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An Animal Model of Flashbulb Memory: Insights into the Time-Dependent Mechanisms of Memory Enhancement

Laura Ashley Bullard
University of South Florida, labullard83@gmail.com

An Animal Model of Flashbulb Memory:

Insights into the Time-Dependent Mechanisms of Memory Enhancement

by

Laura A. Bullard

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Department of Psychology
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Major Professor: David Diamond, Ph.D.
Paul Jacobsen, Ph.D.
Cheryl Kirstein, Ph.D.
Ken Malmberg, Ph.D.
Brent Small, Ph.D.

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ABSTRACT

The vivid memory of an emotional event, as well as memory for incidental details associated with the arousing event, has been referred to collectively as a "flashbulb memory". An important aspect of flashbulb memory in people is that an emotional event enhances memory of contextual details, such as the weather, or clothes one was wearing at the time of the event. Therefore, an emotional event not only produces a detailed memory of the event, itself, but also enhances memory for contextual details that would otherwise not be remembered. The first goal of this work is to describe the development of my animal model of flashbulb memory, including a discussion of the importance of the timing between an emotional event and incidental, contextual cues. The second goal is to address the time-dependent neuroendocrine processes involved in stress-induced memory enhancement in rats. The involvement of brain structures, namely the hippocampus and amygdala, and hormones, including corticosterone and epinephrine, that interact to produce a composite memory of the contextual cues occurring in close temporal proximity to an emotional event are discussed. The results of Experiment 1 validate the animal model of flashbulb memory whereby an emotional event (predator exposure) produced memory for context cues that, under control conditions, would be forgotten. This memory enhancement *only* occurred when the emotional event was close in temporal proximity to training in the task. Experiment 2 provided evidence that epinephrine administration close in time to training mimicked the context memory formation induced by brief predator exposure, while propranolol, a β-adrenergic antagonist, as well as CPP, an NMDA receptor antagonist, blocked this effect. The results of Experiment 3 revealed that propranolol, CPP, and dexamethasone also blocked the brief predator stress-induced context memory formation. The results of Experiment 4 revealed

that cannulated animals infused with aCSF (control) did not show evidence of predator stress-induced memory, therefore methodological issues within this experiment are addressed. Finally, the results of Experiment 5 revealed that adrenalectomy eliminated the predator stress-induced context memory compared to sham operated animals, suggesting that endogenous stress hormones are required for stress-induced context memory formation. Further, adrenalectomized rats supplemented with epinephrine before training did show evidence of context memory enhancement suggesting that epinephrine eliminated the memory impairment produced by adrenalectomy, and was sufficient to enhance memory in the absence of corticosterone. Overall this approach has provided insight into the time-dependent neuroendocrine processes involved in the formation of flashbulb, and potentially traumatic, memories in people.

INTRODUCTION

Emotional events, as well as the circumstances surrounding them, tend to be well remembered. In 1890, William James provided the earliest mention of this phenomenon stating that "an impression may be so exciting emotionally as almost to leave a scar upon the cerebral tissues" (James, 1890). Years later, Brown & Kulik (1977) expanded on the idea that an emotional event could be "burnt' into memory by describing memory for emotional events as so vivid and detailed that they bear resemblance to a photograph. They coined the popular term "flashbulb memory" which is described as memory for the circumstances (or context) in which one learns of an emotional event. Under non-stressful circumstances, these incidental details (the weather, clothing one was wearing, time of day) would be forgotten (Christianson, 1989, 1992; Talarico & Rubin, 2003). They are remembered because they are associated with the emotional event. Therefore, people not only remember an emotional event, itself, but also associative details, such as the weather, that happen to occur close in time to the emotional event.

Emotion-induced context memory, as occurs in the phenomenon of flashbulb memory in people, has been widely debated in the literature. For example, researchers have argued that the context details remembered in flashbulb memories are inaccurate and may be subject to distortion over time (Christianson, 1992; Loftus, 2005; Loftus & Burns, 1982; Loftus & Hoffman, 1989; Loftus et al., 2011; Loftus et al., 1978; Loftus & Bernstein, 2005; Loftus & Palmer, 1974; Loftus, 1979; Talarico & Rubin, 2003). In contrast, other researchers have found that flashbulb memories are quite accurate (Heuer & Reisberg, 1990; Shapiro, 2006). Further,

studies of the weapon focus effect, first described by Elizabeth Loftus and colleagues, have shown that people are highly accurate in their recall of specific details, such as a weapon (the emotional event itself) at the expense of memory for context details (Loftus et al., 1987). Because of these discrepancies in the literature, it is important to dissect, on a more mechanistic level, the conditions in which an emotional event enhances memory for context details that would otherwise not be remembered.

1.1 Factors Involved in Stress-Induced Context Memory

1.1.1 Time is the Critical Variable

From an evolutionary perspective, it is adaptive to remember the details that are associated close in time with an emotional event. If a stressful experience results in enhanced memory for stimuli occurring just before the onset of stress, an organism can react quickly to avoid danger when faced with those stimuli in the future. Research in people has supported this idea that context details occurring close in time to an emotional event are well remembered.

For example, Ehlers et al. (2002) developed a 'warning signal' hypothesis in their study examining intrusive memories in people with post-traumatic stress disorder (PTSD). This disorder develops in some people after exposure to trauma, and includes hallmark features such as intrusive memories, avoidance of reminders of the trauma, negative thoughts, and heightened arousal (American Psychiatric Association, 2013). PTSD can be thought of as a flashbulb memory that is so intense, it is pathological. In the Ehlers et al. (2002) study, people who had experienced severe trauma were asked to identify the content of their intrusive memories. The participants reported visual intrusive memories of stimuli or events that occurred around the time

of the traumatic event, itself. For example, one patient who had "experienced a head-on car crash at night kept seeing headlights coming towards her". Ehlers et al. (2002) suggested that because these context stimuli occurred in close temporal proximity to the traumatic event, they became 'warning signals', or stimuli that, if encountered in the future, would indicate something dangerous is about to happen, which is an adaptive process. The warning signal hypothesis proposed by Ehlers et al. (2002) provides insight into the conditions in which an emotional event enhances memory in that context details occurring just prior to the onset of the emotional event are well remembered.

Under normal conditions, memories of emotional events contain context details that happen to be in the same location of the emotional event, itself. That is, people remember events closely associated with the *place* and *time* of an arousing event. Joëls et al. (2006) proposed a theory of how stress and, specifically, the hormones involved in stress, can enhance memory. They proposed a theory which states that "stress will only facilitate learning and memory processes when stress is experienced in the context and around the time of the event that needs to be remembered and when the hormones and transmitters released in response to stress exert their actions on the same circuits as those activated by the situation, that is, when convergence in time and space takes place" (pg. 152). For example, if stress is an intrinsic part of an experience (e.g. foot shock in a particular location of apparatus), then the resulting increases in stress hormone levels taking place in the same location and around the time of the stress experience enhance memory of that particular event. Therefore, the timing and location of stress are *both* critical components that determine what information will be remembered.

The hypothesis by Joëls et al. (2006) is based on methods where all relevant cues are experienced in the same place as the arousing experience. That is, the location and timing of stress are linked and both become integrated in memory. What if there are two different environments that are encountered close in time? When separated, the timing of stress, independent of the location, appears to be the critical factor in determining whether or not memory will be enhanced.

Experiments conducted by Diamond and colleagues tested the hypothesis that it is time, rather than space, that is the critical factor determining memory enhancement. Diamond et al. (2007) provided the first explicit test of the effects of stress on memory in rats that were exposed to a cat for a brief period of time in one location and then trained in another location in a water maze task. In this task, rats were required to swim to find a hidden platform. The researchers used 4 training trials which resulted in poor memory for the platform location in control animals when tested at 24 h. In order to test whether brief stress occurring close in time could enhance a weak memory, rats were exposed to a cat for 2 min either immediately before or 30 min before training. Exposure to the cat for 2 min immediately before training resulted in enhanced memory for the platform location when tested 24 h later, while 30 min cat exposure did not result in enhanced 24 h memory. Therefore, the timing of stress was critical in that the onset of stress enhanced memory *only* if it occurred immediately before training.

In this study by Diamond et al. (2007), predator exposure took place in a different room than water maze training. Therefore, the location in which stress took place was dissected from the timing of stress. If location was a critical variable in determining memory enhancement, then there would have been no evidence of memory for the platform location in this study. Therefore, timing, and not location, was the critical variable. This study indicated that events occurring at

the onset of stress, but not after a delay, to a stressful event are remembered, even if they are experienced in a different environment.

1.1.2 Effects of Peripheral Epinephrine and Corticosterone

When emotional stimuli are encountered (such as exposure to a predator), activation of the stress response is elicited, which has been well studied (for reviews see McGaugh et al., 1996; McGaugh & Roozendaal, 2002; Rodrigues et al., 2009). Briefly, stressful stimuli are relayed to the amygdala which leads to activation of the sympathetic nervous system including the release of epinephrine from the adrenal medulla. The amygdala also activates endocrine responses via the hypothalamic-pituitary-adrenal (HPA) axis. When activated, the hypothalamus secretes corticotropin-releasing hormone (CRH), which in turn signals the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH then acts on the adrenal cortex which ultimately leads to secretion of glucocorticoid hormones. This activation of the stress response ultimately enables the animal to utilize energy resources to respond to stress. These circulating hormones activated by the stress response also feedback to the brain and activate areas, such as the basolateral amygdala and hippocampus, that are involved in memory storage.

Gold & van Buskirk (1975) conducted one of the earliest studies indicating that exogenous administration of epinephrine, which is released via the sympathetic nervous system in response to stress, enhanced memory for an arousing event in rats. In this study, rats were trained with a weak foot shock in an inhibitory avoidance task. The results revealed that epinephrine administration immediately after training enhanced memory for the shock location when tested 24 h later. Further, animals injected with epinephrine 30 min after training had

latencies similar to control (saline) animals. Therefore, epinephrine administration at a delay after training did not enhance memory for the shock location. In theory, exogenously administered epinephrine enhanced memory for a context cue (shock location) because it mimicked the hormonal consequences of more intense stress (e.g. strong foot shock), which would also enhance memory. This study provided the first evidence that systemic administration of epinephrine, which is endogenously released during stress, modulates memory consolidation for context cues associated with a stressful event.

Liang et al. (1995) conducted a similar study assessing whether or not epinephrine was *required* for memory enhancement in an inhibitory avoidance task using either a weak or strong foot shock. In one experiment, one group of rats underwent an adrenal demedullation (ADMX) procedure which results in a depletion of endogenous epinephrine, while another group underwent a sham operation (adrenal intact). A strong foot shock in sham rats resulted in higher retention scores than the ADMX rats, suggesting that a lack of epinephrine in the ADMX rats impaired memory for context cues associated with the shock. When the ADMX rats were injected with epinephrine immediately after training, performance in the inhibitory avoidance task with a strong foot shock was no different than that of sham rats. Therefore, epinephrine supplementation eliminated the memory impairment resulting from adrenal demedullation.

In a second experiment conducted by Liang et al. (1995), adrenal intact rats were trained in the inhibitory avoidance task with a weak foot shock. Animals then received amygdala infusions of norepinephrine immediately after training which resulted in longer latencies to cross into the dark compartment than control (vehicle) animals. These results suggested that norepinephrine administration enhanced memory for cues associated with the stressful event because it mimicked the hormonal consequences of more intense stress (strong foot shock).

Sandi et al. (1997) provided evidence that a glucocorticoid hormone (corticosterone in rats) that is also endogenously released from the adrenal glands during stress modulates memory for context cues associated with an arousing experience in rats. In this study, different groups of rats were trained in the Morris swim task to escape from the water (using spatial cues) onto a hidden platform. One group was trained in water that was 25°C (weak stress) and another group was trained in water that was 19°C (more intense stress). The results revealed that rats trained in water that was 19°C (more intense stress) had shorter latencies to find the platform than rats trained in water that was 25°C (weak stress) during both acquisition and retention trials. When a stress-level dose of corticosterone was administered to animals trained in water that was 25°C (weak stress) immediately after each session, it significantly improved their performance on both acquisition and retention trials. The same dose of corticosterone did not affect the performance of rats trained in water that was 19°C. These results indicated that stress-level corticosterone administration immediately after weak stress interacted with arousal to strengthen memory for context cues (platform location) associated with the stressful experience.

Okuda et al. (2004) also provided evidence that corticosterone interacts with arousal to enhance memory for context cues associated with arousing events in rats. In this experiment, one group of rats was habituated to an apparatus for several days before training in a novel object recognition task (non-stress group) while another group was not habituated to the apparatus before training (novelty stress group). The results revealed that habituated rats injected with corticosterone immediately after training did not spend more time with the novel object when tested 24 h later, which indicated that there was no evidence of memory. In contrast, non-habituated rats (novelty stress) injected with corticosterone immediately after training did spend more time with the novel object indicating enhanced memory for the training experience.

Therefore, corticosterone interacted with stress occurring under baseline conditions at the time of training to enhance memory. Corticosterone did not enhance memory in rats that had been habituated to the training conditions because there was no arousing event. Therefore, corticosterone only enhanced context memory when it interacted with arousal.

In summary, the results of studies assessing the effects of hormone administration on memory in rodents have revealed that epinephrine and corticosterone administration enhances memory for context cues, such as location in an apparatus, associated with an arousing event in a time-dependent manner. In addition, adrenergic activation of the amygdala is required for this emotion-induced context memory enhancement. Gold and van Buskirk (1975) provided the first evidence that epinephrine administration immediately, but not at a delay, after training enhanced context memory. Sandi et al. (1997) provided evidence that corticosterone administration immediately after a weak learning experience that results in weak performance under control conditions, can strengthen memory for context information associated with a stressful experience. In addition, Okuda et al. (2004) reported that corticosterone administration interacts with emotion as a result of training conditions to enhance memory for context cues. Therefore, administration of hormones that are endogenously released in response to stress enhance memory for context information in a time-dependent manner.

