28} = 1.3360$; $p = 0.1923$).

A second possibility is that the oral dosing procedure itself may have introduced a small, but measurable variability into the behavioral performance of the vehicle + swim group (positive control). Animals in this vehicle + swim group required 14.43 ± 4.15 (average ± SEM) trials to reach criterion in Reversal Training (this study). In comparison, animals in the forced-swim group required 12.00 ± 2.24 (average ± SEM) trials to reach criterion in Reversal Training (see Aim 2). The averages between the vehicle + swim group to the forced-swim group do not appear to be different from each other, and statistical analysis

supports this observation (two-tailed $t_{27} = 0.5245$; $p = 0.6042$). Nonetheless, the variability in the vehicle + swim group has a much higher SEM as compared to that of the forced-swim group (4.15 vs 2.24).

Table 1. T Test Analysis

Post-hoc analysis to compute achieved power.

t tests - Means: Difference between two independent means (two groups) Analysis: Post hoc: Compute achieved power			
Input:	Tail(s)	=	Two
	Effect size d	=	0.1677
	α err prob	=	0.05
	Sample size group 1	=	14
	Sample size group 2	=	13
Output:	Noncentrality parameter δ	=	0.4353984
	Critical t	=	2.0595386
	Df	=	25
	Power (1-β err prob)	=	0.0703352

A priori analysis to compute required sample size.

t tests - Means: Difference between two independent means (two groups) Analysis: A priori: Compute required sample size			
Input:	Tail(s)	=	Two
	Effect size d	=	0.1677
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Noncentrality parameter δ	=	2.8061577
	Critical t	=	1.9620881
	Df	=	1118
	Sample size group 1	=	560
	Sample size group 2	=	560
	Total sample size	=	1120

Actual power = 0.8006055

Table 2. F Test Analysis

Post-hoc analysis to compute achieved power.

F tests - ANOVA: Fixed effects, omnibus, one-way Analysis: Post hoc: Compute achieved power			
Input:	Effect size f	=	0.6138
	α err prob	=	0.05
	Total sample size	=	41
	Number of groups	=	3
Output:	Noncentrality parameter λ	=	15.4467680
	Critical F	=	3.2448184
	Numerator df	=	2
	Denominator df	=	38
	Power (1-β err prob)	=	0.9324054

A priori analysis to compute required sample size.

F tests - ANOVA: Fixed effects, omnibus, one-way Analysis: A priori: Compute required sample size			
Input:	Effect size f	=	0.6138
	α err prob	=	0.05
	Power (1-β err prob)	=	0.95
	Number of groups	=	3
Output:	Noncentrality parameter λ	=	16.9537698
	Critical F	=	3.2199423
	Numerator df		2
	Denominator df		42
	Total sample size	=	45
	Actual power	=	0.9539495

6.5. SUFFICIENCY OF CORTICOSTERONE

Although there have been mixed results and no definitive conclusions, an increasing body of knowledge suggest that administering either stress or exogenous corticosteroids shortly before or shortly after acquisition enhances the process of consolidation of that memory whereas administering either stress or corticosteroids shortly before recall impairs the retrieval of that previously acquired information (McGaugh and Roozendaal, 2002). While the role of corticosteroids around these time points have been extensively studied, there has been little investigation on what corticosteroids do in the absence of any overt acquisition of retrieval.

There are several possibilities as to why delivery of exogenous corticosterone did not produce an enhancement in the expression of a L/R discrimination memory. One explanation is that the dose of exogenous corticosterone produces a variable level of serum corticosterone, which is further influenced by an ultradian cycle. However, with a relatively large sample size and a very low power compared to vehicle (power = 0.0703352), the variable level of serum corticosterone that is further influenced by an ultradian cycle becomes a less likely reason that would explain why corticosterone alone would not induce an enhancement of the expression of a L/R discrimination memory.

A second explanation is that exogenous corticosterone does not mimic the normal physiological response during a forced-swim.

A third explanation is the 3.5 mg/kg dose of corticosterone that was used was an insufficient concentration, and that a higher concentration should have been used.

A fourth explanation is that corticosterone itself does not induce an enhancement of the expression of a L/R discrimination memory. For example, Borrell and colleagues showed that administering corticosterone or dexamethasone alone in the absence of catecholamines to adrenalectomized rats had no effect on the behavioral performance on a passive avoidance task (Borrell et al, 1984). The role of

dexamethasone as well as the possibility that corticosteroids act in conjunction with catecholamines will each be discussed in greater detail in the Discussion section.

6.6. SUMMARY

If corticosterone is necessary for the swim-induced enhancement of memory, is it also sufficient to induce the memory enhancement effect observed subsequent to a forced-swim? This aim was designed to characterize the role of corticosterone alone in modifying the expression of previously acquired memories by delivering corticosteroids with minimal impact on endogenous levels of catecholamines. As such, a 3.5 mg/kg dose of exogenous corticosterone that was used in the oral dosing experiment was selected in part because this dose produces a serum corticosterone level that is higher than that induced by the forced-swim. As such, if there is no significant effect due to administering exogenous corticosterone, delivery of an insufficient dose can be ruled out. In comparison to the vehicle group, the administration of corticosterone itself did not have any appreciable effect (two-tailed $t_{25} = 0.4354$; $p = 0.6670$). The forced-swim experience induces a stress response in which corticosteroids are necessary for the subsequent enhancement of the expression of a L/R discrimination memory as observed during a retention test a day later. The results of the oral dosing behavioral task, which was designed to deliver corticosterone alone without any of the confounding factors such as catecholamines that may also be released during a stress response, suggest that corticosteroids alone are not sufficient to induce the observed memory enhancement effect.

Although the positive control did not reach statistical significance, there is a trend towards enhanced expression of a L/R discrimination memory. The effect size is quite large (effect size $f = 0.6138$), and as such it is plausible that with a few additional samples, this group would reach statistical significance. Nonetheless, the question of whether corticosteroids alone are sufficient to induced an enhanced expression of a L/R discrimination memory still remains. A t-test comparison of the vehicle to corticosterone only group shows a low effect size (effect size $d = 0.1677$) and a very low power (power =

0.0703352). With such a low effect size and power, an incredibly large sample size would be needed (total n > 1000) to reach statistical significance between vehicle and corticosterone alone.

7. Time-Limited Window Of Stress-Induced Modification Of Memories

I investigated whether there is a time-limited window within which swim-stress can enhance the expression of an aversively-conditioned left/right (L/R) discrimination memory. According to the lingering consolidation hypothesis (Dudai and Eisenberg, 2004), an acquired memory progressively undergoes consolidation with time such that the stability of the memory gradually increases and becomes more resilient to disruption in a kind of temporal gradient (Milekic and Alberini, 2002).

Previous experiments showed that propranolol, a non-selective beta-adrenergic receptor antagonist, had no effect on an inhibitory avoidance memory one day after acquisition training, which suggests that the memory was no longer labile (Przybyslawski et al, 1999). Propranolol had no effect if administered in the absence of a forced-swim, but unexpectedly, propranolol had an impairing effect on an inhibitory avoidance memory if administered shortly after a forced-swim. This suggests that the forced-swim had induced a memory to become labile, and consequently, susceptible to the amnesic effects of propranolol (Ježek, Lee et al, 2010).

Moreover, if the L/R discrimination memory is still consolidating, then administering the amnesic treatment electro-convulsive shock (ECS) 24h after training should impair retention (Misanin et al, 1968). As such, ECS was administered to determine whether the L/R discrimination memory was still labile and undergoing consolidation 24h after training. As with propranolol, ECS did not alter the expression of memory in the absence of a forced-swim, which indicates that the L/R discrimination memory was not labile and thus not consolidating 24h after training. But surprisingly, L/R discrimination memory became sensitive to ECS when administered shortly after the forced-swim. This effect of ECS was not observed when ECS was administered 5h after the forced-swim (Ježek, Lee et al, 2010).

These two pieces of evidence suggests that the forced-swim induces a stable memory to become labile such that the memory can be disrupted or influenced by amnesic treatments such as propranolol and

ECS. This raises the question of whether a forced-swim can have a similar impact on the expression of a memory if the time interval between the training and forced-swim is increased.

7.1. T-MAZE RESULTS AND Y-MAZE RESULTS ARE COMPARABLE

The intensive training aversively-conditioned L/R discrimination task had used a Y-maze to create a robust memory of the L/R discrimination task (Ježek, Lee et al, 2010). Moreover, the control group was a non-swim condition that was neither exposed to the same bucket as the forced-swim group nor exposed to any water. I repeated the experiments for three reasons: (1) extend the findings of Aim 1, which had used a T-maze, by using an aversively-conditioned task, (2) use a more appropriate control group (shallow water) rather than a non-swim control that was conducted in the vivarium, and (3) confirm that the observed findings were robust by replicating the results of previous experiments.

The intensive training aversively-conditioned L/R discrimination task is used. Details of the protocol are described in the Materials and Methods section. Briefly, each animal was habituated to a T-maze for 5 min after which they were trained to perform a L/R discrimination task to avoid foot-shocks. After reaching a criterion of 9 of 10 consecutive correct responses, 30 additional trials were given (Day 1, Acquisition Training). One day after learning the L/R discrimination task, one group of rats was forced to swim for 20 min, while one group of control rats was placed in 1 cm of water for 20 min (Day 2, Stressor). One day after the Stressor treatment, rats were tested for retention by Reversal Training in which the rat must escape to the opposite arm as that on Day 1 (Reversal Arm) to the criterion of four consecutive correct responses (Day 3, Reversal Training).

Animals that received the forced-swim needed significantly more trials to switch arms than those that received the shallow water (Figure 9). The forced-swim group showed an enhanced expression of the L/R discrimination similar to the findings previously published (Ježek, Lee et al, 2010), and the results suggest that a forced-swim modifies previously acquired memories towards enhancing the expression of that memory regardless of whether the training regimen was appetitively-conditioned (Figures 3 and 4) or

aversively-conditioned (Figure 9). Moreover, these findings also show that a forced-swim can still enhance the expression of a memory even though the intensive training regimen was much more robust than the short training regimen (Figure 9).

Second, the non-swim control group used in this study (shallow water) did not show an enhancement of the expression of the L/R discrimination memory similar to those that were simply handled in the vivarium as a non-swim control group (Ježek, Lee et al, 2010). Although the shallow water group showed elevated levels of corticosterone (Figure 4), these findings suggest that many of the aspects that the rats in the forced-swim group experienced including the transport to the stressor environment, the novelty of the bucket, and contact with water did not have an appreciable impact on the expression of the previously acquired L/R discrimination memory. Moreover, animals in the shallow water group experience many of the same aspects that the animals in the forced-swim group experience, except for the forced-swimming. Using the same methods as was described earlier, the shallow water group was used as the non-swim control group in these experiments.

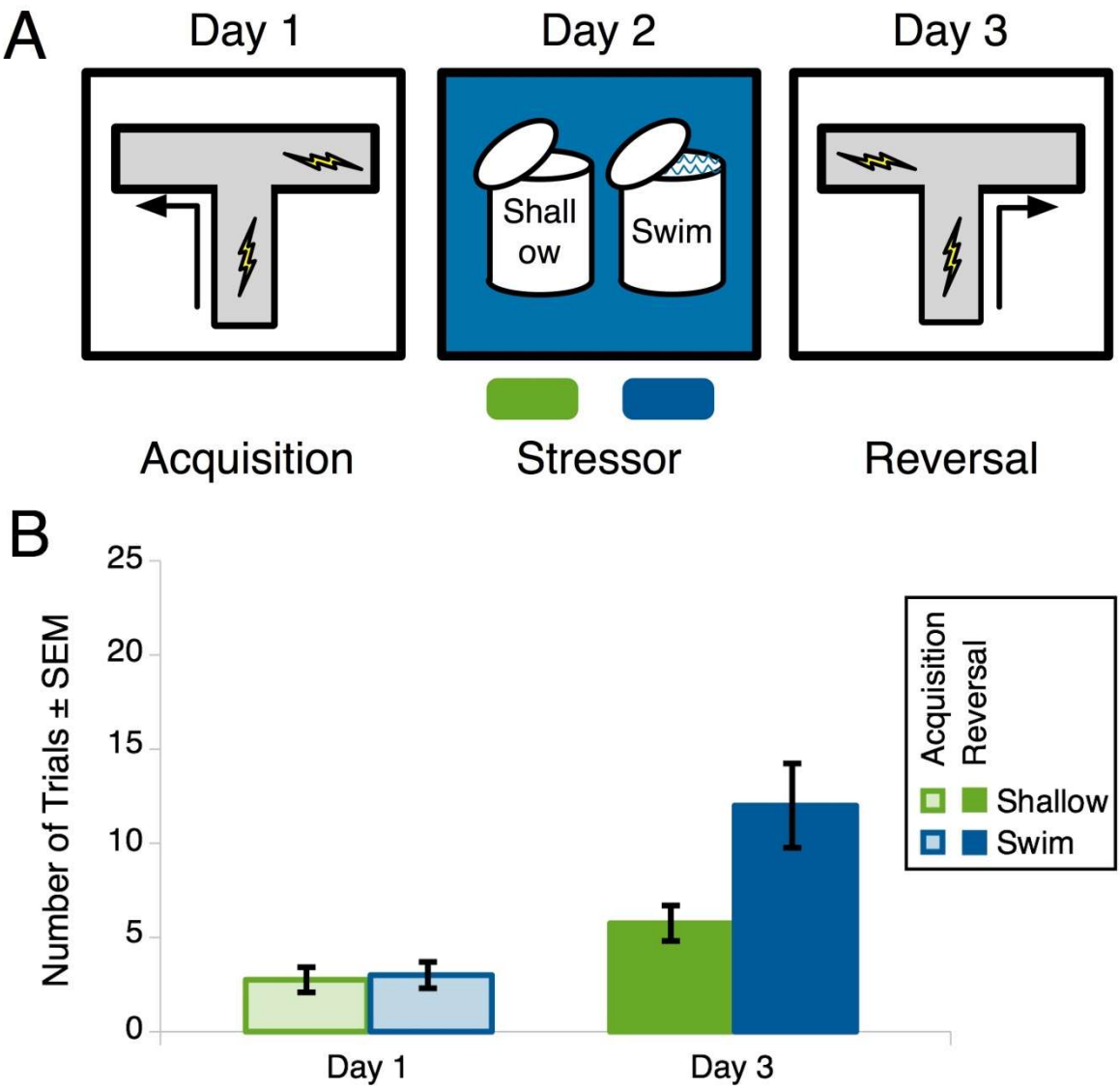


Figure 9. Forced-swim enhanced the expression of an intensive training aversively-conditioned left/right discrimination task.