1.1.3 Glucocorticoid-Noradrenergic Interactions in the Basolateral Amygdala

As an extension of the work by Okuda et al. (2004), Roozendaal et al. (2006) predicted that noradrenergic activation induced by arousal was required for the memory enhancement of context cues as a result of glucocorticoid administration. In this experiment, non-habituated (stressed) animals were trained in a novel object recognition task and then immediately administered saline or corticosterone which was co-administered with propranolol, a β-

adrenergic antagonist. If the corticosterone-induced memory enhancement requires noradrenergic activation, then blockade of noradrenergic mechanisms via propranolol should eliminate the memory enhancement. Indeed, propranolol did block the memory enhancing effects of corticosterone in rats that were not habituated to the experimental conditions.

In addition, habituated (non-stressed) animals given yohimbine, an α_2- adrenoceptor antagonist which increases norepinephrine release, in combination with corticosterone, spent more time with the novel object indicating enhanced memory for context information in the absence of a stressful event. That is, administration of yohimbine mimicked a stressful event by increasing norepinephrine levels which would naturally increase in response to stress. This, in turn, enhanced memory for context information (objects encountered during training).

Additionally, Roozendaal et al. (2006) reported that noradrenergic activation within the basolateral amygdala (BLA) is required for glucocorticoid-induced memory enhancement. When propranolol was administered directly into the BLA, but not the dorsal hippocampus, it blocked the memory enhancement for context cues as a result of corticosterone administration. This series of experiments provided evidence that glucocorticoids act synergistically with noradrenergic activation within the BLA to enhance memory for context information associated with arousing events.

Hatfield and McGaugh (1999) also assessed the effects of norepinephrine in the basolateral amygdala (BLA) on memory for cues associated with an arousing experience in a hippocampal-dependent water maze task (Morris et al., 1982). Rats were given microinfusions of norepinephrine, propranolol (a β-adrenergic antagonist), or vehicle directly into the BLA immediately after training in the task. The next day, the animals were given a retention test to assess memory for the platform location. The results revealed that microinfusions of

norepinephrine into the BLA enhanced memory for the platform location when rats were tested 24 h later. In contrast, microinfusions of propranolol (which blocks noradrenergic activation) into the BLA impaired memory for the platform location when rats were tested 24 h later. These results indicated that norepinephrine infusion to the BLA modulates memory for a context cue associated with an arousing experience in a hippocampal-dependent task.

A related study investigating the influence of the amygdala on hippocampal-dependent memory enhancement was conducted by Packard and Teather (1998). In this study, one group of rats was trained in a water maze task to escape from the water (using spatial cues) onto a submerged platform which is a hippocampal-dependent task (Morris et al., 1982). Another group of rats was trained in a water maze task to escape from the water onto a visible platform, which is a caudate-dependent task (Packard & McGaugh, 1992). In both tasks, rats were given 8 training trials, then immediately administered d-amphetamine (a catecholamine agonist) or saline into the amygdala. Interestingly, d-amphetamine administration into the amygdala immediately after training enhanced memory in both the hidden and visible platform tasks. In contrast, when d-amphetamine was administered 2 h following training, it did not enhance memory, regardless of brain administration site or type of training task. Therefore, Packard & Teather (1998) demonstrated that amygdala activation, via d-amphetamine administration, can modulate memory in other brain regions, specifically the hippocampus and caudate. Additionally, amygdala activation immediately following training enhanced memory for a context cue associated with an arousing experience while amygdala activation 2 h following training did not enhance memory, demonstrating that the memory enhancing effects of amygdala activation are time-dependent.

In a more mechanistic study, Akirav and Richter-Levin (1999) assessed the time-dependent modulation of emotional memory by examining the effect of basolateral amygdala (BLA) priming (which would mimic emotional activation of the amygdala) on hippocampal plasticity. Long-term potentiation (LTP), a form of synaptic plasticity, was first reported by Bliss & Lomo (1973) and is a cellular substrate for processes that occur during learning and memory. In this study, the researchers induced LTP in the dentate gyrus (DG) of the hippocampus of anesthetized rats by applying high frequency stimulation. When priming stimulation was applied to the BLA 1 h before high frequency stimulation to the hippocampus, LTP in the hippocampus was inhibited. Interestingly, when the same priming stimulation was applied to the BLA 30 s before high frequency stimulation to the hippocampus, it enhanced LTP in the hippocampus. In other words, BLA priming enhanced hippocampal synaptic plasticity when occurred close in time, while delayed BLA activation inhibited hippocampal synaptic plasticity.

Akirav & Richter-Levin suggested a biphasic model for the effects of amygdala activation on emotional memory, where there is a fast, short lasting, excitatory phase, followed by a delayed, longer lasting, inhibitory phase. Therefore, when the amygdala is activated close in time to hippocampal activation, memory mechanisms in the hippocampus are enhanced. In contrast, if there is delayed activation of the amygdala before hippocampal activation, memory mechanisms in the hippocampus are impaired.

In summary, this brief overview of studies in rats (for reviews see Cahill & McGaugh, 1996; McGaugh, 2000; McGaugh, 2004; Richter-Levin, 2004; Roozendaal & McGaugh, 2012) has provided evidence that administration of epinephrine and corticosterone (hormones endogenously released in response to stress) enhances memory for context details that are associated with an arousing experience in a time-dependent manner. In addition, the memory

enhancement as a result of corticosterone administration requires noradrenergic activation of the basolateral amygdala. Finally, amygdala activation also modulates hippocampal-dependent memory for context information in a time-dependent manner.

1.2 Temporal Dynamics Model of Emotional Memory

Diamond and colleagues derived a model based on earlier studies by Joëls et al. (2006) and Akirav & Richter-Levin (1999) describing how neurobiological mechanisms involved in the stress response can enhance memory consolidation of context details occurring close in time with an arousing event which can be measured behaviorally and physiologically (Diamond et al., 2007). In their temporal dynamics model of emotional memory processing, the authors stated that stress briefly activates noradrenergic mechanisms in the amygdala which, in turn, rapidly activates memory storage processes in the hippocampus. The model (Figure 1) states that a stressful experience activates hippocampal synaptic plasticity, which is a neural substrate for learning and memory.

The first phase (Phase 1A) in the memory enhancement process in the temporal dynamics model would involve a rapid enhancement in hippocampal synaptic plasticity, which is initiated by an increase in glutamate transmission (Bagley & Moghaddam, 1997; Kole et al., 2002; McEwen et al., 2002; Venero & Borrell, 1999). This would lead to activation of NMDA and AMPA receptors, which are subreceptors for glutamate. The NMDA receptor is of particular importance in that it controls a calcium ion channel that is normally blocked by a magnesium ion. When glutamate is present and when the post-synaptic membrane is depolarized via AMPA receptor activation, the magnesium block is removed which allows calcium ions to enter the cell. This calcium influx leads to a cascade of events (including CaMKII activation and

autophosphorylization) involved in synaptic strengthening (Blair et al., 2001; Lisman et al., 2002; Poser & Storm, 2001; Rongo, 2002; Suenaga et al., 2004). A few minutes after the onset of the stressful experience, corticosterone would also activate mechanisms involved in synaptic plasticity (Phase 1B) (Karst et al., 2005; Wiegert et al., 2006). All of these processes take place in a brief phase that occurs rapidly. In this manner, stress briefly activates the mechanisms involved in the formation of new memories.

Phase 2 of the temporal dynamics model would involve the desensitization of NMDA receptors (in response to elevated calcium levels) (Nakamichi & Yoneda, 2005; Rosenmund et al., 1995; Swope et al., 1999; Zorumski & Thio, 1992) and increase in the threshold for the induction of synaptic plasticity (and memory formation). Therefore, if an event occurs while the hippocampus is in the hypothetical Phase 2 state, memory for that event would be impaired. This phase can be thought of as a period of consolidation for events that occurred when the hippocampus was in the hypothetical Phase 1 state.

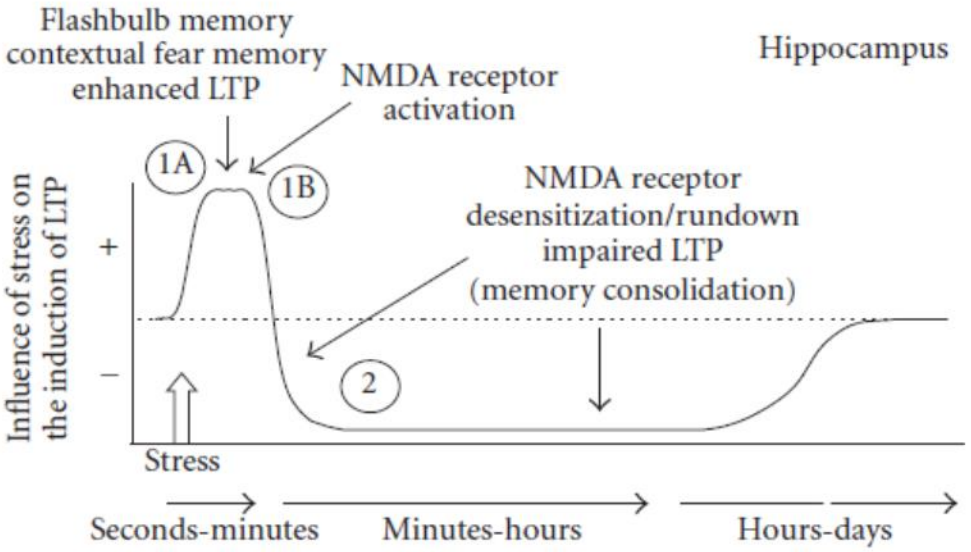

Figure 1. Temporal dynamics model of how stress affects memory processing in the hippocampus. Obtained from Diamond et al., (2007).

The hypotheses provided by Ehlers et al. (2002), Joëls et al. (2006), Akirav & Richter-Levin (1999) and Diamond et al. (2007) can allow specific predictions to be made about the effects of stress on memory for context information in rats. Specifically, the temporal dynamics model by Diamond et al. (2007) predicts that memory for events occurring immediately after stress will be enhanced, while memory for events occurring at a delay after stress will not be enhanced. In flashbulb memory, people remember cues that occur *both* before and after the onset of the emotional event. Therefore, as an extension of the predictions by Diamond et al. (2007), and the warning signal hypothesis by Ehlers et al. (2002), I hypothesized that memory for context cues occurring immediately, but not at a delay, *prior to* as well as *after* the onset of stress would be enhanced (Figure 2). I have developed an animal model which allows the assessment of several aspects of the effect of stress such as timing, hormonal involvement, and amygdala inactivation on memory for context stimuli occurring in close temporal proximity to the stressful experience. By using an animal model, mechanisms involved in the stress response can be manipulated which can provide further insight into the factors involved in stress-induced memory enhancement.

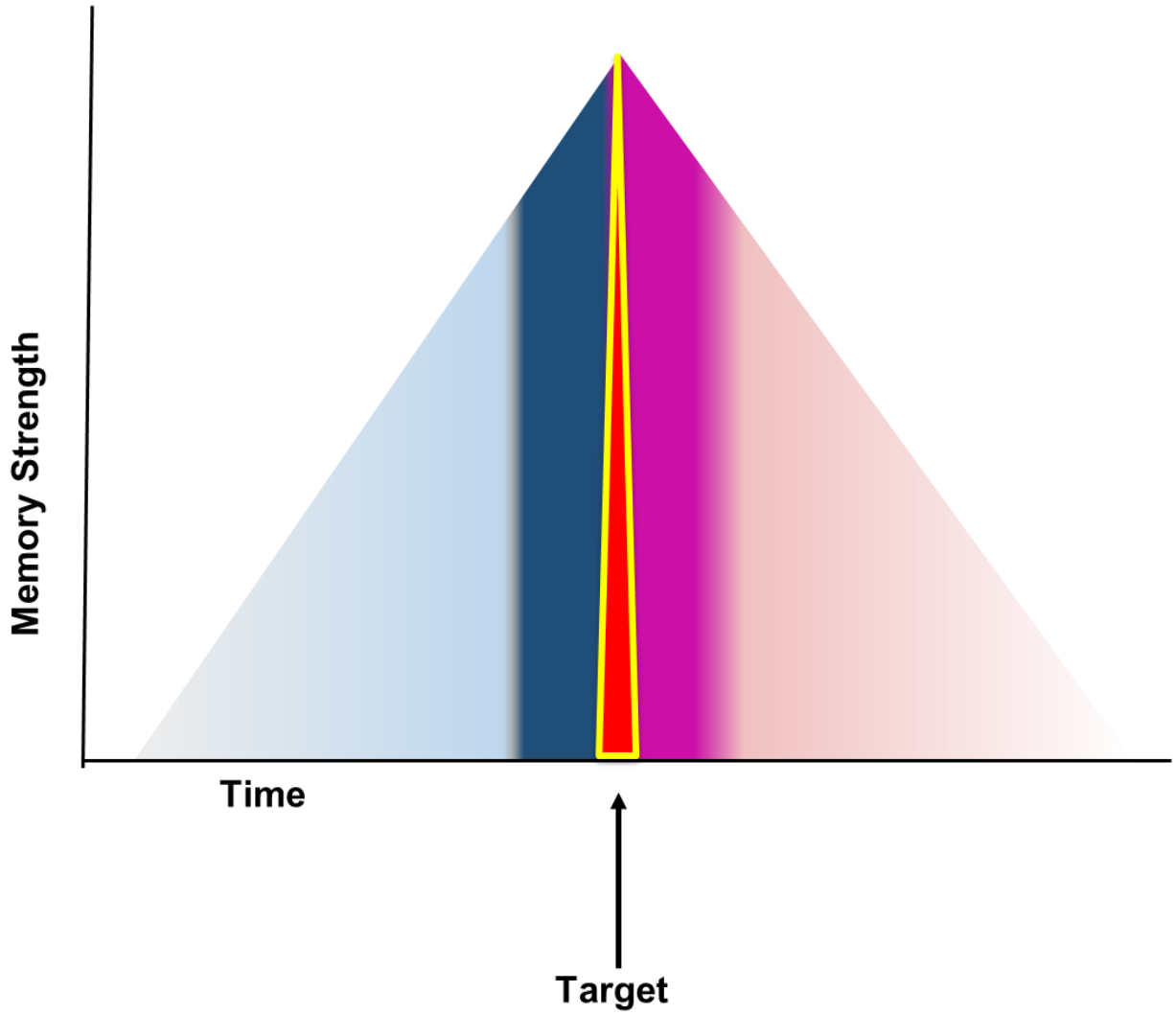

Figure 2. Temporal features of flashbulb memory. The target event (red) is well remembered. Incidental details (darker shades indicate stronger memory strength) occurring close in time to the target event are well remembered whereas incidental details that occur further in time from the target event are not well remembered.. This model is based on the temporal dynamics model of emotional memory (Diamond et al., 2007) where events occurring close in time after stress onset are well remembered and the warning signal hypothesis (Ehlers et al. 2002) where events occurring close in time before stress onset are well remembered.

DEVELOPMENT OF AN ANIMAL MODEL OF FLASHBULB MEMORY

2.1 Conceptual Framework

When assessing flashbulb memory in people, researchers can readily measure memory for context details that occur in the same location as the emotional event itself. The advantage of using an animal model of flashbulb memory is that it can allow a controlled assessment that separates, in time and location, the stressful event from to-be-remembered context cues. Therefore, the conditions in which a stressful experience *produces* memory for context details that would otherwise not be remembered can be assessed. Further, my model can enable a mechanistic-level assessment of factors involved in the stress-induced enhancement of context memory. To address these issues, I developed an animal model, based on a synthesis of the literature on emotional memory in people and animals, which assesses, in rats, the time-dependent effects of stress on memory for context details that are separated, in location, from a stressful event.

The overall goal in the development of this animal model of flashbulb memory was to develop a paradigm that assesses real-life learning surrounding an emotional event. In people, a flashbulb memory is produced after a single exposure to an emotional event in which context details that are attended to both *before* and *after* the onset of the emotional event are well remembered. Similarly, my animal model of flashbulb memory involves a single exposure to a stressful stimulus which produces memory for context stimuli occurring both before and after the onset of stress. That is, there is one-trial learning that occurs between the presentation of the

stressful stimulus and presentation of the to-be-remembered context stimuli, regardless of whether the context cue occurs immediately *prior to* or *after* the onset of stress.

Previous studies by McGaugh and colleagues typically used manipulations that occurred *after* training to test effects of stress or hormones on memory (Cahill & McGaugh, 1996; McGaugh, 2000; McGaugh, 2004; Roozendaal & McGaugh, 2012). The reasoning behind this methodological approach is that manipulations that occur before training can contaminate or interfere with processes involved in learning. However, in flashbulb memory, the emotional event, itself, *enhances* memory for post-event context details. Therefore, to extend the findings of McGaugh and colleagues, my animal model of flashbulb memory assesses the effects of stress both pre- and post-training, which provides a more comprehensive view of how information is processed in a real-life situation. Therefore, this is the first study in rats that is conceptually based on flashbulb memories in people.

In order to explicitly study the effects of stress on memory for context information in rats, a stressful experience was separated, in time and space, from to-be-remembered context stimuli. My first hypothesis was that a stressful event occurring close in time, but separated in location, from context stimuli would result in intact memory for those context stimuli that would otherwise not be remembered. To test this hypothesis, rats were exposed to a cat in one environment and at different time points prior to or after training in a novel object recognition (NOR) task which occurred in another environment. Therefore, the stressful event was separated in time and experienced in a different location than exposure to to-be-remembered context stimuli in the NOR task.

The NOR task is ideal because it has been shown to produce rapid learning with minimal intrinsic arousal that can be used to produce long-term memory in animals. The NOR task was first described by Ennaceur & Delacour (1988) and exploits a rodent's natural tendency to explore novel objects. The procedure consists of first exposing an animal to two identical objects in a familiar environment. After a varying delay, the animal is then exposed to one object that had previously been encountered and one novel object. Time spent with the novel object is used as an index of memory for the familiar object. Ennaceur and Delacour (1988) reported that rats spent more time with the novel object after a short delay (5 min) than after a long delay (24 h). Therefore, rats exhibited strong memory for the familiar object after short retention intervals and no evidence of memory for the familiar object after a long retention interval.

The NOR task is ideal for assessing flashbulb memories in rats in that at a long delay (24 h) there is no evidence of memory for the familiar object. When tested at a short delay (i.e. 5 min or 1 h), rats do show preference for the novel object. Therefore, the intact memory of the objects when tested at short delays degrades over time. Flashbulb memories in people involve memories for context details that would normally be forgotten, but are strengthened because they occur close in time to an emotional event. Therefore, the NOR task is ideal for testing whether a stressful event strengthens memory for context information (the familiar object) that would, under control conditions, be forgotten. A stressful experience occurring close in time to training in the NOR task can be used to enhance the durability of memory for the familiar object so that it exists when animals are tested at 24 h. Therefore, in the experiments outlined below, NOR memory was assessed after a 24 h delay in order to test whether or not a degraded context memory could be strengthened by an arousing experience (cat exposure) occurring close in time.

2.2 General Method

Subjects were male Sprague-Dawley rats obtained from Charles River Laboratories. Rats were approximately 2 months old at the start of the experiment and were pair housed on a 12 h/ 12 h light/dark cycle (lights on at 0700 h) with ad lib access to food and water. Rats were given at least 7 days to acclimate to the housing conditions before the start of experimental manipulations. After the acclimation period, rats were transported to a non-testing room in the laboratory, were given a 30 min acclimation period, and then handled for 2-3 min each for 3 days prior to experimental testing. All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Florida.

2.2.1 Apparatus

The apparatus used for the Novel Object Recognition task was a black Plexiglas box (40 cm × 40 cm × 40 cm). Illumination was provided by a lamp positioned above the apparatus (10 lux). The objects used were 3 identical glass jars filled with sand and 3 identical metal weights (approximately 4 cm × 4 cm × 5 cm). Experimental sessions were video recorded and data were analyzed using ANY-maze™ behavioral tracking software. Animals that were exposed to a cat were placed in a Plexiglas pie-shaped container (with small holes) located in the cat housing room, which insured that the rat could see, hear, and smell the cat without physical contact between the animals.

2.2.2 Procedure

On Days 1-3, subjects were transported to the lab and given a 30 min acclimation period in a non-testing room. Subjects were then individually transported by hand to the NOR room and placed into the empty NOR apparatus for a 5 min habituation session. After the habituation

session, the subject was returned to the home cage. The apparatus was cleaned with diluted ethanol (75% water) after each subject. Subjects were returned to the housing room after completion of each habituation session.

On Day 4 (Training), subjects were transported to the laboratory and given a 30 min acclimation period. All experimental manipulations took place on Day 4. Before or after the experimental manipulation, subjects were individually transported by hand to the NOR room and placed in the apparatus for 5 min. On Day 4, the apparatus contained 2 identical objects (either metal or glass) placed in opposite diagonal corners of the box. Object type and locations were counterbalanced across subjects. After the 5 min Training session, the subject was returned to the home cage and the apparatus was cleaned. Subjects were returned to the housing room after completion of training.

On Day 5 (24 h Recognition Test), subjects were transported to the laboratory and given a 30 min acclimation period. Subjects were individually transported by hand to the NOR room and placed in the apparatus for 5 min. On Day 5, the apparatus contained one copy of the object that the rat had been exposed to on the previous day, and one novel object. After the 5 min Recognition Test, the subject was returned to the home cage and the apparatus was cleaned. Subjects were returned to the housing room after completion of the Recognition Test. No experimental manipulations took place on Day 5.

2.2.3 Data Analysis

The amount of time the subjects spent in the quadrant of the apparatus that contained each object was recorded by ANY-maze™ behavioral tracking software. The percentage of time spent in the quadrant containing the familiar object was then analyzed for each subject and was

averaged across subjects (data were analyzed in the same way for time spent with the novel object). Data were then transformed by dividing the percentage of time each animal spent with the novel object by the average percentage of time spent with the familiar object. In cases where the p-value was less than .1, the number of subjects was doubled to reduce the likelihood of type I or type II error.

2.3 Hypotheses

The first hypothesis was that, based on the flashbulb memory literature in people, memory for context details that occurred close in time (before or after), but independent of location, to the onset of stress would be enhanced in rats. I predicted that brief stress would enhance 24 h memory for the familiar object when training took place in a in a different location immediately after the onset of stress, or when the hippocampus would theoretically be driven into the Phase I state (according to Diamond et al., 2007). For example, brief cat exposure or hormone application immediately before training in the NOR task would theoretically drive the hippocampus into the Phase 1 state and would enhance memory for the objects encountered during training. I also predicted that brief stress occurring immediately after training would enhance hippocampal processing and would result in enhanced memory for context information when tested 24 h later. Additionally, prolonged cat exposure immediately after NOR training was predicted to enhance NOR memory when tested 24 h later because the onset of cat exposure would theoretically enhance hippocampal processing. The duration of post-training stress is not relevant, rather the *onset* of the stressful experience is the important factor.

An additional hypothesis was that memory for events occurring while the hippocampus is in the hypothetical Phase II state (30 min after the onset of stress) would not be enhanced. For example, prolonged or delayed exposure to the cat before NOR training would not result in enhanced memory because theoretically, the objects would be encountered while the hippocampus was in the hypothetical Phase II state. I also predicted that drugs which block noradrenergic activation or interfere with memory storage processes directly would block the cat-induced memory enhancement of context information.

EXPERIMENTAL ASSESSMENT OF AN ANIMAL MODEL OF FLASHBULB MEMORY

3.1 Experiment 1: Does Cat Exposure Affect Context Memory in Rats?

In order to assess the effects of stress on NOR memory, rats were exposed to a cat for different periods of time prior to or after NOR training. In theory, if stress enhances memory for context stimuli that occur close in time to stress, then brief cat exposure immediately prior to or after NOR training, or prolonged cat exposure immediately after training, would enhance memory for the familiar object. Therefore, I hypothesized that rats exposed to the training objects close in time to the *onset* of stress would spend more time with the novel object when tested 24 h later. In contrast, if stress occurs at a delay before or after training, or is prolonged, memory for context stimuli would not be enhanced. Therefore, I hypothesized that brief exposure to the cat 30 min before or after training, or exposure to the cat for 30 min before training would not result in enhanced context memory when tested 24 h later.

3.1.1 Method

Subjects were trained on the NOR task as described previously with the exception that the subjects were transported by the experimenter to the cat housing room and placed into the Plexiglas pie enclosure, which was in close proximity to a female cat, for 2 min or 30 min either prior to or after training on Day 4. Control subjects were trained on the NOR procedures only (no exposure to cat).

3.1.2 Results and Discussion

Control rats did not spend more time with the novel object, t (14) = -.04, p = 0.97, indicating that this group did not show evidence of memory for the training experience when tested 24 h later. In contrast, rats that were exposed to the cat for 2 min immediately *before* NOR training did spend significantly more time with the novel object, t (16) = 2.33, p = 0.03, indicating that 2 min cat exposure before training enhanced memory for the familiar object. Similarly, rats that were exposed to the cat for 2 min immediately *after* NOR training also spent significantly more time with the novel object, t (36) = 2.42, p = 0.02, indicating that 2 min cat exposure after training enhanced memory for the familiar object.

Rats that were exposed to the cat for 2 min followed by a 30 min delay in the home cage *before* training did not spend more time with the novel object, t (14) = 0.88, p = 0.39, indicating that a delay between cat exposure and NOR training did not result in enhanced 24 h memory. Similarly, rats that were exposed to the cat for 2 min after a 30 min delay in the home cage *after* training did not spend more time with the novel object, t (18) = 0.04, p = 0.97, indicating that training followed 30 min later by brief cat exposure did not enhance memory for the familiar object.

Rats that were exposed to the cat for 30 min *before* training did not spend more time with the novel object, t (30) = 1.16, p = 0.26, indicating that prolonged cat exposure before training did not enhance 24 h memory. Interestingly, rats that were exposed to the cat for 30 min *after* training did spend significantly more time with the novel object, t (14) = 2.16, p = 0.05, indicating that prolonged cat exposure after training did enhance 24 h memory. This suggests that weak memory for the objects encountered just prior to cat exposure was enhanced as a result of stress after training. Rats that were exposed to the cat for 30 min before training followed by

immediate exposure to the cat for 30 min after training also did not spend more time with the novel object, t (16) = -0.90, p = 0.38, indicating that prolonged cat exposure before training blocked the 24 h memory enhancement as a result of 30 min cat exposure after training.

Predator exposure lasting for 2 min before or after training enhanced memory when tested 24 h later compared to control animals (which did not show enhanced 24 h memory). In contrast, 30 min of predator immediately before training did not enhance memory. Interestingly, 30 min of predator exposure immediately after training did enhance memory when tested at 24 h. In theory, brief exposure to stress would activate noradrenergic activity in the amygdala which would, in turn, activate information storage processes in the hippocampus. If training in the task takes place immediately before or after stress, the hippocampus would be in the theoretical activation phase and would store information related to the training experience. In contrast, if training takes place 30 min before or after stress, when the activation phase has ended, information related to the training experience would not be stored. This was addressed by exposing rats to a predator for 2 min followed by a 30 min delay in the home cage, which resulted in no memory enhancement when tested 24 h later. It is important to note that predator exposure took place in a different location than NOR training. Therefore, the results of Experiment 1 indicate that predator exposure enhanced memory for context stimuli occurring close in *time*, rather than place, to a stressful experience.