(A) *To determine whether a forced-swim could replicate the observed memory enhancement effect in a Y-maze, an intensive training aversively-conditioned left/right (L/R) discrimination task in a T-maze was used. On Day 1, animals were trained to avoid foot-shocks to a criterion of 9/10 consecutive correct trials followed by an additional 30 Acquisition trials. On Day 2, one group of animals was placed in a bucket with 1 cm of water (Shallow, n = 16) whereas the other group of animals was placed in a bucket and forced to swim (Swim, n = 15). On Day 3, animals were tested for retention by Reversal Training in*

which the safe/shock arms were reversed as compared to that on Day 1. More errors (rats entering Acquisition Arm) during Reversal Training indicate better retention of Day 1 memory.

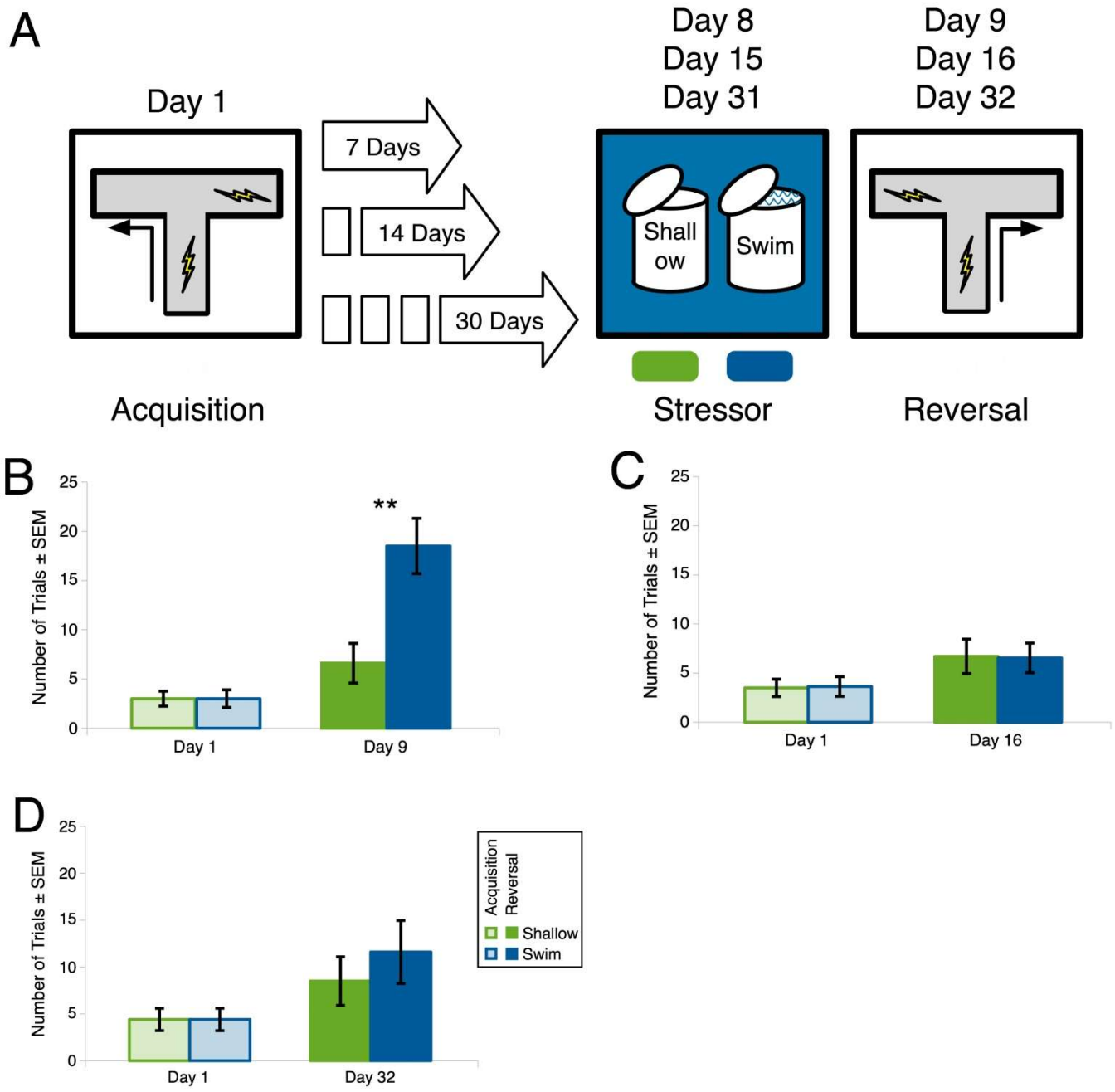
(B) *There was no difference in the number of Acquisition trials between the Shallow group (2.75 ± 0.67) and the Swim group (3.00 ± 0.70) during Acquisition Training on Day 1 (two-tailed t_{29} = 0.2591; p = 0.7974). In contrast, number of Reversal trials was significantly higher in Swim group (12.00 ± 2.24) as compared to that of the Shallow group (5.75 ± 0.94) during Reversal Training on Day 3 (two-tailed t_{29} = 2.6310; p = 0.0135). This finding suggests that the forced-swim enhanced the expression of an intensive training aversively-conditioned left/right discrimination memory in a T-maze, which the observed memory enhancement effect in a Y-maze. The Acquisition and Reversal trials in each group are listed as number of trials ± SEM.*

7.2. SWIM-STRESS CAN MODIFY THE EXPRESSION OF MEMORIES WITHIN A TIME-LIMITED WINDOW

Using an Inhibitory Avoidance (IA) task, Milekic and Alberini showed that anisomycin, a protein synthesis inhibitor, significantly impaired IA memory when that memory was reactivated at 2 days or 7 days after training, but had no effect when IA memory was reactivated at 14 days or 28 days after training (Milekic and Alberini, 2002). These findings predict that swim-stress could enhance the expression of a L/R discrimination memory if that memory is less than 14-days-old. To chart the time course of the memory enhancement effect of the forced-swim, a similar experimental approach was used in the current project in which the following time intervals between the L/R discrimination training and the Stressor treatment were added: 7 days, 14 days, and 30 days.

The group that experienced a 7 day interval between the Acquisition Training and the forced-swim treatment needed significantly more Reversal Training trials on Day 9 as compared to that of the group that received the shallow water treatment (Figures 10 and 12). In contrast, neither group that experienced a 14 day or 30 day interval between the Acquisition Training and the forced-swim treatment showed any difference in the number of Reversal Training trials on Day 16 or Day 32, respectively, as

compared to that of the group that received the shallow water treatment. These results suggest that the memory enhancement effect of the forced-swim on memory occurs within a time-limited window such that memories that are at least one-week-old can become enhanced whereas the expression of memories that are two-weeks and older become more resistant to stress-induced modification. This time-limited window of at least 1 day but less than 2 weeks is consistent with findings in the literature on



consolidation (Milekic and Alberini, 2002; Inda et al, 2011).

Figure 10. Swim-stress enhanced the expression of recent but not remote left/right (L/R) discrimination memories.

(A) *To determine whether a forced-swim could induce the observed memory enhancement for progressively older memories, an intensive training aversively-conditioned left/right (L/R) discrimination task was used in which the time interval between the training and the stressor was modified. On Day 1, animals were trained to avoid foot-shocks to a criterion of 9/10 consecutive correct trials followed by an additional 30 Acquisition trials. On Day 8, 15, or 31, which corresponds to the interval of time between the Acquisition Training and Stressor of 7 days, 14 days, and 30 days, respectively, one group of animals was placed in a bucket with 1 cm of water (Shallow, Day 8 n = 10; Day 15 n = 10; Day 31 n = 10) whereas the other group of animals was placed in a bucket and forced to swim (Swim, Day 8 n = 10; Day 15 n = 11; Day 31 n = 10). On Day 9, 16, or 32, animals were tested for retention by Reversal Training in which the safe/shock arms were reversed as compared to that on Day 1. More errors (rats entering Acquisition Arm) during Reversal Training indicate better retention of Day 1 memory.*

(B) *There was no difference in the number of Acquisition trials between the Shallow group (3.00 ± 0.76) and the Swim group (3.00 ± 0.89) during Acquisition Training on Day 1 (two-tailed $t_{18} = 0.0000$; $p = 1.0000$). In contrast, number of Reversal trials was significantly higher in Swim group (6.60 ± 2.01) as compared to that of the Shallow group (18.50 ± 2.81) during Reversal Training on Day 9 (two-tailed $t_{18} = 3.4466$; $p = 0.0029$). This finding suggests that the forced-swim enhanced the expression of a 7-day-old L/R discrimination memory. The Acquisition and Reversal trials in each group are listed as number of trials ± SEM.*

(C) *There was no difference in the number of Acquisition trials between the Shallow group (3.50 ± 0.89) and the Swim group (3.64 ± 1.00) during Acquisition Training on Day 1 (two-tailed $t_{19} = 0.1011$; $p = 0.9205$). There was also no difference in the number of Reversal trials between the Shallow group (6.70 ± 1.75) and the Swim group (6.55 ± 1.51) during Reversal Training on Day 16 (two-tailed $t_{19} = 0.0672$; $p = 0.9471$). This finding suggests that the forced-swim did not enhance the expression of a 14-day-old L/R discrimination memory.*

(D) *There was no difference in the number of Acquisition trials between the Shallow group (4.40 ± 1.19) and the Swim group (4.40 ± 1.19) during Acquisition Training on Day 1 (two-tailed $t_{18} = 0.0000$; $p = 1.0000$). There was also no difference in the number of Reversal trials between the Shallow group (8.50 ± 2.59) and the Swim group (11.60 ± 3.36) during Reversal Training on Day 32 (two-tailed $t_{18} = 0.7300$; $p = 0.4748$). This finding suggests that the forced-swim did not enhance the expression of a 30-day-old L/R discrimination memory.*

7.3. TRAINING REGIMEN CREATES A ROBUST MEMORY

An alternative interpretation of the findings in which there is no difference in the number of Reversal Training trials on Day 16 or Day 32 is that intensive training regimen on Day 1 did not create a sufficiently robust memory that persists for at least two weeks. If this is the case, then the animals would choose either arm with equal probability. In other words, the animals would behave as if naïve during the Reversal Training on Day 16 or Day 32. Conversely, if the intensive training regimen on Day 1 did create a sufficiently robust memory that persisted for at least two weeks, then the animals would be biased in choosing the arm designated safe during Acquisition Training. As such, an analysis of the first trial during Reversal Training one month after training (Day 32) was conducted to determine whether the intensive training regimen on Day 1 created a sufficiently robust, and persistent, memory.