3.2 Experiment 2: Effects of Hormonal and Drug Manipulations on Context Memory in Rats

In addition to examining the effects of predator exposure on memory for context information, pharmacological manipulations were used to investigate some of the neurobiological mechanisms involved in the effects of stress on the formation of context memory. Roozendaal et al. (2006) suggested that noradrenergic activation is necessary for emotional memory modulation. In addition, Packard and Teather (1998) provided evidence that hippocampal memory enhancement via activation of the amygdala is time-dependent. If stress-induced amygdala activation via noradrenergic mechanisms is necessary for memory enhancement, then exogenous application of stress hormones that, in theory, activate noradrenergic mechanisms in the amygdala immediately before or after training should enhance memory, while blockade of noradrenergic mechanisms should block the hormone effects on memory. Therefore, I hypothesized that systemic administration of epinephrine immediately, but not 30 min before or after NOR training would enhance memory. I also hypothesized that propranolol, a β-adrenergic antagonist administered before epinephrine would block the memory enhancement induced by epinephrine injection administered immediately before training.

In addition, drug manipulations can interfere with memory storage processes. For example, CPP, a competitive NMDA receptor antagonist has been shown to block the induction of LTP and impair memory (Abraham & Mason, 1988; Ward et al., 1990). Therefore, CPP was predicted to block the memory enhancing effects of epinephrine administered immediately before training.

3.2.1 Method

Subjects were trained on the NOR task as described previously with the exception that the subjects were given intraperitoneal (i.p.) injections of either saline, hormone, or drug either prior to or after training on Day 4. Subjects were acclimated to the injection conditions by receiving mock injections (saline) for 5 days prior to the experimental session.

3.2.2 Results and Discussion

Rats that were injected with saline (i.p) 5 min *before* NOR training did not spend more time with the novel object, t (18) = -0.02, p = 0.99. Similarly, rats that were injected with saline immediately *after* NOR training did not spend more time with the novel object, t (18) = 0.64, p = 0.53. Both of these manipulations indicate that the injection procedure, itself, did not affect context memory.

In contrast to the effects of saline, rats that were injected with epinephrine (0.05 mg/kg i.p.) 5 min *before* NOR training spent significantly more time with the novel object, t (14) = 2.53, p = 0.02, indicating that adrenergic activation via systemic epinephrine injection enhanced memory for the familiar object. This epinephrine effect mimicked the 2 min cat-induced memory enhancement immediately before training. Similarly, rats that were administered epinephrine (0.10 mg/kg i.p.) immediately *after* NOR training spent significantly more time with the novel object, t (16) = 2.29, p = 0.04, indicating that adrenergic activation via systemic epinephrine injection immediately after training also enhanced memory for the familiar object. This effect of epinephrine mimicked the 24 h memory enhancement induced by 2 min cat exposure immediately after training.

In contrast to the results found with epinephrine injection that occurred close in time to training, rats injected with epinephrine (0.05 mg/kg i.p.) 30 min *before* training did not spend more time with the novel object, t (16) = 0.08, p = 0.94, indicating that epinephrine 30 min before training did not enhance memory for the familiar object. Similarly, rats injected with epinephrine (0.10 mg/kg i.p.) 30 min *after* training did not spend more time with the novel object, t (16) = 1.28, p = 0.23, indicating that delayed epinephrine administration did not enhance memory for the familiar object. These results of delayed epinephrine injection are similar to those found for delayed cat exposure.

An additional finding of Experiment 2 was that drug manipulations that interfered with noradrenergic, or memory storage processes via NMDA receptor activation blocked the memory enhancement as a result of epinephrine immediately before NOR training. Rats that were administered propranolol (1.0 mg/kg i.p.) 30 min before administration of epinephrine (0.05 mg/kg i.p.) 5 min before training did not spend more time with the novel object, t (14) = -0.61, p = 0.55, indicating that the β-adrenergic antagonist blocked the memory enhancing effects of epinephrine. In addition, rats that were administered CPP (5.0 mg/kg i.p.) 30 min before epinephrine administration (0.05 mg/kg i.p.) 5 min before NOR training did not spend more time with the novel object, t (18) = -0.79, p = 0.44, indicating that the NMDA receptor antagonist blocked the memory enhancing effects of epinephrine.

One hypothesis of Experiment 2 was that rats injected with epinephrine immediately before or after, but not 30 min before or after NOR training would exhibit enhanced memory 24 h later. The results of this manipulation indicated that rats injected with epinephrine immediately before or after training, but not 30 min before or after training, showed enhanced memory for the familiar object when tested 24 h later. Another hypothesis was that blockade of noradrenergic

mechanisms (e.g. propranolol) would block the memory enhancing properties of epinephrine. This hypothesis was tested by injecting rats with propranolol, a β-adrenergic antagonist, 30 min before injection of epinephrine. The results indicated that propranolol did block the epinephrine-induced memory enhancement when tested 24 h later. In addition, CPP, a NMDA receptor antagonist, also blocked the epinephrine-induced memory enhancement, suggesting that NMDA receptor activation is necessary.

3.3 Experiment 3: Effects of Cat Exposure in Combination with Drugs on Context Memory in Rats

Experiment 3 assessed the effects of drugs in combination with predator exposure. My first hypothesis was that blockade of noradrenergic mechanisms (e.g. propranolol) would block the memory enhancing effects of brief predator exposure. This was assessed by injecting rats with propranolol, a β-adrenergic antagonist, 30 min before exposure to the cat. In addition, I hypothesized that because CPP blocked the memory enhancing effects of epinephrine, that it would also block the memory enhancement as a result of brief predator exposure. Another hypothesis was that suppression of corticosterone would prevent the memory enhancement via brief predator exposure. Dexamethasone is a synthetic glucocorticoid that, through actions at the pituitary gland, suppresses corticosterone secretion (De Kloet, 1974). Therefore, this drug was used to assess the effects of corticosterone suppression on brief predator exposure-induced memory enhancement.

3.3.1 Method

Subjects were trained on the NOR task as described previously with the exception that the subjects were exposed to a predator as well as given intraperitoneal (i.p.) or subcutaneous (Dexamethasone) injections of saline or drug either prior to or after training on Day 4. Subjects were acclimated to the injection conditions by receiving mock injections (saline) for 5 days prior to the experimental session.

3.3.2 Results and Discussion

Rats that were injected with saline (i.p.) 30 min before 2 min cat exposure spent significantly more time with the novel object, t (16) = 2.23, p = 0.04, indicating that the injection procedure, itself, did not block the 2 min cat-induced memory enhancement. In contrast, when rats were administered propranolol (1.0 mg/kg i.p.) 30 min before 2 min cat exposure, they did not spend more time with the novel object, t (18) = 0.05, p = 0.96, indicating that the β-adrenergic antagonist blocked the 2 min cat-induced memory enhancement. In addition, rats injected with saline 30 min before training, followed by 30 min cat exposure after training spent more time with the novel object, t (16) = 2.25, p = 0.04, indicating that the injection procedure, itself, did not block the memory enhancement resulting from 30 min cat exposure after training. In contrast, rats injected with propranolol 30 min before training, followed by 30 min cat exposure after training did not spend more time with the novel object, t (18) = 0.6407, p = 0.5297, indicating that propranolol blocked the memory enhancement resulting from 30 min cat exposure after training.

In addition, rats that were injected with CPP (5.0 mg/kg i.p.) 30 min before 2 min cat exposure did not spend more time with the novel object, t (18) = 1.10, p = 0.29, indicating that the NMDA receptor antagonist blocked the 2 min cat-induced memory enhancement.
Rats injected with Dexamethasone (50 µg/kg s.c.) 3 h before 2 min cat exposure also did not spend more time with the novel object, t (18) = 0.80, p = 0.44, indicating that drug-induced suppression of corticosterone at the time of cat exposure did not result in enhanced 24 h memory.

Because propranolol blocked the 2 min cat-induced 24 h memory enhancement, these results indicate that activation of noradrenergic mechanisms are required for the brief stress-induced memory enhancement. In addition, propranolol blocked the memory enhancement resulting from 30 min of cat exposure after training, suggesting that noradrenergic mechanisms are also required for the prolonged stress-induced memory enhancement. Dexamethasone also blocked the predator stress-induced memory enhancement, suggesting that corticosterone may also be a necessary component. In addition, CPP blocked the predator stress-induced memory enhancement, indicating that NMDA receptor activation is necessary for the predator stress-induced memory enhancement.

3.4 Experiment 4: Effects of Temporary Amygdala Inactivation on Predator Stress-Induced Context Memory in Rats

It is important to note that in Experiments 1-3, both drugs and hormones were administered systemically, which would affect many brain regions. Therefore, it is important to further assess the brain structures that are critically involved in stress-induced memory enhancement of context information. The amygdala is a critical brain region necessary for the formation of emotional memory (for reviews, see McGaugh, 2004; LeDoux, 2003). The

basolateral amygdala (BLA) is particularly important for the modulation of emotional memory. For example, Roozendaal et al. (2006) provided evidence that noradrenergic activation within the BLA is required for glucocorticoid-induced memory enhancement in rats that were not habituated in an NOR task. Additionally, propranolol administration directly to the BLA impaired the memory enhancement as a result of corticosterone administration in non-habituated rats. Hatfield & McGaugh (1999) also provided evidence that propranolol infusion to the BLA impaired performance in a water maze task compared to control animals, which provides further evidence of the importance of this brain region in stress-induced memory.

The BLA has also been shown to play an important role in predator-stress induced memory. For example, previous work conducted by Zoladz et al. (2011) has shown that temporary BLA inactivation blocked predator stress-induced memory impairment. In this study, rats were given eight trials to find a hidden platform in a radial arm water maze. Training was followed by 30 min of cat exposure followed by a memory test trial. Stressed rats infused with artificial cerebrospinal fluid (aCSF) into the BLA exhibited impaired memory for the platform location compared to a non-stress condition in which rats spent the delay in the home cage. In contrast, rats infused with muscimol, a GABA agonist, exhibited intact memory for the platform location. Therefore, inactivation of the BLA via muscimol blocked the memory-impairment as a result of predator exposure in this task.

The results of the studies by Roozendaal et al. (2006), Hatfield and McGaugh (1999), and Zoladz et al. (2011) have all provided evidence that the BLA is critically involved in the modulation of emotional memory. Therefore, based on these results, as well as the results of Experiments 1-3, I hypothesized that the BLA is critically involved in the predator stress-induced memory enhancement of context information. Therefore, BLA inactivation via muscimol

infusion would block memory enhancement as a result of brief predator exposure in the NOR task.

3.4.1 Method

Subjects were thirty male Sprague-Dawley rats obtained from Charles River Laboratories. Rats were approximately 2 months old at the start of the experiment and were pair housed on a 12 h/ 12 h light/dark cycle (lights on at 0700 h) with ad lib access to rat chow and water. Rats were acclimated to the vivarium conditions for at least 7 days prior to surgery. After the vivarium acclimation period, subjects were transported to a non-testing room in the laboratory, given a 30 min acclimation period, and then handled for 2-3 min each for 3 consecutive days prior to surgery. After surgery, rats were singly housed. All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Florida.

3.4.2 Surgery

On the day of surgery, rats were transported to the lab, deeply anesthetized with isoflurane and oxygen delivered through a nose cone. Animals were treated with Ketoprofen (5 mg/kg, s.c.) every 12 hours for 48 hours following surgery. All surgical procedures were conducted under aseptic conditions. Rats' heads were shaved, cleansed, and placed in a stereotaxic frame. The skull was then exposed and the location of bregma was recorded. Stainless steel guide cannulae (26 gauge, Plastics One Inc. Roanoke, VA) were then positioned bilaterally just above the basolateral amygdala (BLA) using coordinates of 2.8mm posterior to bregma, 5.0mm lateral to bregma, and 6.0mm ventral to the skull surface. The guide cannulae were lowered and then fixed to four skull-screws using dental cement. After the cement

hardened, removable stylets cut 2mm longer than the guide cannulae, were inserted (to prevent clogging) and secured with a dust cap (Plastics One Inc. Roanoke, VA). Rats recovered for 7 days before the start of experimental manipulations

3.4.3 Intracerebral Infusions

For three consecutive days, rats were brought to the lab and given a 30 min acclimation period. On the first of the three consecutive days, after the acclimation period, animals were habituated to the infusion procedures by undergoing a mock infusion in which the dust cap was removed and a mock injection tube placed on the cannula pedestal. The stylet and dust caps were replaced after each habituation session. On the second and third day, the dust cap and stylet were removed and 25 µl syringe injectors were placed into the guide cannulae. On the third day, a Harvard Apparatus pump (Holliston, MA) was connected to the injectors by plastic tubing (Plastics One) and rats were infused with aCSF at a rate of 0.1 µl/min for 5 min. The following day (Day 4) aCSF was infused bilaterally at a rate of 0.1 µl/min for 5 min. After the infusion, the injectors were left in place for an additional 1 min to allow diffusion of vehicle away from the injector tip, after which the stylet and dust cap were replaced.

3.4.4 Histology

After completion of the 24 h memory test, all animals were euthanized, brains were extracted and flash frozen using methylbutane and dry ice. The tissue was then sliced in coronal sections in 40µm increments on a Cryostat held at -19°C, mounted on microscope slides, and stained with cresyl violet in order to determine injector tip location. Animals in which placement was outside of the target area were excluded from analysis.

3.4.5 Behavioral Procedure

Rats were trained on the NOR task as described previously with the exception that rats were infused with aCSF 30 min or 4 hours before brief predator exposure occurring immediately prior to training on Day 4. Subjects were acclimated to the infusion procedure by receiving mock infusions which took place 30 min or 4 hours before habituation sessions.

3.4.6 Results and Discussion

My first hypothesis was that that control rats (infused with aCSF) into the BLA in combination with brief predator exposure immediately before training would spend more time with the novel object indicating intact memory for the familiar object. Therefore, infusion of aCSF would not block the memory enhancement resulting from brief predator exposure immediately before training in the NOR task. The results revealed that animals infused with aCSF 30 min before predator exposure did not spend significantly more time with the novel object, t (12) = 1.17, p = 0.27. This indicated that some factor, such as cannula or injector site, interfered with the cat-induced context memory. Therefore, an additional experiment was conducted in which the cannulae were placed 0.3mm ventral to the skull surface (well above the location of the BLA). The results of this additional experiment revealed that these animals also did not spend significantly more time with the novel object, t (16) = 0.65, p = 0.52., indicating that some aspect of the experiment other than the placement of the cannulae near the BLA interfered with the cat-induced context memory.

One possibility for the lack of predator-stress induced memory enhancement after infusion of aCSF was that the infusion procedure, itself, was stressful. The infusion occurred 30 min prior to cat exposure by which time, theoretically, the hippocampus would be driven into the

Phase II state. Therefore, a final experiment was conducted in which the cannulae were placed 0.5mm ventral to the skull surface (near the BLA) and aCSF was infused 4 hours prior to cat exposure. The results of this experiment also revealed that animals infused with aCSF 4 hours prior to cat exposure did not spend significantly more time with the novel object, t (14) = 0.97, p = 0.34. These results indicated that some other aspect of the procedure interfered with the cat-induced memory for context information. Therefore, more research is needed to rule out possible confounding variables that can account for the blockade of predator stress-induced memory enhancement under control (aCSF) conditions.

3.5 Experiment 5: Effects of Adrenalectomy on Predator Stress-Induced Context Memory in Rats

Several studies outlined above have provided evidence that both epinephrine and corticosterone are necessary for stress-induced memory enhancement (for reviews see Rodrigues et al., 2009; McGaugh et al., 1996; McGaugh & Roozendaal, 2002;). For example, the results of the study by Liang et al. (1995) showed that removal of endogenous epinephrine via adrenal demedullation impaired memory in an inhibitory avoidance task in rats indicating that endogenous epinephrine is a necessary component involved in the formation of emotional memory. In addition, previous work from our lab has shown that adrenalectomy (ADX) prevents memory enhancement in a hippocampal-dependent task as a result of brief predator exposure (unpublished data). In this study, rats were given weak training (4 trials) in a radial arm water maze (RAWM) task. Sham operated animals exposed to a cat for 2 min immediately before training had significantly fewer errors than sham rats that were not exposed to the cat, indicating that the brief predator exposure enhanced memory in sham operated rats. In contrast, ADX rats

exposed to the cat did not have fewer errors compared to sham rats exposed to the cat, indicating that ADX prevented the stress-induced memory enhancement as a result of brief predator exposure. Further, stressed and non-stressed ADX rats given an injection of corticosterone before cat exposure or training did not display enhanced memory suggesting that corticosterone alone cannot account for the memory enhancing properties of brief predator stress. Therefore, an interaction of epinephrine and corticosterone appear to be necessary for stress-induced memory enhancement.

In addition to studies assessing the effects of adrenalectomy on stress-induced memory enhancement, the results of Experiments 2 and 3 revealed that systemic administration of epinephrine immediately before or after training in the NOR task enhanced memory when animals were tested 24 h later. Additionally, propranolol, a β-adrenergic antagonist, blocked the memory enhancement as a result of both predator exposure and epinephrine administration in the NOR task. Administration of dexamethasone, which results in a suppression of corticosterone secretion via actions on the HPA axis, blocked the memory enhancement as a result of brief predator exposure. Together, these results provide evidence that an interaction of both epinephrine and corticosterone appear to be necessary for the predator stress-induced memory enhancement in the NOR task. Therefore, I hypothesized that removal of endogenous epinephrine and corticosterone via adrenalectomy would prevent memory enhancement in the NOR task as a result of brief predator exposure.

An important point to address is that adrenalectomy removes *both* endogenous sources of corticosterone and epinephrine. Therefore, an important question to consider is whether the memory enhancement effect in the NOR task is due to the combined action of these hormones or to the action of one individually. Preliminary findings from our lab (unpublished data) has shown

that ADX rats (both stressed and non-stressed) given corticosterone injections did not show memory enhancement in the RAWM task, indicating that corticosterone, alone, was not sufficient for memory enhancement. However, the effects of epinephrine administration were not assessed in this task. To address the role of epinephrine on context memory, ADX rats were administered epinephrine immediately before training in the NOR task.

3.5.1 Method

Subjects were twenty male Sprague-Dawley rats obtained from Charles River Laboratories. Rats were approximately 2 months old at the start of the experiment and were pair housed on a 12 h/ 12 h light/dark cycle (lights on at 0700 h) with ad lib access to rat chow and water. Rats were acclimated to the vivarium conditions for at least 7 days prior to surgery. After the acclimation period, the rats were transported to a non-testing room in the laboratory, given a 30 min acclimation period, and then handled for 2-3 min each for 3 days prior to surgery. Rats receiving epinephrine injection were acclimated to the injection procedure as described previously. All procedures were approved by the Institutional Animal Care and Use Committee at the University of South Florida.

3.5.2 Surgery

On the day of surgery, rats were deeply anesthetized with isoflurane and oxygen which were delivered through a nose cone and adrenalectomized (ADX) or sham adrenalectomized (SHAM). All surgical procedures were conducted under aseptic conditions. Briefly, rats were shaved and cleansed and a small incision was made through the dorsal skin (posterior to the rib cage). Bilateral incisions were then made in the muscle wall above the location of the kidneys and the adrenal glands were located and extracted using circular forceps and small surgical

scissors. After the adrenal glands were removed the muscle walls and skin were sutured. Sham operated animals underwent all surgical procedures with the exception that the adrenal glands were left intact. Animals were treated with Carprofen (5 mg/kg, s.c.) every 12 hours for 48 hours following surgery. Rats recovered for 10 days before the start of experimental manipulations. Research has found that adrenalectomy in rats leads to a loss of neurons in the dentate gyrus (Sapolsky et al., 1991; Sloviter et al., 1993) which can be reversed with a low dose of corticosterone (Gould et al., 1990). Therefore, after surgery, ADX rats were maintained on rat chow and 20 µg corticosterone 0.9% saline water available ad libitum.

3.5.3 Behavioral Procedure

Subjects were trained in the NOR task as described previously. Briefly, subjects were habituated to the NOR apparatus for 3 consecutive days. On Day 4 (training), subjects were placed in close proximity to a cat for 2 min or given an injection of epinephrine (0.05 mg/kg i.p.) immediately before training with two identical objects. On Day 5 (memory test) subjects were placed back into the NOR apparatus and allowed to explore one familiar (encountered the previous day) and one novel object for 5 min.

3.5.4 Corticosterone Assay

After completion of behavioral testing, subjects underwent 20 min of restraint stress (decapicone) in order to test for stress-induced increases in corticosterone. Following the 20 min restraint stress, animals were decapitated and trunk blood was collected for assay of serum corticosterone. The blood was spun in a centrifuge and the serum was collected and stored at -80 degrees until the time of assay. Assays will be performed by a collaborator at a future date.

3.5.5 Results and Discussion

I hypothesized that sham operated rats exposed to the cat for 2 min immediately before NOR training would spend more time with the novel object providing evidence of intact memory for the familiar object when tested at 24 hours. Indeed, rats that underwent sham operation procedures did spend significantly more time with the novel object, t (12) = 2.59, p = 0.02, indicating that the sham operation, itself, did not interfere with predator stress-induced context memory.

In contrast, I hypothesized that adrenalectomized (ADX) rats would not show preference for the novel object. That is, the lack of stress hormones (epinephrine and corticosterone) via adrenalectomy would prevent the memory enhancement as a result of brief predator stress. The results of this experiment revealed that ADX rats did not spend significantly more time with the novel object, t (18) = 0.42, p = 0.7, indicating no evidence of memory for the familiar object when tested at 24 hours. These results provided evidence that removal of endogenous epinephrine and corticosterone eliminated the memory enhancement produced by brief predator exposure before training.

Additionally, ADX rats injected with epinephrine immediately before training spent significantly more time with the novel object t (18) = 2.07, p = 0.05, indicating enhanced memory for the familiar object when tested 24 h later. These results provided evidence that replacement of epinephrine, in the absence of circulating corticosterone, resulted in enhanced memory for context information in ADX rats.

SUMMARY

Overall this work has provided an important framework in which to further study flashbulb memory where memory for only those context details that occur close in time to the onset of an emotional event are well remembered. In my animal model, I have separated, in time and location, a stressful event from to-be-remembered context information. Therefore, the conditions in which stress *produces* memory for context details that would otherwise not be remembered could be assessed.

The first hypothesis in my study was that memory for context details that occur close in time (before or after), but independent of location, to the onset of stress would be enhanced. For example, I hypothesized that brief stress would enhance 24 h NOR memory when training took place in a in a different location immediately after the onset of stress, because the hippocampus would theoretically be driven into the Phase I state (according to Diamond et al., 2007). That is, brief stress occurring just before training would enhance memory for that training experience that would otherwise not be remembered. For example, cat exposure for a brief period of time or hormone administration immediately before training in the NOR task would theoretically drive the hippocampus into the Phase 1 state which would enhance memory for the objects encountered during training. An additional hypothesis was that the brief stress occurring immediately after training would enhance hippocampal processing which would also result in enhanced memory for context information when tested 24 h later. Further, prolonged cat exposure immediately after NOR training would enhance NOR memory when tested 24 h later because the onset of cat exposure (occurring immediately after training) would enhance

hippocampal processing. The duration of post-training stress is not relevant, rather it is the *onset* of the stressful experience that is the important factor.

Though the duration of cat exposure immediately *after* training is not an important factor, the duration of cat exposure *prior* to training is important because the onset of stress must occur close in time to training. Therefore, I hypothesized that prolonged cat exposure immediately *before* training would not enhance memory. Prolonged or delayed exposure to the cat before NOR training would not result in enhanced memory because theoretically, the objects would be encountered while the hippocampus was in the phase II state. Finally, I hypothesized that drugs which block noradrenergic activation or interfere with memory storage processes directly would also block the cat-induced memory enhancement.

There were several main findings of my experiments examining the effects of predator exposure on NOR memory. First, rats exposed to brief stress (cat exposure) immediately before or after an incidental event (exposure to objects), spent more time with the novel object, indicating enhanced memory for that incidental event compared to control animals which showed no evidence of memory when tested 24 h later. That is, brief cat exposure occurring either immediately before or after training enhanced memory for the objects that would otherwise not have been remembered. These results are consistent with the flashbulb memory literature in people where memory for incidental context events occurring close in time with an emotional event is enhanced. It is important to note that exposure to the objects occurred in a different room than cat exposure. Therefore, time, rather than location, was the critical variable determining whether context cues would be well remembered.

My results are also consistent with the temporal dynamics model of emotional memory proposed by Diamond et al. (2007) which hypothesized that memory for events occurring

immediately after stress would be enhanced because arousal would activate storage processes in the hippocampus. The results of Experiment 1 revealed that brief predator exposure immediately before an incidental event (exposure to objects) occurring in a different environment, enhanced memory for that incidental event in rats because theoretically, the objects were encountered when the hippocampus was in the activated (Phase 1) state. In addition, brief predator exposure occurring at a delay before training did not enhance memory because the hippocampus was theoretically in the Phase II state at the time the objects were encountered which is also consistent with the temporal dynamics model.

As an extension of the temporal dynamics model, I hypothesized that context cues occurring close in time before the onset of stress would also be well remembered. The results of Experiment 1 revealed that *brief* predator exposure immediately after an incidental event (NOR training) enhanced memory for that event, which is consistent with flashbulb memory literature in people. The results also provided evidence that *prolonged* predator exposure immediately after training enhanced memory in the NOR task. In this case, the duration of predator exposure after training was irrelevant in that the *onset* of stress occurred immediately after training which would theoretically boost hippocampal processing so that context information encountered at the onset of stress would be well remembered.

In addition to the timing of stress, mechanisms involved in the stress-induced enhancement of context memory in rats were assessed. Results of other studies of emotional memory in animals have provided evidence that noradrenergic activation is required for emotional memory enhancement (Gold & van Buskirk, 1975; Liang et al., 1995; Roozendaal et al., 2006). Similarly, the results Experiments 2 and 3 revealed that the administration of epinephrine immediately, but not 30 min, prior to as well as after training in the NOR task

enhanced memory when rats were tested 24 h later. Additionally, propranolol, a drug that blocks noradrenergic activation, blocked the memory enhancing effects of epinephrine as well as of brief predator exposure. These results provide strong evidence that noradrenergic mechanisms are required for the memory enhancing effects of brief cat exposure and epinephrine administration occurring before or after training in the NOR task.

Another hormone that plays a role in the formation of stress-induced context memory is corticosterone. The results of other studies in animals addressing the effects of corticosterone on emotional memory have revealed that memory is only enhanced when corticosterone administration occurs in combination with an arousing experience (Sandi et al., 1997; Okuda et al., 2004). In Experiment 3, dexamethasone, which suppresses endogenous corticosterone via actions on the pituitary, was administered to examine whether corticosterone is necessary for the memory enhancing properties of an arousing event (brief cat exposure). Dexamethasone did block the memory enhancement resulting from brief cat exposure suggesting that corticosterone secretion (or actions of the ACTH component of the stress response) appears to be a necessary component of the brief stress-induced memory enhancement. The results of my study are also consistent with the temporal dynamics model which states that as a delayed component of Phase 1, corticosterone would begin to activate synaptic plasticity mechanisms thereby leading to memory enhancement of context events occurring during the Phase 1 state. Therefore, corticosterone appears to be a delayed component involved in the predator stress- induced memory enhancement in rats.