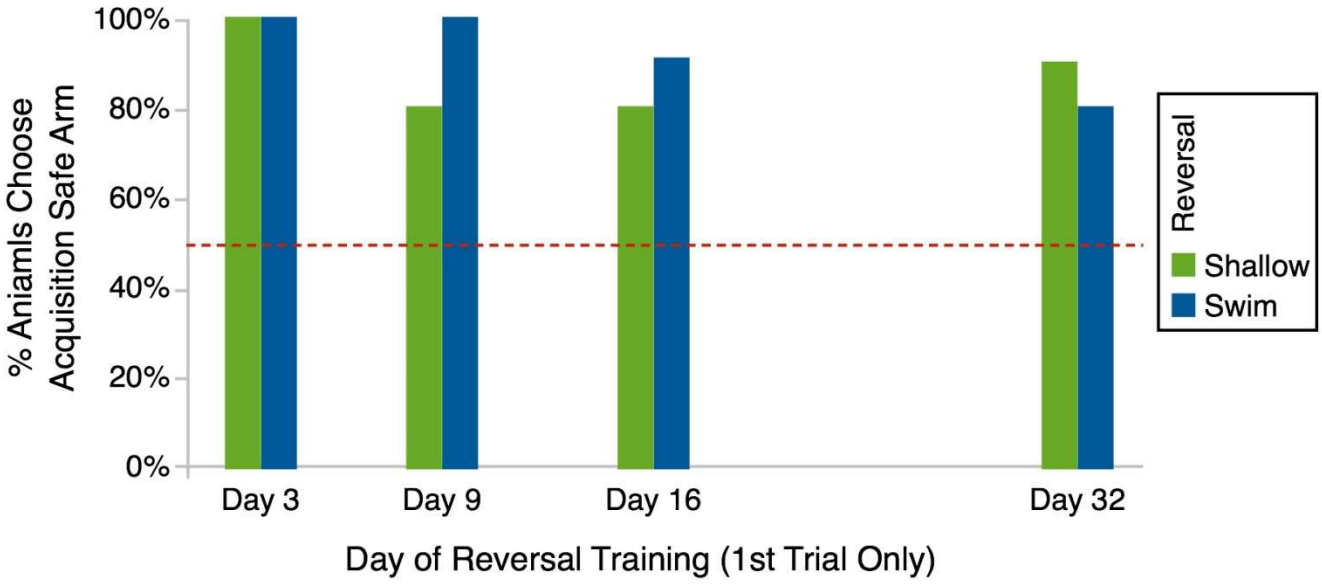


Figure 11. Intensive training aversively-conditioned left/right discrimination task creates a robust memory that persist for a month.

To determine whether the intensive training aversively-conditioned left/right (L/R) discrimination task produces a sufficiently robust memory that persist for a month, the first trial during Reversal Training from the data presented in Figure 10 was analyzed. During Reversal Training on Day 3, 31/31 animals

(Shallow group = 16/16, Swim group = 15/15) chose the same arm as that acquired during Acquisition Training on the first trial (test of proportions, two-tail z = 4.5337; p = 0.0000). During Reversal Training on Day 9, 18/20 animals (Shallow group = 8/10, Swim group = 10/10) chose the same arm as that acquired during Acquisition Training on the first trial (test of proportions, two-tail z = 2.7603; p = 0.0058). During Reversal Training on Day 16, 18/21 animals i(Shallow group = 8/10, Swim group 10/11) chose the same arm as that acquired during Acquisition Training on the first trial (test of proportions, two-tail z = 2.4564; p = 0.0139). During Reversal Training on Day 32, 17/20 animals (Shallow group = 9/10, Swim group = 8/10 (test of proportions, two-tail z = 2.3631; p = 0.0183). These findings suggest that the intensive training aversively-conditioned L/R discrimination regimen produces a robust memory that is sufficient to persist for at least one month after Acquisition. This eliminates the possibility that the forced-swim had no enhancement effect on Day 16 and Day 32 as compared to that of the shallow water group because the rats did not remember what they had previously acquired. As such, the remaining possibility is that the forced-swim had no impact on memories outside of a time-limited window. A red dashed line is set at the level of chance, which indicates that the memory produced during Acquisition Training on Day 1 was not sufficiently robust to persist for that particular time interval.

7.4. SUMMARY

I investigated whether swim-stress could enhance the expression of recent and remote L/R discrimination memories. The age of the memory may play a role in whether a memory can be activated and subsequently enhanced (Suzuki et al, 2004). It is possible that the memory could still be made labile, but that the forced-swim only strengthened memories that were younger than two-weeks-old. These findings are consistent with the lingering consolidation hypothesis and the findings of the Alberini lab (Milekic and Alberini, 2002; Inda et al, 2011).

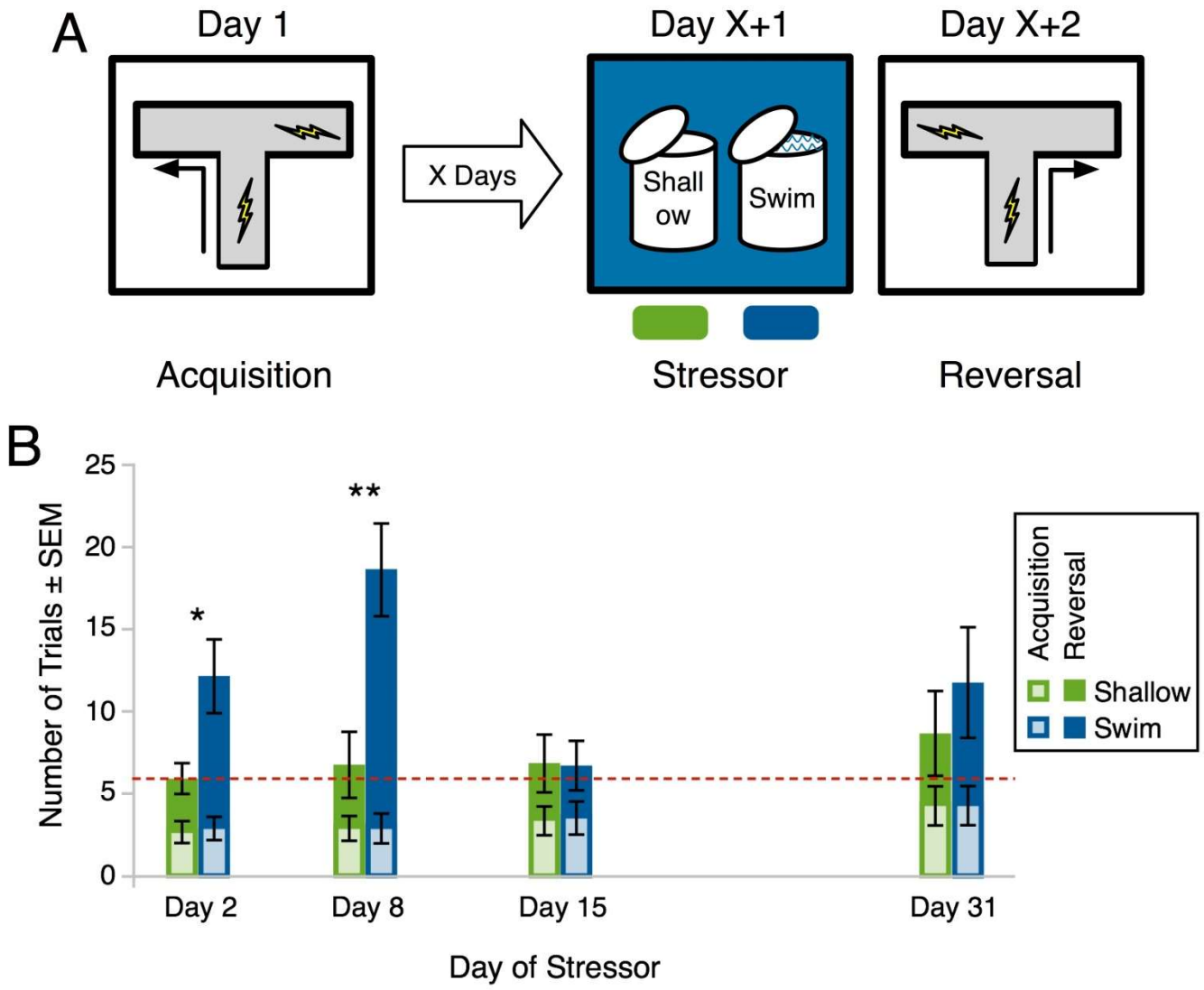


Figure 12. Swim-stress enhanced the expression of recent memories but not remote memories.

(A) *Diagram of the behavioral protocol in which the interval between Acquisition Training and the Stressor varied from 1 day, 7 days, 14 days, and 30 days.*

(B) *The number of trials needed to reach criterion of a left/right (L/R) discrimination task during Acquisition Training and Reversal Training are shown. Translucent bars show the number of trials needed to reach criterion during Acquisition Training on Day 1 (criterion of 4 consecutive correct responses). The day of the Stressor (x-axis) is conducted one or more days after Acquisition (Day 2, Day 8, Day 15, or Day 31, respectively). Solid bars show the number of trials needed to reach criterion*

during Reversal Training one day after the Stressor (Day 3, Day 9, Day 16, or Day 32, respectively) (criterion of 4 consecutive correct responses). These findings show that swim-stress can modify recent memories within a time-limited window up to at least one week after the original learning experience (forced-swim 1 day after training: $t_{28} = 2.7127$; $p = 0.0113$; forced-swim 7 days after training: $t_{18} = 3.4466$; $p = 0.0029$) but had no observable impact on remote memories that were two-weeks-old and older (forced-swim 14 days after training: $t_{19} = 0.0672$; $p = 0.9471$; forced-swim 30 days after training: $t_{18} = 0.73$; $p = 0.4748$) at the time of the forced-swim.

8. Swim-Stress Effects On Hippocampus-Dependent Memory Expression

I investigated whether corticosteroids that are released during swim-stress, which act on the hippocampus, can enhance the expression of hippocampus-dependent memories. Cognitive theory asserts that animals solve a L/R discrimination task by approaching the arm in the same spatial location that was reinforced during training whereas stimulus-response theory asserts that animals solve a L/R discrimination task by approaching the arm with the same body turn that was reinforced during training (Tolman, 1948; Restle, 1957). The seminal work by Packard and McGaugh who showed that rats initially prefer using a spatial strategy and later switch to response strategy even as both spatial learning and response learning occur concurrently (Packard and McGaugh, 1996).

Previous findings show that temporarily inactivating the hippocampus bilaterally with TTX prior to the forced-swim blocked the observed memory enhancement effect, which suggests that the hippocampus is necessary for the swim-induced enhancement of memory expression (Ježek, Lee et al, 2010). Moreover, temporarily inactivating the hippocampus bilaterally with TTX prior to acquisition or retrieval of the memory did not impair the performance of a L/R discrimination task (Ježek, Lee et al, 2010), suggesting that a response strategy can be employed for either acquisition or retrieval of the task. These findings are consistent with the conclusions put forth by Packard and McGaugh (Packard and McGaugh, 1996) as well as the theory of multiple parallel memory systems in the rat brain proposed by White and McDonald (White and McDonald, 2002).

Since two different strategies can be employed to solve a L/R discrimination task, this leads to four possible outcomes:

Table 3. Enhanced expression of hippocampus-dependent or striatum-dependent memories

The different possible scenarios in which the stress induced by the forced-swim enhances the expression of memories that are either hippocampus-dependent or striatum-dependent.

Scenario	Hippocampus-dependent memories become enhanced	Striatum-dependent memories become enhanced
Scenario 1	√	√
Scenario 2	√	X
Scenario 3	X	√
Scenario 4	X	X

- Scenario 1: Swim-stress enhances the expression of both hippocampus-dependent and striatum-dependent memories.
- Scenario 2: Swim-stress enhances the expression of hippocampus-dependent but does not enhance the expression of striatum-dependent memories.
- Scenario 3: Swim-stress does not enhance the expression of hippocampus-dependent but enhances the expression of striatum-dependent memories.
- Scenario 4: Swim-stress does not enhance the expression of either hippocampus-dependent or striatum-dependent memories.

During the retention test, the escape arm could be selected with either the spatial or response strategy, and since the forced-swim raises corticosteroid levels that would target multiple brain regions simultaneously, the aversively-conditioned L/R discrimination task makes it difficult to determine whether the forced-swim enhanced the expression of memory for spatial learning (scenario 2) or enhanced the memory for response learning (scenario 3). The solution that Packard and McGaugh devised to distinguish between memory for place learning and memory for response learning was to conduct a probe trial in which the T-maze was rotated 180° with respect to the room (Packard and McGaugh, 1996). However, this method is inadequate at distinguishing the possibility that swim-stress enhances the expression of both memories (scenario 1) from the other possibility that swim-stress enhances neither memory (scenario 4). In other words, even if swim-stress enhanced the expression of both

memories, the results would appear the same as if swim-stress enhanced neither the expression of spatial memories or response memories (scenario 4).

Alternately, one may want to use a task that is predominantly dependent on one of the two strategies, and its attributed brain region. Since the findings suggest that the hippocampus is necessary for the swim-induced enhancement of memory (Ježek, Lee et al, 2010), a task that depends on the hippocampus, rather than a task that depends on the striatum, would be a better approach. An ideal paradigm is the active place avoidance task.