NMDA receptor function is also a necessary component involved in memory formation. The NMDA receptor, a subreceptor for glutamate, is a critical receptor for hippocampal-dependent learning and memory. Because NMDA receptor antagonists have been shown to block

hippocampal-dependent memory (Morris et al., 1986; Baker & Kim, 2002), it was hypothesized that NMDA receptor antagonists would also block the memory-enhancing properties resulting from brief predator exposure or epinephrine administration immediately before training. The results of Experiment 3 revealed that CPP, an NMDA receptor antagonist which interferes with memory storage processes in the hippocampus, blocked the memory enhancing effects of pre-training epinephrine administration as well as pre-training brief cat exposure. Therefore, activation of NMDA receptors is also required for the memory enhancing effects observed in this study.

Overall, the results of Experiments 1-3 provided evidence that context cues occurring close in time prior to or after the onset of stress are remembered independent of the location in which the stress and context cues occurred. Therefore, timing, rather than location is the critical variable involved in the stress-induced context memory enhancement. In addition, these results provided evidence that noradrenergic activation, corticosterone secretion, and NMDA receptor function all appear to be required for the time-dependent memory enhancement as a result of brief predator exposure.

Another goal of this work was to extend the findings of Experiments 1-3 by assessing the role of the amygdala in the brief predator stress-induced memory enhancement of context information. I hypothesized that temporary amygdala inactivation in rats would block the formation of memory for context stimuli occurring immediately after exposure to a predator. The results from the first control group of Experiment 4, which were infused with aCSF into the BLA, indicated that rats did not show preference for the novel object, suggesting no evidence of memory for the familiar object.

One explanation as to why the aCSF infused animals did not show predator stress-induced memory enhancement was that the cannula or injector position interfered with amygdala function, thereby disrupting memory formation. Therefore, a second manipulation infusing aCSF was conducted in which the cannulae were placed just below the cortex (well above the coordinates for the BLA). The results of this experiment also revealed that animals did not show preference for the novel object, therefore it is unlikely that the position of the cannulae interfered with the stress-induced memory formation. An additional explanation was that the infusion procedure, itself, was stressful. Therefore, a third manipulation was conducted in which the infusion procedure took place 4 hours prior to cat exposure. The results of this experiment revealed that animals did not show a preference for the novel object, indicating no evidence of memory for the familiar object.

An additional explanation for why the aCSF infused animals did not show evidence of enhanced memory as a result of cat exposure was that the animals were singly housed after surgery. Therefore, this methodological change could have affected the predator-induced memory enhancement. Another possibility is that there may be some feature of the surgery, itself, that has an influence on cannulated animals. These issues should be addressed in future studies.

A final goal of this work was to assess the effects of adrenalectomy on predator stress-induced memory for context information. I hypothesized that sham operated animals briefly exposed to a cat before training would show intact memory for the familiar object. In contrast, I hypothesized that ADX rats would not show intact memory in the NOR task. The results of Experiment 5 revealed that sham operated animals exposed to a cat for 2 min immediately before NOR training did show preference for the novel object which indicated that the surgical

procedure, itself, did not interfere with the predator stress-induced memory enhancement. In contrast, ADX rats exposed to a cat for 2 min immediately before NOR training did not show preference for the novel object, which indicated that removal of the endogenous source of epinephrine and corticosterone prevented the predator stress-induced memory enhancement. These results are consistent with other studies assessing the effects of ADX on memory as well as previous work from our lab that has shown that ADX prevents memory enhancement in the RAWM task as a result of brief predator exposure.

It is important to note that adrenalectomy removes the endogenous source of both corticosterone and epinephrine. Therefore, it is difficult to assess whether the memory enhancement from brief predator stress is due to a combination of these hormones or if the effect is due to the action of one hormone individually. There is preliminary evidence from our lab that corticosterone supplementation in ADX rats does not result in memory enhancement in a hippocampal dependent task (unpublished data). Therefore, I conducted an additional experiment in which ADX rats were supplemented with epinephrine immediately before training in the NOR task. The results of this experiment indicated that in ADX rats, epinephrine injection, which was the same dose that was given to adrenal intact animals in my previous experiments, enhanced memory for the familiar object in the absence of the endogenous source of corticosterone.

The results revealing that epinephrine, alone, enhances context memory are intriguing in that studies assessing the effects of corticosterone on emotional memory suggest that corticosterone is a necessary component of emotional memory formation. However, many studies assessing the effects of corticosterone have reported that corticosterone, alone, does not enhance memory; it only enhances memory when under conditions of arousal (Cahill & McGaugh, 1996; McGaugh, 2000; McGaugh, 2004; Okuda et al. 2004; Roozendaal et al., 2006)

Therefore, it appears that noradrenergic activation via the action of peripheral epinephrine is sufficient for predator-stress induced memory enhancement in the absence of corticosterone.

In summary, this work has provided evidence that predator stress-induced context memory requires noradrenergic activation that is time-dependent. Brief predator stress occurring immediately before or after exposure to context stimuli enhances memory for those stimuli, which would otherwise be forgotten. In addition, epinephrine administered immediately, but not at a delay, before or after exposure to context stimuli, also enhances memory for those stimuli indicating that epinephrine administration mimics noradrenergic activation induced by predator stress. Additional evidence that supports the necessity of noradrenergic activation is that propranolol, a β-adrenergic antagonist, blocked memory enhancement of epinephrine injection and brief predator exposure occurring immediately before exposure to context stimuli. Further, removal of endogenous stress hormones via adrenalectomy prevented predator stress-induced memory enhancement, and replacement of epinephrine via systemic injection in ADX rats eliminated the memory deficit due to adrenalectomy. Overall, this work has provided further insight into the time-dependent, noradrenergic mechanisms involved in the formation of flashbulb, and potentially traumatic, memories in people.

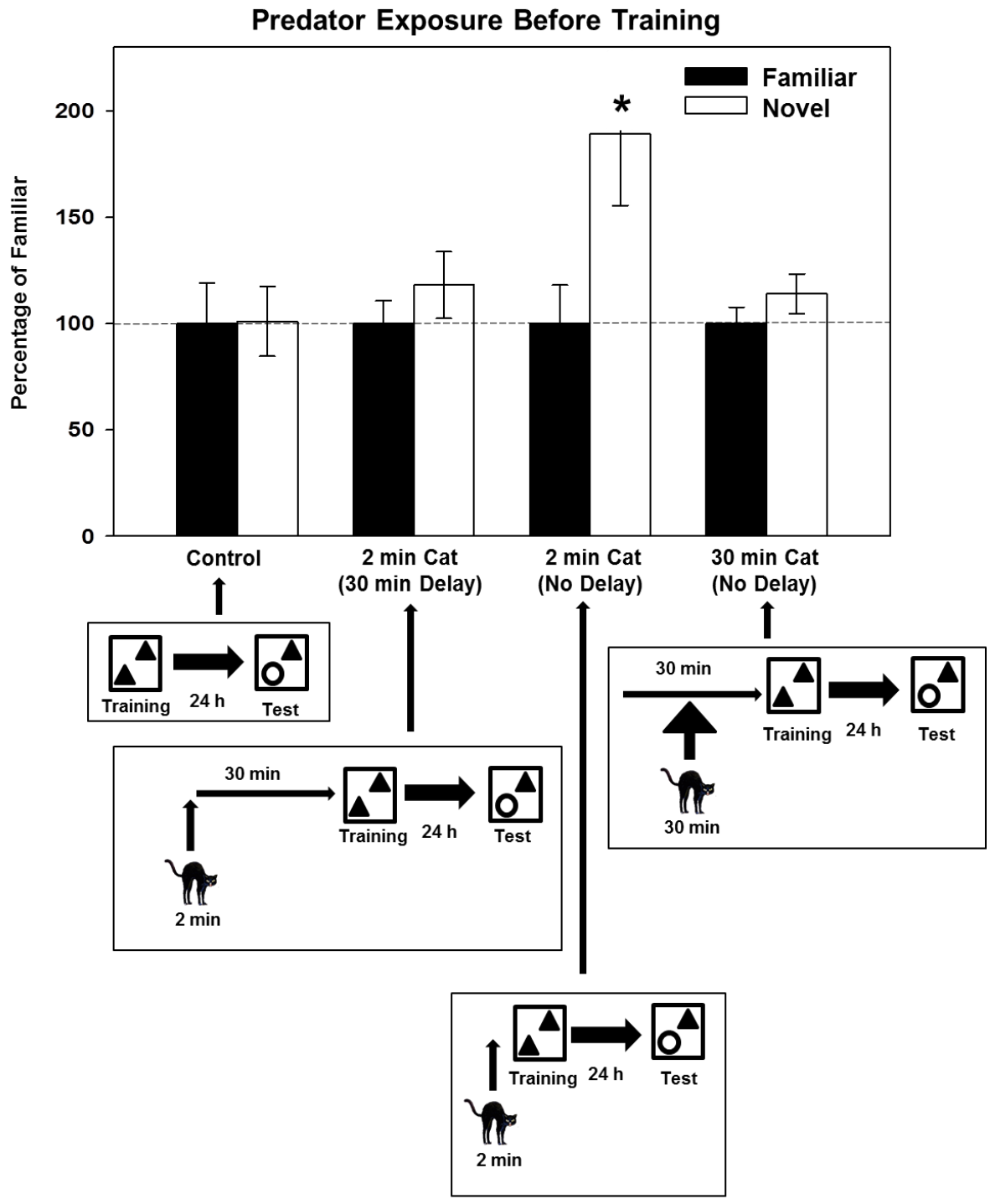

Figure 3. Effects of predator exposure before novel object recognition training. Predator exposure for a 2 min duration immediately before training enhanced 24 h memory relative to control. Predator exposure for 2 min a duration followed by a delay and predator exposure for a 30 min duration did not enhance 24 h memory. Solid bars represent time spent with the familiar object. Open bars represent time spent with the novel object. Asterisks indicate statistical significance ($p < .05$).

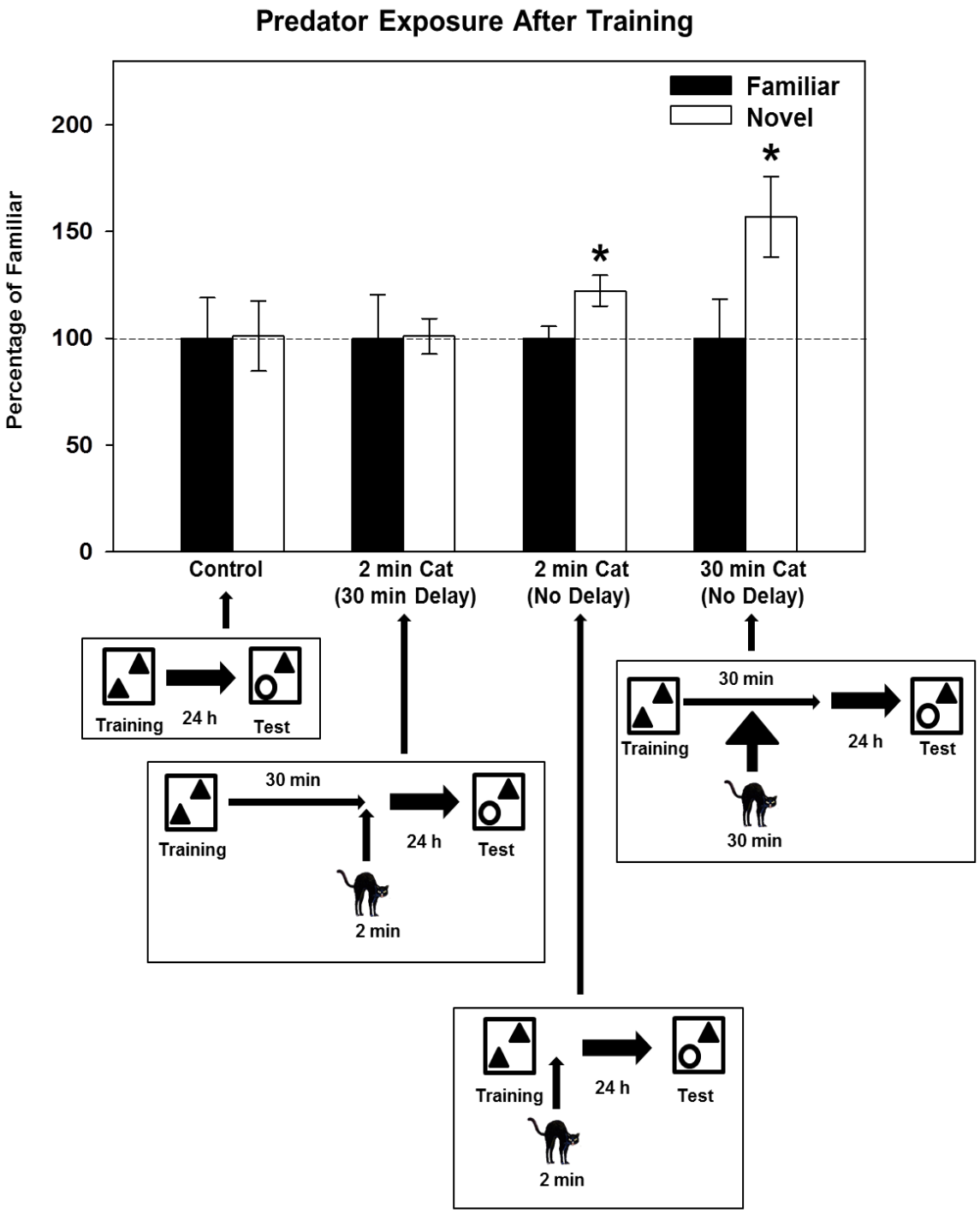

Figure 4. Effects of predator exposure after novel object recognition training (n range 8-19). Predator exposure for a 2 min or 30 min duration immediately after training enhanced 24 h memory relative to control. Predator exposure for a 2 min duration following a delay after training did not enhance 24 h memory. Solid bars represent time spent with the familiar object. Open bars represent time spent with the novel object. Asterisks indicate statistical significance ($p < .05$).

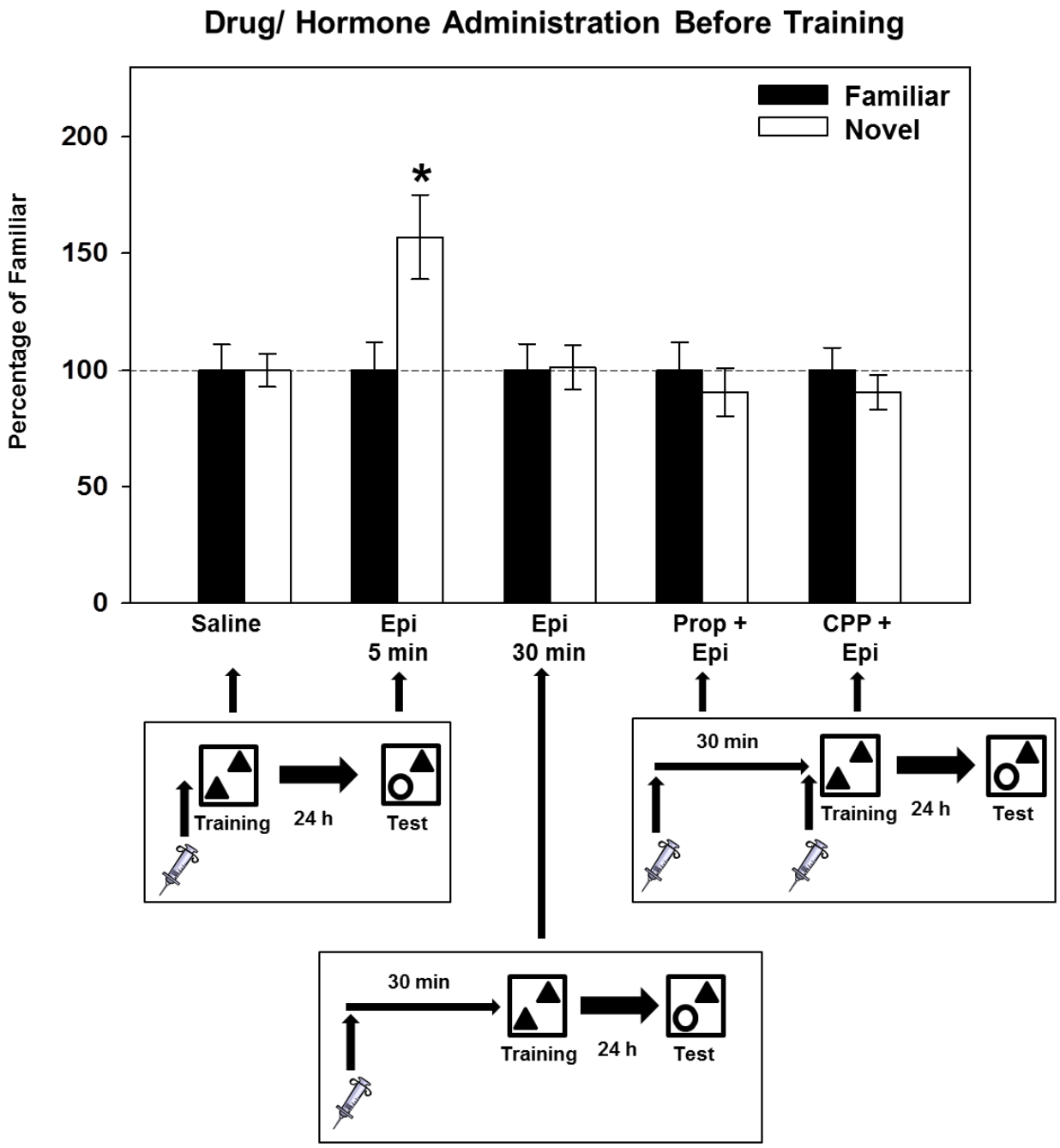

Figure 5. Effects of drug or epinephrine administration before novel object recognition training. Epinephrine administered immediately before training enhanced 24 h memory relative to saline. Epinephrine administered 30 min before training did not enhance 24 h memory. Propranolol and CPP blocked the memory enhancement resulting from epinephrine administration immediately before training. Solid bars represent time spent with the familiar object. Open bars represent time spent with the novel object. Asterisks indicate statistical significance ($p < .05$).

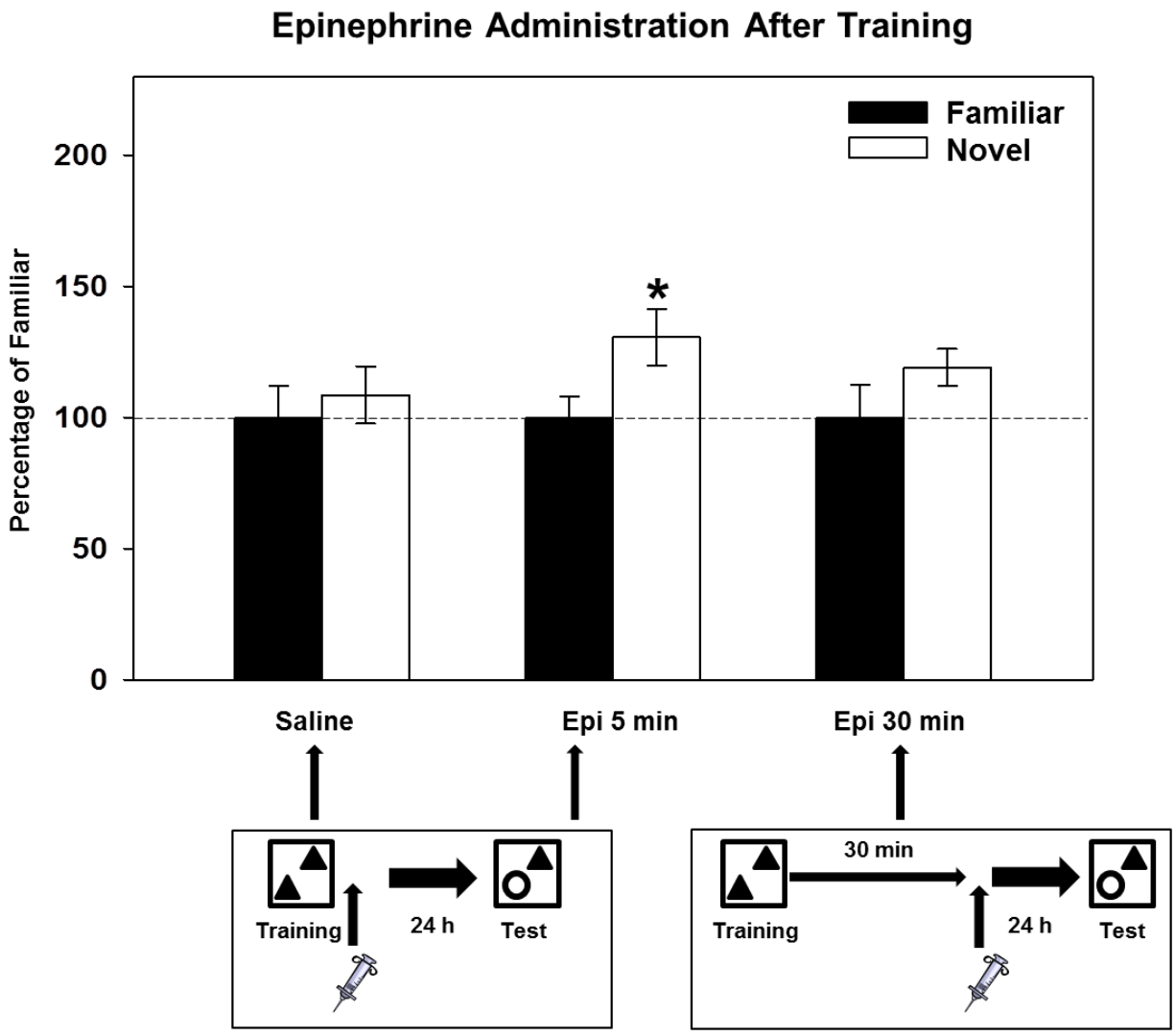

Figure 6. Effects of epinephrine administration after novel object recognition training. Epinephrine administered immediately after training enhanced 24 h memory relative to saline. Epinephrine administered 30 min after training did not enhance 24 h memory. Solid bars represent time spent with the familiar object. Open bars represent time spent with the novel object. Asterisks indicate statistical significance ($p < .05$).

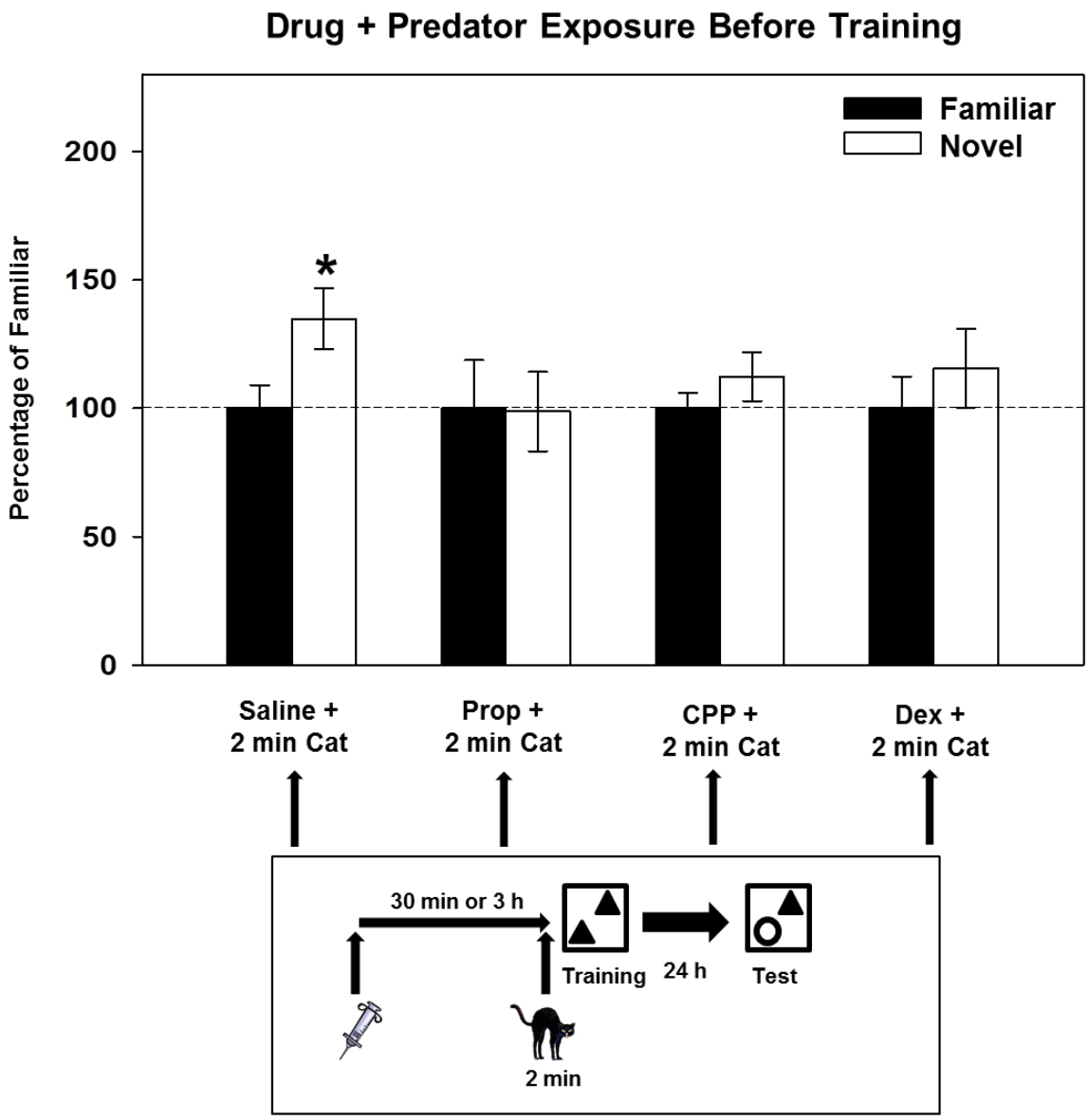

Figure 7. Effects of drug administration in combination with brief predator exposure before novel object recognition training. Propranolol, CPP, and Dexamethasone blocked the 2 min cat-induced memory enhancement. Solid bars represent time spent with the familiar object. Open bars represent time spent with the novel object. Asterisks indicate statistical significance ($p < .05$).