The active place avoidance task requires an animal to avoid a specific spatial location on a rotating arena that are demarcated by cues on the room wall. Hippocampal activity is necessary for effective performance on this task as pre- and post-training bilateral (Cimadevilla et al, 2000) or unilateral inactivation (Cimadevilla et al, 2001) of the hippocampus with TTX impairs acquisition and retention of this paradigm. Moreover, Fajnerova and colleagues showed that in the absence allocentric cues, rats were not able to efficiently solve the task. A small number of animals developed an alternative strategy that relied on idiothetic navigation and/or stereotypic motor behavior after extensive training, but only if the animal had received prior training with visible room-bound cues (Fajnerova et al, 2014).

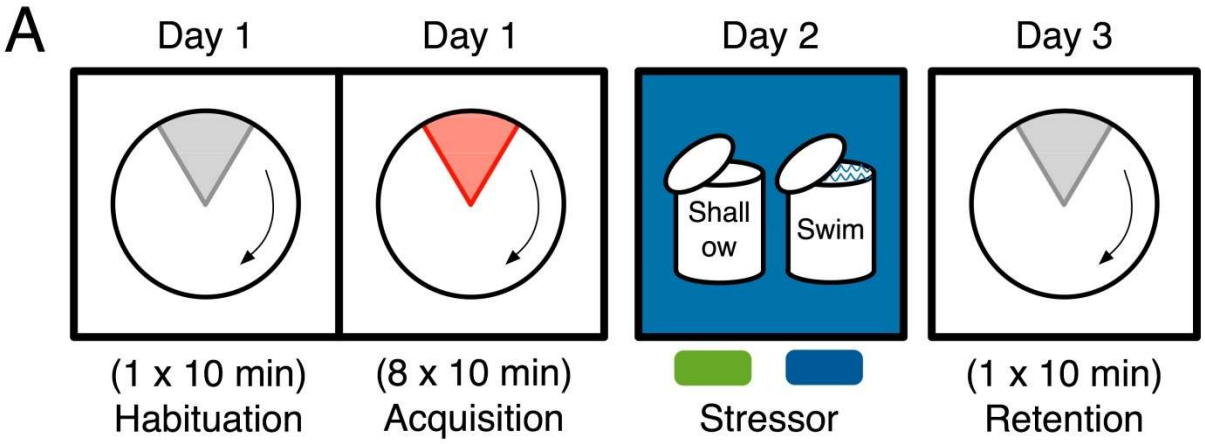
The working hypothesis is that if swim-stress enhances the expression of all memories including both place strategy and response strategy memories, then the expectation is that swim-stress will enhance the expression of a spatial strategy memory for the active place avoidance task like it did for the L/R discrimination task.

8.1. SWIM-STRESS DOES NOT ENHANCE ROBUST HIPPOCAMPUS-DEPENDENT MEMORY EXPRESSION

Although hippocampal activity is necessary during the forced-swim to induce the enhancement of memory (Ježek, Lee et al, 2010), it is unknown whether the forced-swim specifically enhances the expression of hippocampus-dependent memories. The active place avoidance task, a task in which the acquisition (Cimadevilla et al, 2001) and retention (Fajnerova et al, 2014; Pastalkova et al, 2006) critically depend upon the hippocampus, will facilitate the assessment as to whether the forced-swim enhances the expression of spatial memories.

The intensive training (eight trials) active place avoidance task (Pastalkova et al, 2006) was used (Figure 13). Details of the protocol are described in the Materials and Methods section. Briefly, each animal was habituated to the rotating arena without foot-shock for a single 10-min trial after which they were trained to avoid an invisible and stationary 60° sector shock area oriented according to distal cues on the walls of the room (Day 1, Acquisition). One day after learning the active place avoidance task, one group of rats was forced to swim for 20 min, while one group of control rats was placed in 1 cm of water for 20 min (Day 2, Stressor). One day after the Stressor treatment, rats were tested for retention by a single 10-min trial without foot-shock (Day 3, Retention).

The results show no differences between the forced-swim or shallow water groups in either the number of entrances into the shock zone (Figure 13), or in the latency-to-1st-entrance into the shock zone during retention (Figure 13).


A
Day 1
Day 1
Day 2
Day 3
Shall ow
Swim
(1 x 10 min)
Habituation
(8 x 10 min)
Acquisition
Stressor
(1 x 10 min)
Retention

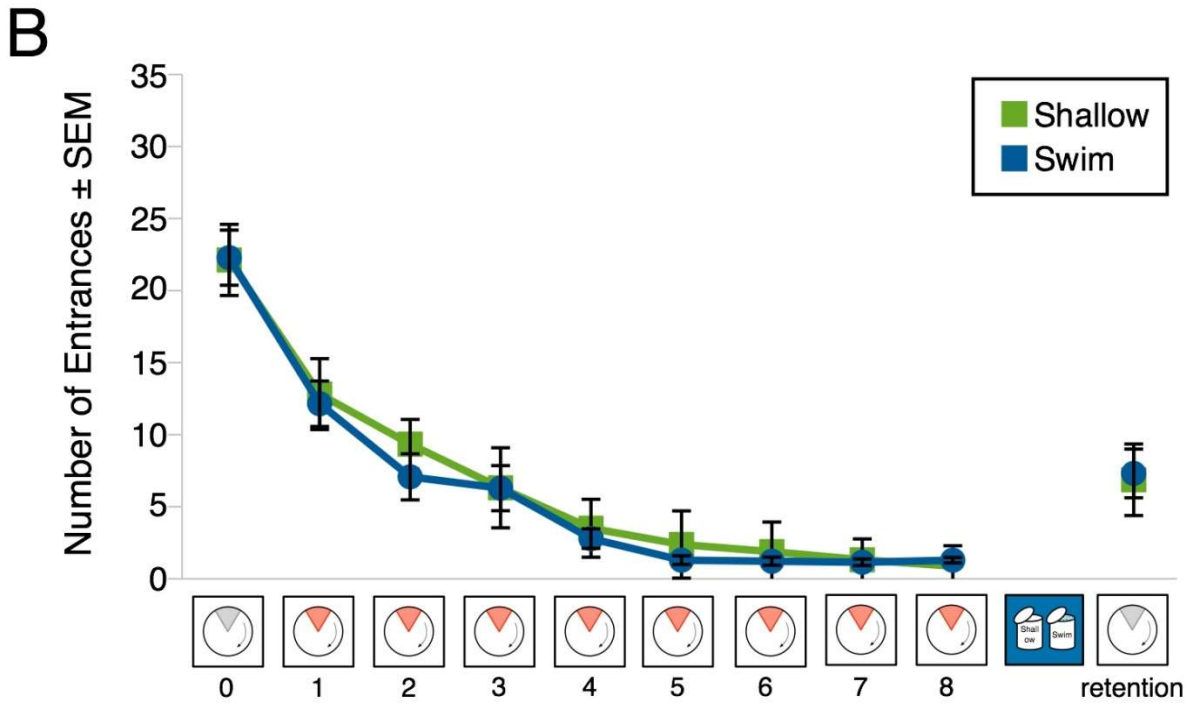
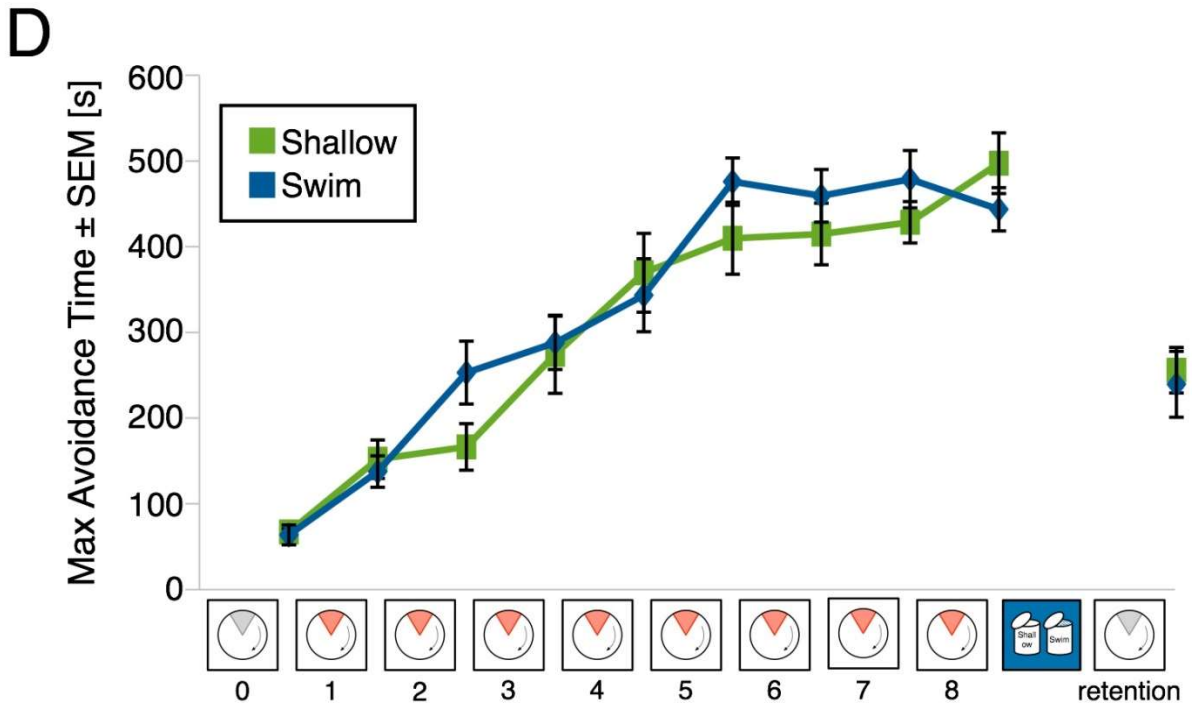
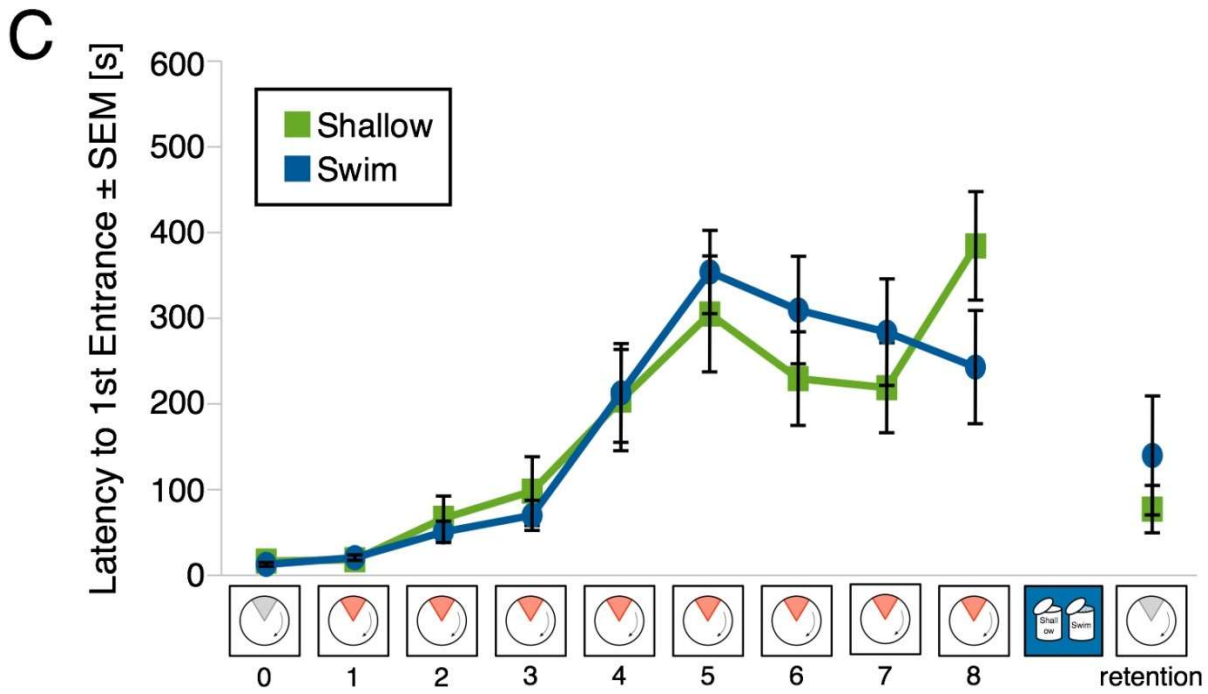

B
Number of Entrances ± SEM
35
30
25
20
15
10
5
0
Shallow
Swim
0
1
2
3
4
5
6
7
8
retention

Figure 13. Forced-swim did not enhance memory expression of an intensive training aversively-conditioned active place avoidance task.