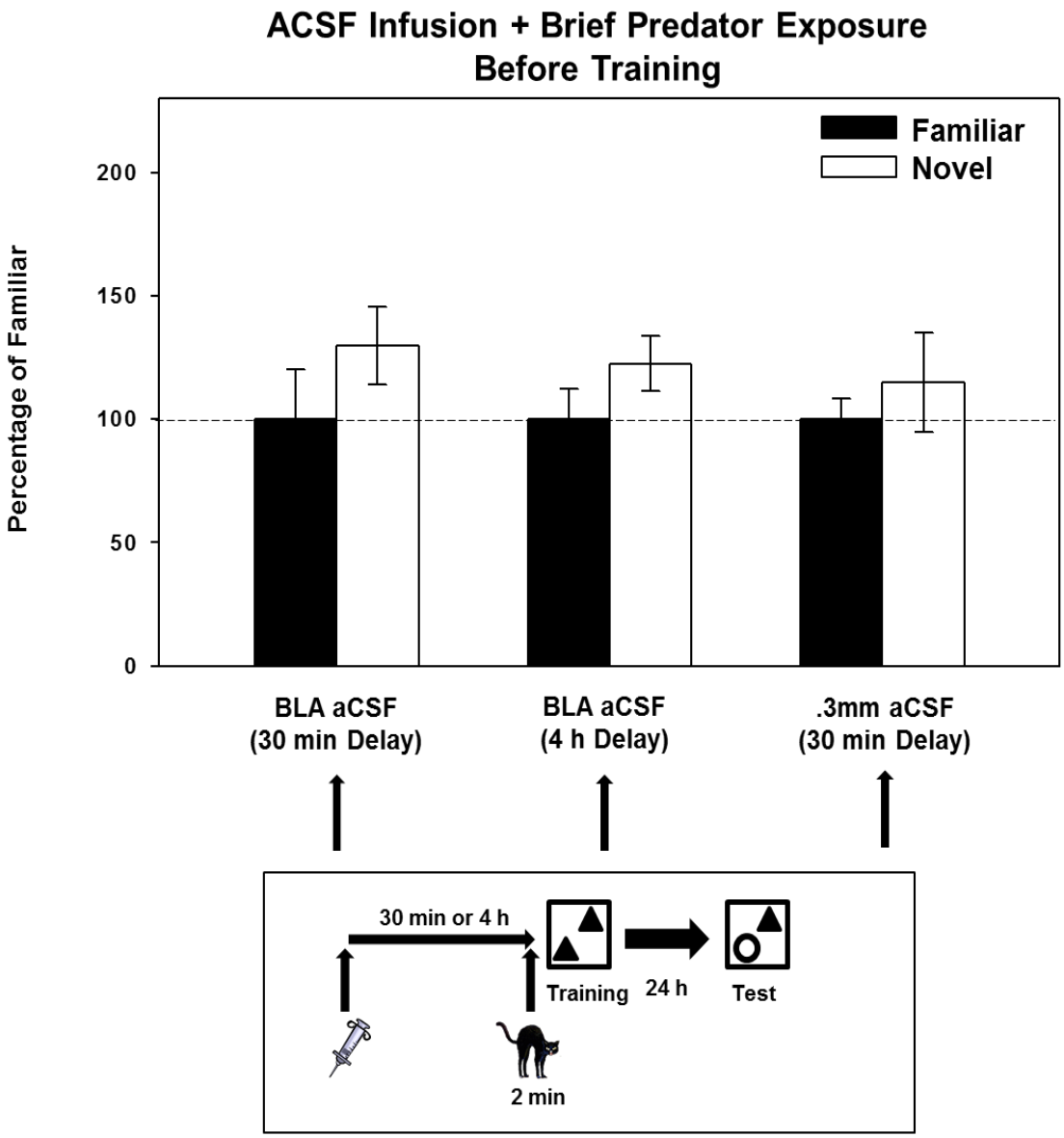

Figure 8. Effects of aCSF infusion in combination with brief predator exposure immediately before novel object recognition training. Infusion of aCSF into the BLA 30 min and 4 h prior to cat exposure prevented the 2 min cat-induced memory enhancement. Infusion of aCSF .3mm below the cortex 30 min prior to cat exposure prevented the 2 min cat-induced memory enhancement. Solid bars represent time spent with the familiar object. Open bars represent time spent with the novel object. Asterisks indicate statistical significance ($p < .05$).

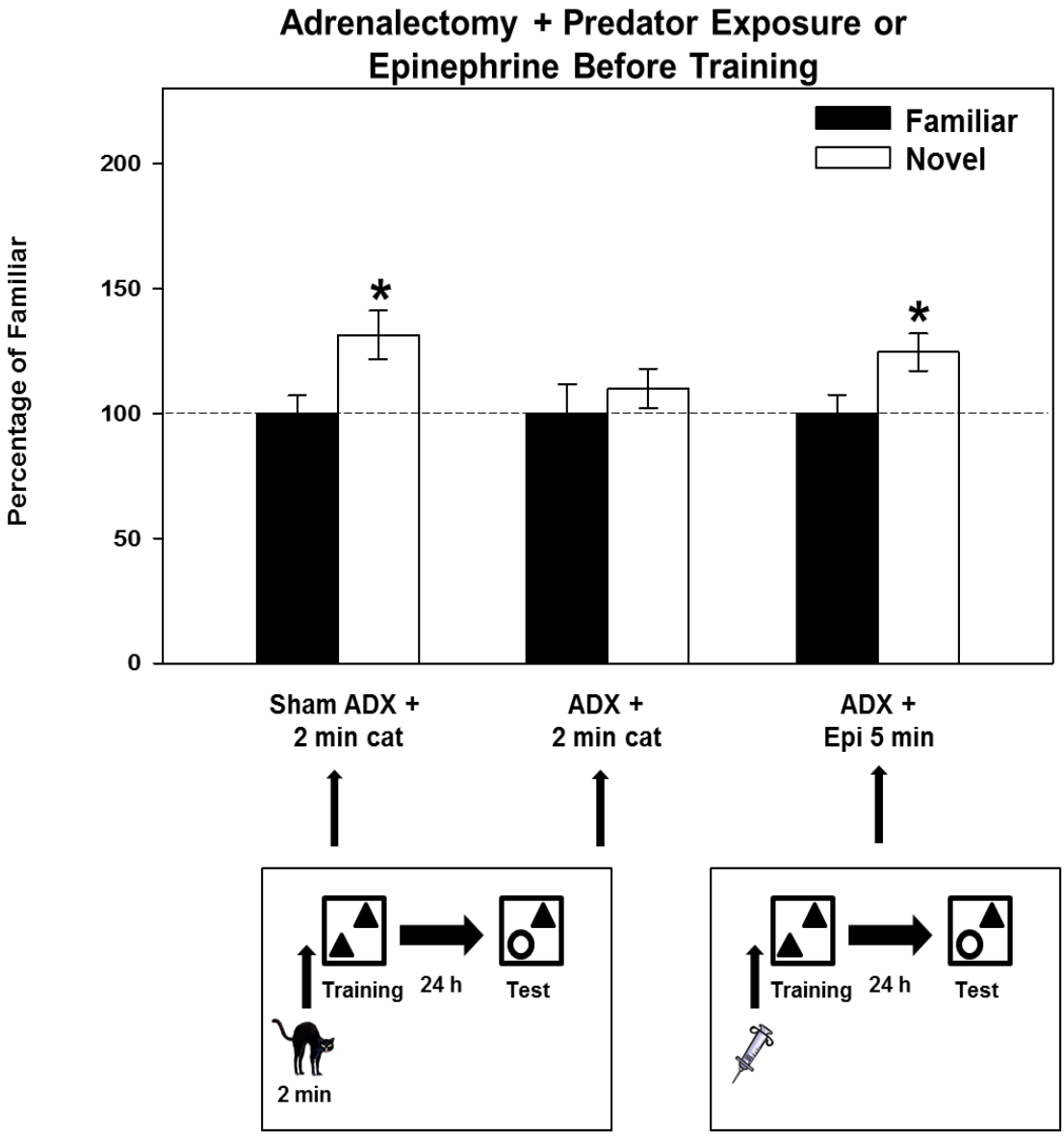

Figure 9. Effects of adrenalectomy in combination with brief predator exposure or epinephrine immediately before novel object recognition training. Sham ADX resulted in intact 2 min cat-induced memory enhancement. Adrenalectomy (ADX) prevented the 2 min cat-induced memory enhancement. Epinephrine blocked the memory impairment produced by adrenalectomy. Solid bars represent time spent with the familiar object. Open bars represent time spent with the novel object. Asterisks indicate statistical significance ($p < .05$).

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Appendix A: IACUC Approval Rat

USF UNIVERSITY OF SOUTH FLORIDA

DIVISION OF RESEARCH INTEGRITY AND COMPLIANCE
INSTITUTIONAL ANIMAL CARE USE COMMITTEE

MEMORANDUM

TO:	David Diamond, Ph.D. Dept. of Psychology PCD4118-G
FROM:	Jay B. Dean, Ph.D, Chairperson Institutional Animal Care & Use Committee Division of Research Integrity and Compliance
DATE:	2/6/2013
PROJECT TITLE:	Neuroendocrine Mechanisms and Therapeutics in an Animal Model of PTSD – Rat
AGENCY/SOURCE OF SUPPORT:	Dept. of Veterans Affairs
IACUC PROTOCOL#:	**V 4385**
PROTOCOL STATUS:	**APPROVED**

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC requested modifications/further information in response to that review and has received the required information. The IACUC **APPROVED** your request to use the following animals in your protocol for a one-year period **beginning 2/5/2013 :**

- 2592 Rats, Male

Please reference the above IACUC protocol number in all correspondence regarding this project with the IACUC, Comparative Medicine, or the Division of Research Integrity and Compliance. In addition, please take note of the following:

- **IACUC approval is granted for a one-year period at the end of which, an annual renewal form must be submitted for years two (2) and three (3) of the protocol.** After three years all continuing studies must be completely re-described in a new application and submitted to IACUC for review.
- **All Comparative Medicine pre-performance safety and logistic meetings must occur prior to implementation of this protocol** [IACUC policy V.10]. Please contact the program coordinator at compmed@research.usf.edu to schedule a pre-performance meeting.
- **All changes to the IACUC-Approved Protocol must be pre-approved by the IACUC [IACUC policyIII.11].** Minor changes can be submitted to the IACUC for review and approval as an amendment or procedural change, whereas major changes to the protocol require submission of a new IACUC application. Minor changes are changes considered to be within the scope of the original research hypothesis or involve the original species and are submitted to the IACUC as an Amendment or Procedural change. Any change in the IACUC–approved protocol that does not meet the latter definition is considered a major protocol change and requires th submission of a new application. More information on what constitutes a minor versus major protocol change and procedural steps necessary for IACUC review and approval are available on the Comparative Medicine web site at http://www.research.usf.edu/cm/amendments.htm
- **All costs invoiced to a grant account must be allocable to the purpose of the grant [IACUC policies IV.5 and V.10].** Costs allocable to one protocol may not be shifted to another in order to meet deficiencies caused by overruns, or for other reasons of convenience. Rotation of charges among protocols by month without establishing that the rotation schedule credibly reflects the relative benefit to each protocol is unacceptable.

For more information on IACUC policies and procedures, please visit the Comparative Medicine web site at http://www.research.usf.edu/cm/default.htm.

cc: Comparative Medicine
Division of Research Grants

OFFICE OF RESEARCH · DIVISION OF RESEARCH INTEGRITY AND COMPLIANCE
INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
PHS No. A4100-01, AAALAC No.58-15, USDA No. 58-15
University of South Florida · 12901 Bruce B. Downs Blvd., MDC35 · Tampa, FL 33612-4799
(813) 974-7106 · FAX (813) 974-7091

Appendix B: IACUC Approval Cat

USF UNIVERSITY OF SOUTH FLORIDA

DIVISION OF RESEARCH INTEGRITY AND COMPLIANCE
INSTITUTIONAL ANIMAL CARE USE COMMITTEE

MEMORANDUM

TO:	David Diamond, Ph.D. Dept. of Psychology PCD4118-G
FROM:	Jay B. Dean, Ph.D, Chairperson Institutional Animal Care & Use Committee Division of Research Integrity and Compliance
DATE:	2/5/2013
PROJECT TITLE:	Neuroendocrine Mechanisms and Therapeutics in an Animal Model of PTSD - Cat
AGENCY/SOURCE OF SUPPORT:	Dept. of Veterans Affairs
IACUC PROTOCOL#:	**V 4384**
PROTOCOL STATUS:	**APPROVED**

The Institutional Animal Care and Use Committee (IACUC) reviewed your application requesting the use of animals in research for the above-entitled study. The IACUC requested modifications/further information in response to that review and has received the required information. The IACUC **APPROVED** your request to use the following animals in your protocol for a one-year period **beginning 2/5/2013 :**

- 4 Cat-Adult, Female

Please reference the above IACUC protocol number in all correspondence regarding this project with the IACUC, Comparative Medicine, or the Division of Research Integrity and Compliance. In addition, please take note of the following:

- **IACUC approval is granted for a one-year period at the end of which, an annual renewal form must be submitted for years two (2) and three (3) of the protocol.** After three years all continuing studies must be completely re-described in a new application and submitted to IACUC for review.
- **All Comparative Medicine pre-performance safety and logistic meetings must occur prior to implementation of this protocol** [IACUC policy V.10]. Please contact the program coordinator at compmed@research.usf.edu to schedule a pre-performance meeting.
- **All changes to the IACUC-Approved Protocol must be pre-approved by the IACUC [IACUC policyIII.11].** Minor changes can be submitted to the IACUC for review and approval as an amendment or procedural change, whereas major changes to the protocol require submission of a new IACUC application. Minor changes are changes considered to be within the scope of the original research hypothesis or involve the original species and are submitted to the IACUC as an Amendment or Procedural change. Any change in the IACUC–approved protocol that does not meet the latter definition is considered a major protocol change and requires th submission of a new application. More information on what constitutes a minor versus major protocol change and procedural steps necessary for IACUC review and approval are available on the Comparative Medicine web site at http://www.research.usf.edu/cm/amendments.htm
- **All costs invoiced to a grant account must be allocable to the purpose of the grant [IACUC policies IV.5 and V.10].** Costs allocable to one protocol may not be shifted to another in order to meet deficiencies caused by overruns, or for other reasons of convenience. Rotation of charges among protocols by month without establishing that the rotation schedule credibly reflects the relative benefit to each protocol is unacceptable.

For more information on IACUC policies and procedures, please visit the Comparative Medicine web site at http://www.research.usf.edu/cm/default.htm.

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