(A) *To determine whether a forced-swim could induce the observed memory enhancement for a hippocampus-dependent task, an intensive training aversively-conditioned place avoidance task was used. On Day 1, animals were trained to avoid an invisible and stationary 60° sector shock area on a rotating arena that was oriented according to distal cues on the walls of the room during eight 10-min trials. On Day 2, one group of animals was placed in a bucket with 1 cm of water (Shallow, n = 14) whereas the other group of animals was placed in a bucket and forced to swim (Swim, n = 13). On Day 3, animals were tested for retention by a single 10-min trial without foot-shock.*





(B) There was no difference in the number of entrances into the shock zone during Habituation between the Shallow group (21.79 ± 1.47) and the Swim group (23.00 ± 1.71) (two-tailed t_{25} = 0.5418; p = 0.5927). Also, there was no difference in the number of entrances into the shock zone during Acquisition on trial 1 between the Shallow group (13.43 ± 1.72) and the Swim group (12.77 ± 1.44) (two-tailed t_{25} = 0.2916; p = 0.7730), on trial 3 between the Shallow group (7.15 ± 1.69) and the Swim group (6.54 ± 1.54) (two-tailed t_{25} = 0.2696; p = 0.7898), and on trial 8 between the Shallow group (0.86 ± 0.27) and the Swim group (1.38 ± 0.18) (two-tailed t_{25} = 1.5796; p = 0.1268). Also, there was no difference in the number of entrances into the shock zone during Retention between the Shallow group (6.64 ± 1.22) and the Swim group (7.31 ± 1.75) (two-tailed t_{25} = 0.3151; p = 0.7553). The numbers in each group are listed as the number of entrances ± SEM.

***(C)** There was no difference in the latency-to-1st-entrance into the shock zone during Habituation between the Shallow group (17.82 ± 4.43) and the Swim group (13.24 ± 2.46) (two-tailed t_{25} = 0.8851; p = 0.3845). Also, there was no difference in the latency-to-1st-entrance into the shock zone during Acquisition on trial 1 between the Shallow group (19.72 ± 4.78) and the Swim group (21.83 ± 4.12) (two-tailed t_{25} = 0.3310; p = 0.7434), on trial 3 between the Shallow group (103.80 ± 42.87) and the Swim group (74.27 ± 19.96) (two-tailed t_{25} = 0.6086; p = 0.5483), and on trial 8 between the Shallow group*

(373.23 ± 66.37) and the Swim group (218.12 ± 52.64) (two-tailed t_{25} = 1.8127; p = 0.0819). Also, there was no difference in the latency-to-1st-entrance into the shock zone during Retention between the Shallow group (80.11 ± 27.64) and the Swim group (142.79 ± 48.28) (two-tailed t_{25} = 1.1476; p = 0.2620). The numbers in each group are listed as the seconds ± SEM.

(D) *There was no difference in the maximum time of avoiding the shock zone during Habituation between the Shallow group (67.21 ± 4.05) and the Swim group (63.77 ± 5.89) (two-tailed t_{25} = 0.4879; p = 0.6299). Also, there was no difference in the maximum time of avoiding the shock zone during Acquisition on trial 1 between the Shallow group (153.36 ± 21.39) and the Swim group (138.69 ± 16.93) (two-tailed t_{25} = 0.5320; p = 0.5994), on trial 3 between the Shallow group (277.64 ± 45.32) and the Swim group (291.23 ± 37.07) (two-tailed t_{25} = 0.2300; p = 0.8200), and on trial 8 between the Shallow group (503.79 ± 38.23) and the Swim group (449.23 ± 28.30) (two-tailed t_{25} = 1.1327; p = 0.2681). Also, there was no difference in the maximum time of avoiding the shock zone during Retention between the Shallow group (258.79 ± 26.99) and the Swim group (242.08 ± 37.16) (two-tailed t_{25} = 0.3677; p = 0.7162). The numbers in each group are listed as the seconds ± SEM.*

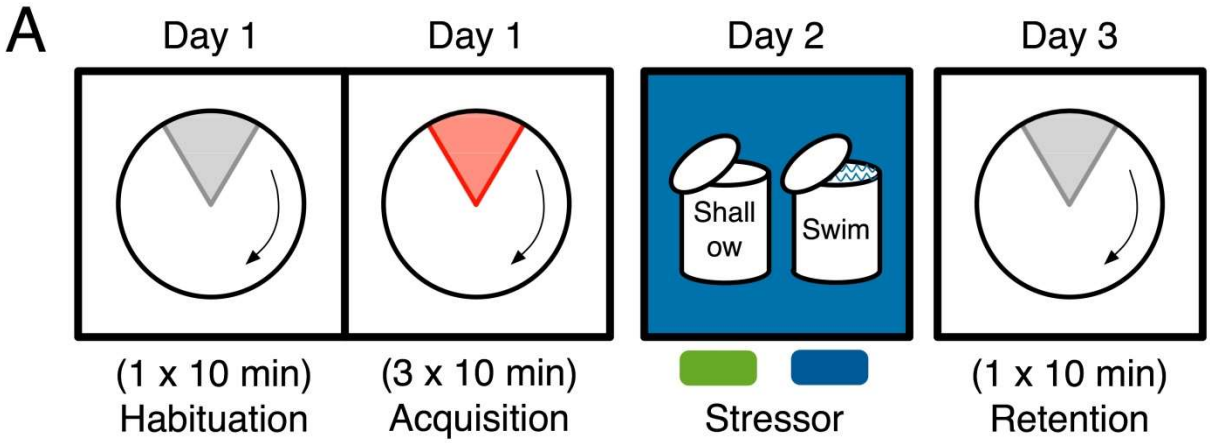
8.2. SWIM-STRESS DOES NOT ENHANCE WEAK HIPPOCAMPUS-DEPENDENT MEMORY EXPRESSION

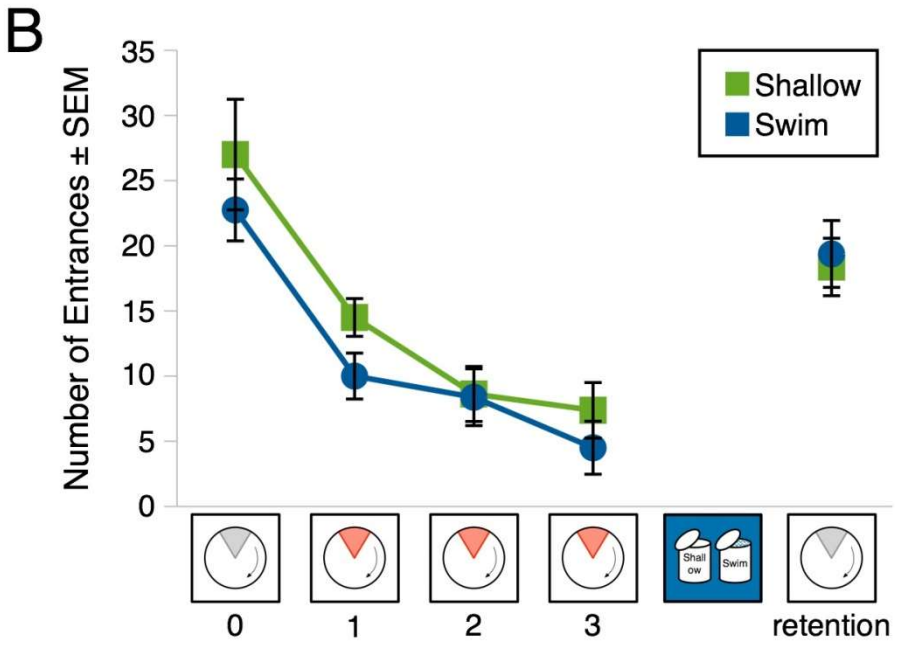
The results from the intensive training (eight trials) active place avoidance task show no differences in both the number of entrances and latency-to-1st-entrance during retention between the two groups. Possible explanations for such findings include: (1) the behavioral regimen used created a robust memory such that swim-stress had no observable enhancement effect (i.e., a ceiling effect), or (2) swim-stress does not enhance the expression of hippocampus-dependent spatial memories.

To distinguish between these two possibilities, I repeated the active place avoidance task but with fewer trials to create a relatively weak memory. The maximum latency-to-1st-entrance is 355.65 s and 386.95 s for the forced-swim group and non-swim group, respectively. The results of the first retention trial on the next day demonstrate what the animal remembers at the end of 8 training trials acquired the previous day. Using this learning curve, the number of training trials needed to create a memory that is about 50% of the strength of the original eight 10-min trial task occurs at the end of three training trials as demonstrated by average latency-to-1st-entrance into the shock zone of a little more than 200 s on trial 4 for both the forced-swim and non-swim groups.

The experimental approach of Study 3A was repeated with some modifications. The short training (three trials) active place avoidance task is used (Figure 14).

The results show no differences between the forced-swim or shallow water groups in either the number of entrances into the shock zone (Figure 14), or in the latency-to-1st-entrance into the shock zone during retention (Figure 14).


A
Day 1
Day 1
Day 2
Day 3
Shall ow
Swim
(1 x 10 min)
Habituation
(3 x 10 min)
Acquisition
Stressor
(1 x 10 min)
Retention


B
Number of Entrances ± SEM
35
30
25
20
15
10
5
0
Shallow
Swim
0
1
2
3
retention

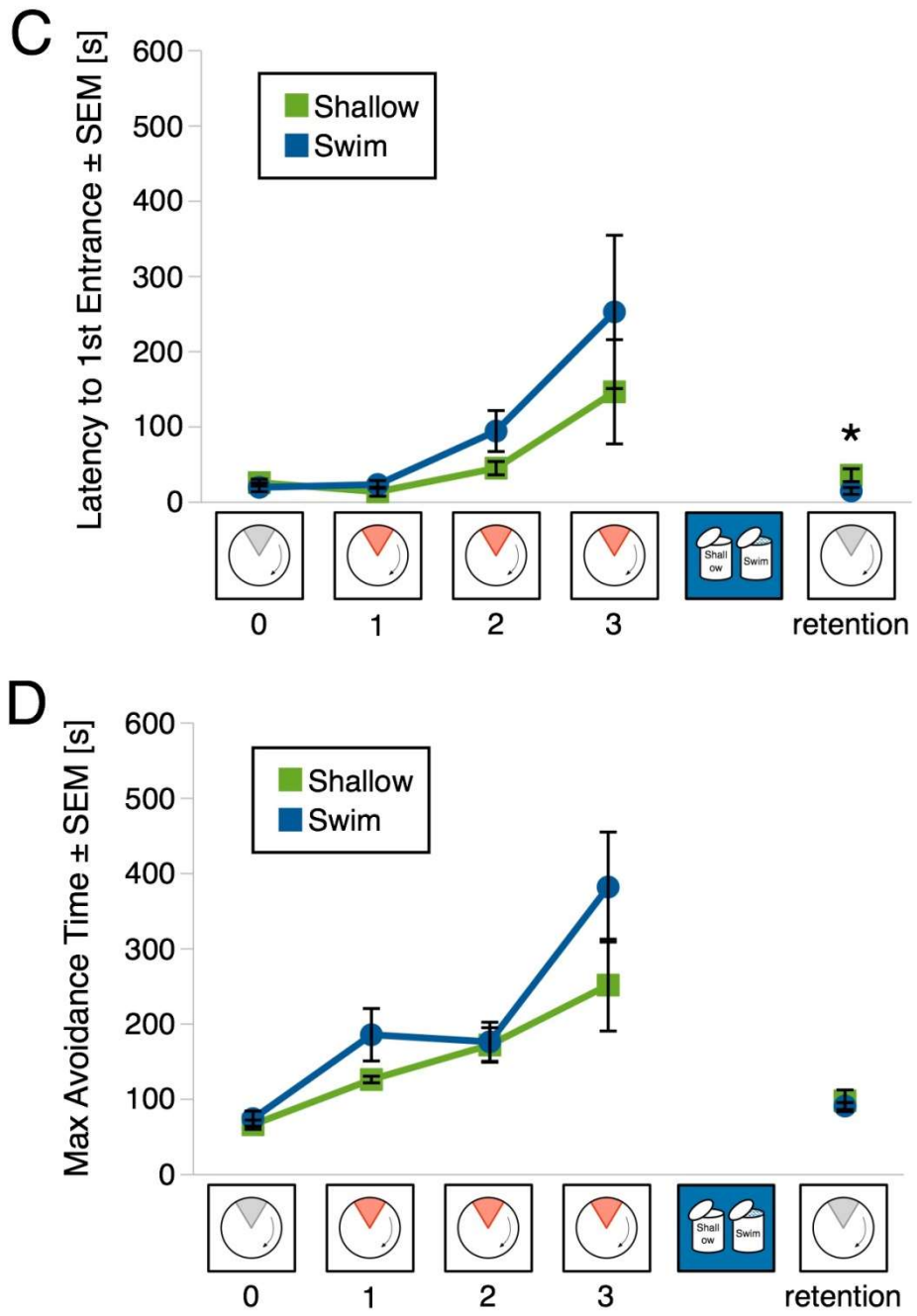


Figure 14. Forced-swim did not enhance memory expression of a short training aversively-conditioned active place avoidance task.

(A) *To determine whether the intensive training place avoidance task created a robust memory such that swim-stress had no observable enhancement effect (i.e., a ceiling effect), a short training version of the behavioral paradigm was used. On Day 1, animals were trained to avoid an invisible and stationary 60° sector shock area on a rotating arena that was oriented according to distal cues on the walls of the room during three 10-min trials. On Day 2, one group of animals was placed in a bucket with 1 cm of water*

(Shallow, n = 8) whereas the other group of animals was placed in a bucket and forced to swim (Swim, n = 8). On Day 3, animals were tested for retention by a single 10-min trial without foot-shock.

(B) *There was no difference in the number of entrances into the shock zone during Habituation between the Shallow group (27.00 ± 4.24) and the Swim group (22.75 ± 2.37) (two-tailed t_{14} = 0.8749; p = 0.3964). Also, there was no difference in the number of entrances into the shock zone during Acquisition on trial 1 between the Shallow group (14.50 ± 1.45) and the Swim group (10.00 ± 1.76) (two-tailed t_{14} = 1.9707; p = 0.0689), and on trial 3 between the Shallow group (7.38 ± 2.13) and the Swim group (4.50 ± 2.04) (two-tailed t_{14} = 0.9760; p = 0.3456). Also, there was no difference in the number of entrances into the shock zone during Retention between the Shallow group (18.38 ± 2.20) and the Swim group (19.38 ± 2.56) (two-tailed t_{14} = 0.2959; p = 0.7717). The numbers in each group are listed as the number of entrances ± SEM.*

(C) *There was no difference in the latency-to-1st-entrance into the shock zone during Habituation between the Shallow group (26.01 ± 4.63) and the Swim group (19.75 ± 5.89) (two-tailed t_{14} = 0.8354; p = 0.4175). There was no difference in the latency-to-1st-entrance into the shock zone during Acquisition on trial 1 between the Shallow group (13.85 ± 5.92) and the Swim group (23.50 ± 5.16) (two-tailed t_{14} = 1.2291; p = 0.2393), and on trial 3 between the Shallow group (146.66 ± 69.27) and the Swim group (252.87 ± 101.88) (two-tailed t_{14} = 0.8621; p = 0.4031). In contrast, there was a difference in the latency-to-1st-entrance into the shock zone during Retention between the Shallow group (35.82 ± 8.50) and the Swim group (14.86 ± 4.62) (two-tailed t_{14} = 2.1669; p = 0.0480). The numbers in each group are listed as the seconds ± SEM.*

(D) *There was no difference in the maximum time of avoiding the shock zone during Habituation between the Shallow group (65.50 ± 6.16) and the Swim group (73.50 ± 10.05) (two-tailed t_{14} = 0.6786; p = 0.5085). Also, there was no difference in the maximum time of avoiding the shock zone during Acquisition on trial 1 between the Shallow group (125.50 ± 4.40) and the Swim group (185.00 ± 34.83) (two-tailed t_{14} = 1.6948; p = 0.1122), and on trial 3 between the Shallow group (251.00 ± 61.08) and the Swim group (381.38 ± 73.03) (two-tailed t_{14} = 1.3694; p = 0.1924). Also, there was a difference in the maximum time of avoiding the shock zone during Retention between the Shallow group (97.25 ± 14.35)*

and the Swim group (90.25 ± 18.44) (two-tailed $t_{14} = 0.2996$; $p = 0.7689$). The numbers in each group are listed as the seconds ± SEM.

8.3. SUMMARY

Adequate performance on the active place avoidance task critically depends on the hippocampus. The results demonstrate that the forced-swim experience did not enhance the expression of the memory for the active place avoidance task regardless of whether the training was intensive (8-trial training) or short (3-trial training). This finding, along with the previous result for bilateral inactivation of the hippocampus with tetrodotoxin point towards the interpretation that forced-swim experience does not enhance memories that depend on the hippocampus for its expression.

DISCUSSION

9. Effects Of Corticosteroids On Memory Enhancement

9.1. RESULTS AND INTERPRETATION ON THE ROLE OF CORTICOSTEROIDS ON MEMORY ENHANCEMENT

Our previous work reported that dexamethasone blocked the memory enhancement effect induced by a forced-swim (Ježek, Lee et al, 2010). The findings presented in this thesis show that a forced-swim induces a significant increase in circulating corticosterone levels (see Figure 4). Combining these two results suggest that corticosteroids are a necessary component of the stress response that lead to a memory enhancement effect.

Additional findings presented in this thesis show that corticosterone itself did not induce the memory enhancement effect as that observed subsequent to swim-stress (see Figure 8). This suggests that corticosteroids are a necessary but not a sufficient component of the stress response that lead to a memory enhancement effect.

This section discusses the role of corticosteroids itself and as a component of the stress response in reaching brain targets to influence the observed behavior.

9.2. CORTICOSTEROIDS MODULATE MEMORY UPDATING

Our previous work reported that swim-stress induced a stable memory to become activated (labile) (Ježek, Lee et al, 2010), and if this activated memory was modified by swim-stress, that modification was towards an enhancement of expression rather than an impairment of retrieval. It is possible that subsequent to memory activation, the circulating hormones that are released in response to stress, which includes corticosteroids, could then act on that activated memory and facilitate the enhancement of its expression. Returning a stable memory into an active state is an important step in modifying that

memory (Piñeyro et al, 2014), and stress has been shown to enhance the reconsolidation of reactivated memory (Bos et al, 2014; Coccoz et al, 2011; Coccoz et al, 2013; Marin et al, 2010). Alternatively, blocking the effects of corticosteroids or reducing its levels after memory reactivation impairs the reconsolidation of a fear memory in rats (Nikzad et al, 2011) and reduce the strength of an emotional memory in humans (Marin et al, 2011). However, other studies show that stress impairs subsequent retrieval (Abrari et al, 2008; de Quervain et al, 1998; Hupbach and Dorskind, 2014; Roozendaal et al, 2003; Yang et al, 2013)

The memory enhancement effect of swim-stress may be due to the lack of similarity (i.e., incongruence) between the swim experience and the training experience, which avoids an effect referred to as retroactive interference (Gordon and Spear, 1973). The forced-swim and the environment in which the procedure was conducted were novel experiences for the animal, and both of these aspects would contribute to starting the consolidation of a separate, new memory. Concurrently, reactivation of a previously acquired memory triggers reconsolidation to restabilize that memory (Alberini, 2011; Inda et al, 2011). Since the experiences of the forced-swim and that of the L/R discrimination training had no physical or contextual similarity with each other, these experiences cannot involve the same consolidation and reconsolidation process. Both sets of information, the swim and the training, can coexist with one another, and a consequence of memory reactivation is to enhance the accessibility of a particular memory or to induce the malleability of that memory (Gisquet-Verrier and Riccio, 2012). As such, enhanced accessibility of a memory would lead to an improvement in the subsequent retention performance.

It has been proposed that a function of memory reactivation is to allow that memory to be modified or to incorporate new information (Alberini, 2005; Pedreira et al, 2004; Sevenster et al, 2012). As such, an alternative possibility is that during swim-stress, the previously acquired memory is activated and is then subsequently updated such that the two distinct experiences of the swim and the training become linked together (Hupbach, 2011). The resulting hybrid memory trace may not undergo the process of

reconsolidation (Tronel et al, 2005), in which the current understanding of the process is to strengthen an already existing memory trace and prevent its forgetting (Alberini, 2011; Inda et al, 2011). Moreover, if swim-stress does trigger the retrieval of a previously acquired memory, then this method of triggering retrieval may not undergo extinction either (Monfils et al, 2009; Suzuki et al, 2004) since the previously acquired memory has no physical or contextual similarity to the current swim-stress experience. It is possible that this hybrid memory has become a new memory that undergoes a stress-induced facilitation of consolidation (Abrari et al, 2009; Akirav et al, 2004; Conboy and Sandi, 2010; Hui et al, 2004; Roozendaal, 1999; Roozendaal, 2002; Taubenfeld et al, 2001; Tronel et al, 2005).

To address some of the limitations of behavioral studies, molecular markers for memory may provide a better tool with which to evaluate the impact of swim-stress on a previously acquired memory. For example, it has been showed that the transcription factor CCAAT enhancer-binding protein (C/EBP) (Taubenfeld et al, 2001) and brain-derived neurotrophic factor (BDNF) (Lee JLC et al, 2004) both play a role in consolidation, but neither of these molecules are involved in reconsolidation. In contrast, the transcription factor Zif268 (also known as egr-1) (Bozon et al, 2003; Lee JLC et al, 2004) plays a role in reconsolidation, but is not involved in consolidation. However, a significant drawback to using Zif268 as a marker for reconsolidation is that stress itself induces a rise in Zif268 levels (Cullinan et al, 1995), making it difficult to ascertain whether any manipulations of Zif268 were due to an induction of memory-related processes or to an induction of stress-related processes. This is also true for other molecular markers in which rising corticosterone levels increase the diffusion (Martin et al, 2009) or upregulate and increase synaptic surface expression (Conboy and Sandi, 2010) of GluA2-containing α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid receptors (AMPARs).

Currently, it remains unknown as to whether the swim-stress experience induced a stable memory to become activated and subsequently underwent reconsolidation, another round of consolidation, or some other process.

9.3. COMBINED CORTICOSTEROID AND CATECHOLAMINE INPUTS

Our previous work reported that propranolol, a non-selective beta-adrenergic receptor antagonist, had no observable impact on an inhibitory avoidance memory one day after acquisition training (Ježek, Lee et al, 2010). Since the memory was not susceptible to disruption by propranolol, an amnesic treatment, this suggests that the memory was not labile (Przybyslawski et al, 1999). However, our previous work also reported that propranolol had an impairing effect on an inhibitory avoidance memory if administered shortly after swim-stress. This suggests that swim-stress had induced a stable memory to become activated, and consequently, susceptible to the amnesic effects of propranolol (Ježek, Lee et al, 2010), and that it is the combined effects of corticosteroids and catecholamines within a restricted time window that induced a stable memory to become activated (Joëls et al, 2011).

Before delving further into the idea of concurrent catecholamine and corticosteroid inputs, one problem that needs to be addressed is that the kinetic properties of the adrenergic system are considerably faster than that of the corticosteroid system, the latter of which require about 20 minutes after the onset of stress to reach peak levels in the brain (Droste et al, 2008). Moreover, catecholamine levels were not measured in any of my experiments, and the expectation is that swim-stress would induce a sufficient increase in catecholamine levels that persists such that the time window of the effects of catecholamines overlap with those of corticosteroids. A study by de Boer and colleagues addressed this situation in which they showed that rats subjected to water immersion stress (rats were submerged in water but not forced to swim) had a significant increase in the levels of both corticosterone and norepinephrine that remained elevated at least 30 minutes after the termination of the stressor (de Boer et al, 1990). Moreover, after a stressful situation, norepinephrine levels show an additional secondary rise that persist for at least another 30 minutes (de Boer et al, 1990) and up to 2h (McIntyre et al, 2002). These time points fit with our 20 min swim-stress protocol, and as such, the concurrent activity of catecholamine and corticosteroid can be considered (see Figure 15 for a schematic on the window of overlapping effects of norepinephrine and corticosteroids).

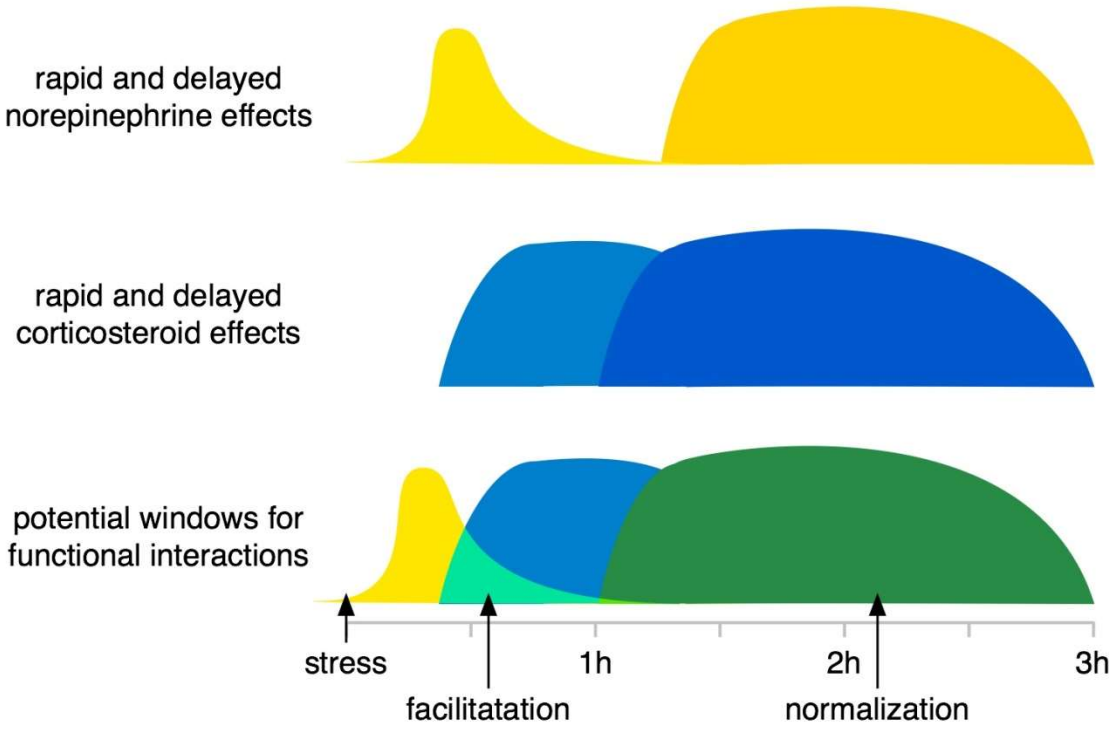


Figure 15. Overlap of norepinephrine and corticosteroids.

A model on the effects of stress on memory over time (Joëls et al, 2006) proposes that the short-term effects of corticosterone act via a non-genomic pathway to facilitate learning and memory formation whereas the long-term effects of corticosterone act via a gene-mediated pathway to suppress memory processing of new or unrelated information. The top time course shows both the rapid and delayed effects of norepinephrine. There is an initial surge in norepinephrine activity shortly after the onset of stress (lemon yellow) that is short-lived, which is then followed by a more prolonged norepinephrine effect (gold yellow). The middle time course shows both the rapid and delayed effects of corticosteroids. There is an initial rise corticosteroid activity that exert its effects on membrane-bound mineralocorticoid receptors (cyan blue), which is then followed by a more prolonged corticosteroid activity that exert its effects on intracellular glucocorticoid receptors (navy blue). Moreover, during this entire time, intracellular mineralocorticoid receptors are constitutively active. The bottom time course shows the overlap of norepinephrine and corticosteroids. This overlap of the rapid effects of both norepinephrine and corticosteroids act in an additive manner to facilitate the encoding of information. In contrast, the overlap of the delayed effects of both norepinephrine and corticosteroids act in an additive manner to

block encoding of new information, to solidify recently acquired information, and to normalize activity in preparation to receive new information. Figure modified from Joëls et al, 2011.

If blocking the adrenergic response with propranolol shortly after swim-stress has an impairing effect (Ježek, Lee et al, 2010), this suggests that catecholamines have a role in modifying an active memory. Moreover, since there was no concurrent adrenergic input, this may be why corticosterone itself did not have an observable impact on the expression of a L/R discrimination memory (see Figure 8) as that induced by swim-stress. One possibility is that catecholamines play a permissive role in that concurrent catecholamine activity allow corticosteroids to exert its effects. In support of this idea, it was previously shown that antagonism of beta-adrenergic receptors block the facilitating effects of corticosteroids on consolidation (Roozendaal et al, 2006a; Roozendaal et al, 2006b), and alternatively, antagonism of beta-adrenergic receptors block the impairing effects of corticosteroids on retention (Roozendaal et al, 2004a; Roozendaal et al, 2004b).

Another possibility on the combination of inputs from both corticosteroids and catecholamines is that corticosteroids do not modify the expression of a memory but play a role in modulating the effects of catecholamines, and that it is catecholamines that are responsible for modifying an active memory. Corticosteroids may augment the effects of catecholamines as it was previously shown that the administration of metyrapone, an inhibitor of 11beta-hydroxylase activity that results in the inhibition of corticosterone and cortisol synthesis, attenuated the enhancement effects of amphetamine and epinephrine (Liu et al, 1999; Roozendaal et al, 1996a; Roozendaal et al, 1996b). Alternatively, corticosteroids may attenuate the effects of catecholamines as it was previously shown that the corticosterone reduced the efficacy of epinephrine such that a 10,000-fold greater dose of adrenaline was needed to exert similar effects as that of epinephrine alone in adrenalectomized rats on the behavioral performance on a passive avoidance task (Borrell et al, 1984).

A possible mechanism by which the combined corticosteroid and noradrenergic inputs could converge is AMPAR signaling, which is a common target for both corticosteroids and norepinephrine in the hippocampus (Hu et al, 2007; Groc et al, 2008). Norepinephrine induces phosphorylation of GluA1 subunit of AMPARs (GluA1-AMPAR), which lowers the threshold for GluA1 synaptic incorporation during LTP (Hu et al, 2007). In addition to a delayed increase in dwell time in the postsynaptic density that is mediated by GRs in the postsynaptic membrane, corticosterone acts on membrane-bound MRs to cause a rapid increase in the surface diffusion of the GluA2 subunit of AMPARs (GluA2-AMPAR), and to a lesser extent GluA1-AMPAR (Groc et al, 2008). And to further complicate the matter, the type of adrenergic receptors that are stimulated can subsequently regulate the effects that corticosteroids exert by altering the expression of corticosteroids receptors. Work done by Kabbaj and colleagues showed that stimulation of beta-adrenergic receptors increases the expression of hippocampal GRs and MRs whereas stimulation of alpha-adrenergic receptors decreases the expression of hippocampal GRs and MRs (Kabbaj et al, 1995).

The concomitant effects of corticosteroids and catecholamines would explain the swim-stress enhancement of a L/R discrimination memory (see Figures 3, 9, 10, and 12) as well as the trend towards an enhanced expression of a previously acquired memory in the Swim group. In comparison, animals that received exogenous corticosterone were already familiar with the oral dosing procedure as they were habituated to the process so as to avoid eliciting any endogenous stress response due to the procedure itself or to novelty of a new experimental room. As such, it is expected that animals receiving exogenous corticosterone would not have a concomitant rise in catecholamines that would coincide with the rise in systemic corticosterone levels. This would explain the absence of observable behavioral effects in the oral dosing of exogenous corticosterone group (see Figure 8).

9.4. SUMMARY

Many promising pre-clinical animal studies fail to achieve significant effects when translated into clinical applications (Wood et al, 2015). A better understanding of the role that corticosteroids play in modifying

previously acquired memories, not just the memories that are involved with the currently experienced stress, may provide some clarity into the often contradictory findings in the PTSD literature. In particular, the inadequate pharmacological effects could result from inadvertent memory activation, which is subsequently enhanced (Bos et al, 2014; Coccoz et al, 2011; Coccoz et al, 2013; Marin et al, 2010) prior to intervention. Additionally, the inadequate pharmacological effects could also result from focusing on the role of corticosteroids to the exclusion of catecholamines as described by the glucocorticoid toxicity hypothesis (McEwen and Sapolsky, 1995; Reagan and McEwen, 1997; Sapolsky, 1996) and the enhanced negative feedback sensitivity of the HPA axis (Yehuda, 2009).

10. Stress Levels And Its Timing

10.1. RESULTS AND INTERPRETATION ON STRESS AND MEMORY INTERVAL

The findings presented in this thesis show that swim-stress can induce a memory enhancement effect for L/R discrimination memories that are at least one-week-old, but swim-stress did not have any observable enhancement effect for memories that are two-weeks-old and older (see Figures 9 and 10). This suggest that there is a time-limited window within which swim-stress modify previously acquired memories.

Moreover, our previous work reported that amnesic treatments including propranolol, a non-selective beta-adrenergic receptor antagonist, and electro-convulsive shock (ECS) had no effect on a memory one day after acquisition training. However, both treatments showed amnesic effects if administered shortly after swim-stress up to 5h afterwards (Ježek, Lee et al, 2010). This suggests that the memory-related effects induced by swim-stress are rapid and short-lived.

This section discusses the findings in relation to the reconsolidation literature as well as the rapid effects of stress, both of which were presented in the introduction.

10.2. TIME-LIMITED WINDOW OF STRESS-INDUCED MEMORY ENHANCEMENT

The findings presented in this thesis show that there is a time-limited window within which swim-stress can modify a previously acquired L/R discrimination memory (see Figure 10). This finding lends support for the lingering consolidation hypothesis (Dudai and Eisenberg, 2004) in which an acquired memory progressively becomes more stable and more resilient to disruption (Milekic and Alberini, 2002). This finding is in contrast to the work by Debiec and colleagues who used a contextual fear conditioning paradigm to show that memories up to 45-days-old could still be made labile upon reactivation (Debiec et al, 2002).

Moreover, Nader and colleagues would argue that if reconsolidation effects are not observed, one cannot conclude that a limit or boundary has been found, and that any temporal limit or boundary with respect to the age of the memory is merely an experimental limitation (Nader and Einarsson, 2010). Unfortunately, the findings presented in this thesis do not directly address whether swim-stress induced a previously acquired memory became activated because the studies were designed to determine whether swim-stress can enhance the expression of progressively older memories. As such, what is known is that there is a time-limited window within which swim-stress can modify a previously acquired memory towards enhancement, but it is not known whether there is there is a time-limited window within which swim-stress can activate a previously acquired memory towards enhancement.

In addition, if swim-stress induced a previously acquired memory to undergo a reconsolidation process, and this resulted in an enhanced expression of a previously acquired memory, then expression of this memory is not similar to that before swim-stress but rather the expression is stronger than that before swim-stress. This finding is in contrast to the viewpoints of Nader and colleagues who argue that consolidation and reconsolidation are intracellular molecular events in which new memories and reactivated memories exist in similar, but not identical, states (Nader et al, 2000). Moreover, our previous work reported that swim-stress not only enhanced the expression of a short training aversively-conditioned L/R memory, but that this enhanced expression persisted for up to 6 days (Ježek, Lee et al, 2010). Both of these findings lend support to the idea that the role of reconsolidation is to strengthen a memory trace and prevent forgetting (Inda et al, 2011).

Overall, the findings presented in this thesis as well as those of our previous work are more consistent with the view that reconsolidation is a manifestation of a consolidation process that proceeds slowly rather than being a recapitulation of a finished process. Moreover, the time-limited window within which swim-stress can modify a L/R discrimination memory may reflect an increased resilience to disruption or modification with increased cortical representation or distribution (Nadel and Moscovitch, 1997; Frankland and Bontempi, 2005) Future work needs to separate memory reactivation from an observable

behavioral output by taking advantage of the susceptibility of a memory trace to protein synthesis inhibitors shortly after memory reactivation (Bailey et al, 1996; Lee SH et al, 2008; Nader et al, 2000).

10.3. RAPID EFFECTS OF STRESS

Our previous work reported that propranolol, a non-selective beta-adrenergic receptor antagonist, had no effect on an inhibitory avoidance memory one day after acquisition training if administered in the absence of swim-stress, but propranolol had an impairing effect if administered shortly after a forced-swim (Ježek, Lee et al, 2010). And, the amnesic effects of electro-convulsive shock (ECS) had no effect on a L/R discrimination memory one day after acquisition training if administered in the absence of swim-stress, but ECS blocked the enhancement effect if administered shortly after a forced-swim (Ježek, Lee et al, 2010). Moreover, neither amnesic treatment (propranolol and ECS) had any effect if either were administered 5h after swim-stress (Ježek, Lee et al, 2010).

Both of these findings suggest that the memory activation effects of swim-stress occur rapidly, and that the time window of these rapid memory activation effects closes at 5h. The kinetic properties of the adrenergic system occur quickly, which can result in a surge in norepinephrine activity in the brain shortly after the onset of stress (see Figure 15). Since propranolol is lipophilic and can easily penetrate the blood-brain barrier, this would explain the amnesic effects of propranolol shortly after swim-stress. Moreover, the 5h window of efficacy for propranolol may reflect the drop norepinephrine activity in the brain (de Boer et al, 1990).

It is uncertain whether corticosteroid receptor antagonist would also exhibit amnesic effects like that of propranolol. Peak corticosteroids levels in the rat brain require about 20 min after significant increases in systemic levels (Droste et al, 2008), and the duration of the swim-stress is also 20 min, and as such, it is expected that the fast effects of corticosteroids are mediated by membrane-bound MRs. An infusion of spironolactone, an MR antagonist, could help clarify whether a corticosteroid receptor antagonist also exhibit amnesic effects like that of propranolol. Moreover, 5h after swim-stress would be a sufficient time

to allow corticosteroid levels to return back to baseline levels (Connor et al, 1997; Linthorst et al, 2008; Shors et al, 1999), which would suggest that spironolactone could no longer exert amnesic effects. As such, this 5h window of efficacy for spironolactone would mimic the 5h window of efficacy for propranolol.

10.4. SUMMARY

Swim-stress can induce the enhancement of a previously acquired memory that is at least one-week-old but less than two-weeks-old. Whether this finding is a constraint due to the age of the memory itself or an experimental limitation remains unknown, this finding there is a time-limited window within which swim-stress can enhance the expression of a memory is consistent the lingering consolidation hypothesis (Dudai and Eisenberg, 2004). In addition, the memory-related effects induced by swim-stress occur rapidly and are short-lived.

11. Effects Of Stress On Multiple Memory Systems

11.1. RESULTS AND INTERPRETATION ON STRESS AND HIPPOCAMPUS-DEPENDENT MEMORIES

Our previous work reported that animals with bilateral injection of tetrodotoxin (TTX) into the hippocampus prior to acquisition or prior to retention of a L/R discrimination task performed the task as well as their saline injected counterparts (Ježek, Lee et al, 2010). This suggests that animals are able to perform a L/R discrimination task independent of the hippocampus.

Our previous work also reported that inactivation of the hippocampus with TTX prior to swim-stress blocked the memory enhancement effect induced by swim-stress (Ježek, Lee et al, 2010). This suggests that the hippocampus must be functional during the swim-stress for the memory enhancement effect to occur. The findings presented in this thesis show that swim-stress did not induce a memory enhancement effect for memories that are dependent on the hippocampus for its expression (see Figures 13 and 14). Combining these two results suggest that swim-stress does not enhance hippocampus-dependent memories, but that memories that are dependent on other memory systems for its expression are enhanced, which in turn influences subsequent behavior.

This section discusses the role of multiple memory systems in the expression of a memory, and the role of the hippocampus in mediating the observed memory enhancement effect subsequent to swim-stress.

11.2. MULTIPLE MEMORY SYSTEMS AND MULTIPLE CHOICES

Active place avoidance is a task in which the acquisition (Cimadevilla et al, 2001) and retention (Fajnerova et al, 2014; Pastalkova et al, 2006) are critically depend upon the hippocampus. The findings presented in this thesis show that swim-stress did not induce any observable memory enhancement effect (see Figures 13 and 14) as that seen subsequent to swim-stress in a L/R discrimination task (see Figures 3, 9, 10, and 12). In one instance, the latency-to-1st-entrance during retention in the Swim group

was significantly lower than that of the Shallow group. One interpretation of the findings presented in this thesis is that swim-stress had an impairing effect on a hippocampus-dependent spatial memory. This interpretation is not surprising as many studies on the effects of stress on memory show that stress has an impairing effect on spatial working memory in rodents (Diamond et al, 1996; Diamond et al, 1999; Woodson et al, 2003), on retrieval in rodents (Abrari et al, 2008; Cai et al, 2006; de Quervain et al, 1998; Roozendaal, 2002; Roozendaal et al, 2003; Roozendaal et al, 2004), and on retrieval in humans (de Quervain et al, 2000; Kirschbaum et al, 1996).

However, there was no difference in the max avoidance time during retention between the Swim and Shallow groups, and as such, an alternate interpretation of the findings presented in this thesis is that swim-stress had no significant impairing effect on a hippocampus-dependent spatial memory. Regardless of how the data on the active place avoidance is interpreted, the salient feature is that swim-stress did not induce an enhancement in the expression of an active place avoidance memory (see Figures 13 and 14). This finding in the active place avoidance is in contrast to other findings presented in this thesis that shows an enhanced expression of a L/R discrimination memory subsequent to swim-stress (see Figures 3, 9, 10, and 12).

One explanation is that unlike the active place avoidance task, which is critically dependent on one structure for both its acquisition and retention, the L/R discrimination task does not have such a dependence on any one structure. Packard and McGaugh showed that animals preferentially use a spatial strategy during the initial acquisition of a L/R discrimination task, but can acquire the task using either the hippocampus or the dorsal striatum (Packard and McGaugh, 1996). Moreover, our previous work reported that animals with bilateral infusions of tetrodotoxin (TTX) into the hippocampus can perform a L/R discrimination task as well as their saline injected counterparts on both acquisition and retention (Ježek, Lee et al, 2010). Combined, this suggests that multiple memory systems can influence behavior on a L/R discrimination task.

In addition to multiple memory systems, a L/R discrimination task allows for multiple choices. These two features distinguish the L/R discrimination task from other tasks that are commonly used to study stress and memory. In classical conditioning behavioral paradigms, such as auditory fear conditioning or contextual fear conditioning, animals rapidly associate a conditioned stimulus (CS) (e.g., auditory tone or environment) with an unconditioned stimulus (US) (e.g., foot-shock), and express this learned association by exhibiting the limited repertoire of freezing behavior upon presentation of the CS. In instrumental conditioning behavioral paradigms, such as inhibitory avoidance (IA), passive avoidance, step-through avoidance, and step-down avoidance, animals rapidly associate a particular chamber or floor with foot-shocks (US), and express this learned association by remaining in the safe compartment so as to avoid receiving foot-shocks. Even if multiple memory systems may be involved, these conditioning behavioral paradigms limit the range of choices such that the multiple memory systems that are engaged act cooperatively towards the same goal.

Tasks such as the Morris water maze (Morris et al, 1982) and the circular hole board task (Barnes maze)(Barnes, 1979) can be modified into a spatial version or into a cued-version (Packard and McGaugh, 1992; Schwabe et al, 2010b) such that select lesions or the administration of stress or corticosterone can preferentially affect the ability of an animal to perform the task in one of the two versions. For example, animals were subjected to a restraint stress, a corticosterone injection, or a vehicle injection show an impairment in the performance on a spatial version of the circular hole board task in which the exit hole with a home cage located underneath the board is oriented according to distal visual cues. However, there was no impairment effect on a stimulus-response version of the circular hole board task in which the exit hole is oriented according to proximal (intra-maze) cues (Schwabe et al, 2010b).

Tasks that use multiple memory systems that also allow for multiple choices present a better representation of the effects due to stress on memory and the underlying brain regions that control behavior by putting these memory systems in competition or by lesioning a brain component of a neural

circuit that controls a specific type of behavior. The idea of studying multiple memory systems that acquire information in parallel has been already been proposed by White and McDonald (White and McDonald, 2002). It is possible that many studies that show stress-induced or corticosteroid-induced impairment of retrieval (Abrari et al, 2008; de Quervain et al, 1998; McGaugh and Roozendaal, 2002; Nikzad et al, 2011; Roozendaal et al, 2003; Roozendaal et al, 2004; Schwabe et al, 2012; Yang et al, 2013) is merely a reflection of the limited behavioral expression of that memory (Schwabe and Wolf, 2012).

11.3. HIPPOCAMPUS MEDIATES STRESS-INDUCED EFFECTS

Corticosteroids play a prominent role in the stress response (McEwen et al, 1968; McEwen et al, 1986), and measuring corticosterone levels as a physiological marker of stress supports this assertion (see Figure 4). Corticosteroid receptors have a wide distribution in the brain, and a factor that influences the effects that corticosteroids exert is the location of its receptors in the brain (Lupien and McEwen, 1997; Reul and de Kloet, 1985). However, if localized infusions of corticosteroids itself were sufficient to induce the behavioral alterations, then swim-stress alone would be sufficient to induce these behavioral alterations with or without a functional hippocampus during the swim. Our previous work reported that inactivation of the hippocampus bilaterally prior to swim-stress blocked the enhancement of the expression of a L/R discrimination memory and also blocked the interhemispheric transfer of a lateralized memory (Ježek, Lee et al, 2010).

There is accumulating evidence that during swim-stress, the hippocampus plays two roles. One role is to modify previously acquired memories towards an enhanced expression. Our previous work reported that inactivation of the hippocampus bilaterally with tetrodotoxin (TTX) prior to swim-stress blocked the enhancement of the expression of a L/R discrimination memory (Ježek, Lee et al, 2010). The findings presented in this thesis show that swim-stress enhanced the expression of a L/R discrimination memory, which replicated the results of our previous work (Ježek, Lee et al, 2010). Additional findings presented in this thesis show that swim-stress did not enhance a hippocampus-dependent memory trace itself (see

Figures 13 and 14), which suggests that the memory enhancement effect modified the expression of a memory located outside of the hippocampus.

A second role of the hippocampus is to mediate the shift in the storage site in which a memory is expressed. Our previous work reported that swim-stress was able to induce the interhemispheric transfer of a lateralized memory (Ježek, Lee et al, 2010). Animals that are trained in a task with one hemicortex temporarily inactivated acquire the task with the remaining functional hemicortex to create a lateralized memory such that an animal behaves naïve if tested with the trained hemicortex inactivated (Bures,1959; Fenton and Bures, 1993; Nadel and Buresova, 1968). Upon return to the learning context with both hemispheres functional, the lateralized memory is activated and no longer remains lateralized (Bures,1959; Fenton and Bures, 1994; Russell and Ochs, 1961). As such, inducing the interhemispheric transfer of a lateralized memory suggests that a stable memory was activated and that the storage of that memory was copied to another location (Ježek, Lee et al, 2010). Importantly, inactivation of the hippocampus bilaterally with lidocaine prior to swim-stress blocked the interhemispheric transfer of a lateralized memory (Ježek, Lee et al, 2010).

These two roles of enhancing memory expression and shifting the storage site in which a memory is expressed may actually be intertwined such that the observed enhanced expression of a L/R discrimination memory induced by swim-stress is also consistent with a shift in control of the expression of that memory from one memory system to another (Schwabe and Wolf, 2009; Schwabe and Wolf, 2010; Schwabe and Wolf, 2011). In particular, modulating the control of instrumental action in a manner that favor habit-based control of behavior (Schwabe and Wolf, 2011), and this would explain why the animals in the Swim group needed more trials to reach criterion during Reversal Training as compared to the animals in the Shallow group (see Figures 9, 10, and 12). Moreover, similar findings have been shown using human subjects in which stress prior to learning promoted habit processes at the expense of goal-directed processes in instrumental learning (Schwabe et al, 2007; Schwabe and Wolf, 2009), and stress after learning promote habit behaviors over goal-directed behavior (Schwabe and Wolf, 2010).

The behavioral differences were observed between the L/R discrimination training and the active place avoidance training in that the former showed an enhanced expression whereas the latter showed no enhanced expression (see Figures 13 and 14). These differences could be attributed the localization or distribution of that memory (Schiller and Phelps, 2011), and this would explain why swim-stress did not induce a memory enhancement effect for an active place avoidance memory. Specifically, because active place avoidance memory is critically dependent on the hippocampus for its expression (Cimadevilla et al, 2000; Cimadevilla et al, 2001; Pastalkova et al, 2006), neither enhancing memory expression and nor shifting the storage site in which that memory is expressed would have had an impact.

The mechanism by which how swim-stress can with a shift in control of the expression of that memory from one memory system to another remains unknown. One possibility is that the hippocampus is primarily responsible for mediating this enhancement or shift in the location of a memory. Evidence in support of this possibility comes from the work by Packard, who extended the findings of his earlier work with McGaugh. Packard repeated the behavioral experiment but used post-training infusions of saline or glutamate into either the hippocampus or the dorsolateral caudate. Rats that received saline infusions displayed spatial learning on a probe trial on day 8, and shifted to response learning with extended training on a probe trial on day 16, similar to his earlier work with McGaugh (Packard and McGaugh, 1996). In contrast, rats that received glutamate infusions into the hippocampus bilaterally showed spatial learning on both days 8 and 16, which suggest that the shift to response learning was inhibited. Moreover, rats received glutamate infusions into the dorsolateral caudate bilaterally showed response learning on both days 8 and 16, which suggests that the shift to response learning was facilitated (Packard, 1999).

Another possibility is that this enhancement or shift in the location of a memory requires inputs from both the hippocampus as well as that from stress hormones, in particular corticosteroids. Evidence in support

of this possibility comes from the work by Medina and colleagues found that post-training infusion of corticosterone or dexamethasone into the dorsal striatum facilitated the consolidation of a memory for an inhibitory avoidance task, as evidence by an increase in the latency to enter the dark shock chamber. However, the features of the task that are normally attributed to the hippocampus and amygdala, including the contextual and motivational aspects of the foot-shock, respectively, were not enhanced (Medina et al, 2007; Quirarte et al, 1997). Additionally, post-training infusions of corticosterone into the dorsal striatum enhanced a cued version of the Morris water maze (Quirarte et al, 2009).

Currently there is insufficient evidence that supports one possibility over the other, but this idea can be investigated further by infusing a corticosteroid receptor antagonist into the dorsolateral striatum prior to swim-stress followed by a retention test in a L/R discrimination task.

11.4. SUMMARY

Using behavioral tasks in which multiple memory systems are engaged during learning (White and McDonald, 2002) that also allow for multiple choices (Packard and McGaugh, 1992) may be a better reflection of the actual behavioral alterations subsequent to a stressful experience. One such stress-induced change is the shift from a flexible, declarative learning system that is hippocampus-dependent (a “cognitive system”) to an inflexible procedural learning system that is striatum-dependent (a “habit system”) (Schwabe and Wolf, 2012; Schwabe and Wolf, 2013). Our previous work as well as the findings presented in this thesis support the idea that the hippocampus may play an important role in mediating this stress-induced shift of behavioral control, and recent research in both animals and humans suggest that stress alters the contributions of these memory systems to learning, but the underlying mechanism by which this process takes place remains unknown (Goodman et al, 2012).

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