# THE ASSOCIATIVE REPRESENTATION OF FEAR MEMORIES MEDIATED BY THE AMYGDALA

by

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This dissertation is dedicated to

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Tebe ar vždycky v můj srdce.

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**CHAPTER I**

**INTRODUCTION**

Fear and the expression of fear behaviors are evolutionarily advantageous and necessary for survival. Evolution may have promoted fear mechanisms to help animals escape or avoid life-threatening situations (Fanselow, 1994; LeDoux, 1996; Mineka & Ohman, 2002). However, the inability to suppress inappropriate fear responses can be very debilitating and ultimately develop into numerous psychiatric disorders, such as panic, anxiety, obsessive-compulsive, post-traumatic stress (PTSD), and phobia disorders (LeDoux, 1996; Rosen & Schulkin, 1998; Wolpe, 1981). Over 19 million Americans each year suffer from an anxiety disorder, such as panic disorder or PTSD making it one of the most prevalent psychiatric disorders. Recently, the prevalence of PTSD in the United States has drastically increased beginning with the tragic events on September 11th, 2001 and has continued to increase among soldiers after deployment to Iraq (Hoge et al., 2004). An example of the tragedy of PTSD is illustrated in the following quote from a Marine who served 3 tours in Iraq.

> My brother decided to join the National Guard to get some money for college and they promised he wouldn't go to Iraq. Instead after enlisting he was sent to Iraq. Since he has been home...he refuses to talk to anyone...He called me a few weeks ago for the first time and told me he's having nightmares...about picking up the pieces of his fellow soldiers after a car bomb hit...Every one of the

Marines I served with has broken down crying…saying all they can do since they got back is bounce from job to job, drink, do drugs, and contemplate suicide to end the pain…soldiers with wounds you can't see (Richards, 2007).

As this example illustrates, many anxiety disorders are triggered by aversive or traumatic experiences, and considerable interest exists for understanding how the brain records memories of these experiences.

One of the most fruitful behavioral paradigms for exploring the neurobiology underlying the development and maintenance of fear learning and memory is Pavlovian fear conditioning. In this form of learning, animals or humans (Bechara et al., 1995; LaBar et al., 1998; Olsson & Phelps, 2004) learn that an innocuous conditioned stimulus (CS), such as a tone, predicts an aversive unconditioned stimulus (US), such as a mild electric shock. As a consequence, CS presentations alone elicit a conditioned fear response (CR), which includes increases in heart rate (Antoniadis & McDonald, 1999), arterial blood pressure (Romanski & LeDoux, 1992), hypoalgesia (Chance et al., 1978), potentiated acoustic startle (Davis, 2001), stress hormone release (Sullivan et al., 2004), ultrasonic vocalizations (Antoniadis & McDonald, 1999; Blanchard et al., 1991; Borszcz & Leaton, 2003) and freezing behavior (somatomotor immobility) (Antoniadis & McDonald, 1999; Fanselow, 1980; Fanselow & Bolles, 1979; Fendt & Fanselow, 1999). Moreover, animals also come to fear the place or context in which conditioning occurs (Maren, 2001). Pavlovian fear conditioning is a robust and long-lasting form of learning that can be acquired after as little as a single CS-US pairing (Blanchard & Blanchard, 1972; Davis, 1989; Maren, 2001a) and the CS can elicit fear CRs for at least 1 year after initial acquisition (Gale et al., 2004). An important question that has emerged from this basic behavioral paradigm is: what are the neural substrates underlying aversive learning?

**Neurobiology of Pavlovian Fear Conditioning**

In 1888 Brown and Schäfer began a series of experiments examining the behavioral consequence of temporal lobe lesions in monkeys. They removed the entire temporal lobe bilaterally in a group of monkeys and discovered a profound change in one monkey's emotional disposition. Specifically, they noticed that prior to the surgery the monkey was aggressive and wild, however upon removal of the temporal lobes the monkey became very docile and failed to show any evidence of fear even when attacked by a wild monkey (Brown & Schafer, 1888). However, the importance of these findings were overlooked until about 44 years later when work in the 1930s by Kluver and Bucy also revealed similar changes in the emotional responses of monkeys after temporal lobe lesions (Kluver & Bucy, 1937). These effects can be attributed to a collection of anatomically and functionally distinct nuclei known as the amygdala, buried deep within the temporal lobes of the brain. Since then the amygdala has long been appreciated as having a critical role in emotion and emotional memory.

These changes in emotion found by Kluver and Bucy (1937) were later shown in animals with amygdala damage alone and linked to deficits in assigning emotional significance to biologically relevant stimuli (Weiskrantz, 1956). Indeed, humans with amygdala damage show similar deficits in emotional processing. For example, patient S.M. who has a rare genetic disorder that causes selective and complete bilateral damage to her amygdalae, is impaired in recognizing facial expressions of emotion, particularly fear (Adolphs et al., 1994; Adolphs et al., 1995). Furthermore, when presented with fear-inducing stimuli, such as a movie clip, S.M. is unable to produce normal emotional

responses, although she can explain that most people would feel afraid when watching these clips (Adolphs et al., 1999; Adolphs et al., 1994; Adolphs et al., 1995). Consistent with this, normal subjects that view facial expressions of fear exhibit increased regional cerebral blood flow in the amygdala (Breiter et al., 1996; Morris et al., 1996; Morris et al., 1998; Phillips et al., 1998; Phillips et al., 1997; Zald et al., 1998; Zald & Pardo, 1997).

The amygdala is clearly an important brain region for encoding and storing fear memories (Davis & Whalen, 2001; LeDoux, 2000; Maren, 2001b) (Figure 1.1). Buried deep within the temporal lobe, the amygdala is comprised of a collection of anatomically and functionally distinct nuclei. The two main divisions of the amygdala are the basolateral amygdala complex (BLA), which can be further divided into the lateral (LA), basolateral (BL), and basomedial (BM) nuclei of the amygdala and the central nucleus of the amygdala (CEA) which, is made up of a medial (CEAm) and lateral (CEAl) divisions. All of these nuclei are made up of a heterogenous population of cells. Within the BLA three major neuronal classes have been morphologically identified and are similar to cortical cells. Class I cells are primarily large in size, spine-dense, pyramidal or stellate in appearance, and contain glutamate. These cells also appear to be the primary projection neurons of the BLA. Class II and III are typically small in size, identified as spine-sparse, interneurons or neurogliaform, respectively, and contain GABA, acetylcholine, and neuropeptides (Davis et al., 1994; Krettek & Price, 1978; Maren, 1996; McDonald, 1998; Rainne et al., 1993; Washburn & Moises, 1992; Womble & Moises, 1992).

Unlike the BLA the CEA is organized similar to the ventral and dorsal striatopallidal system. The CEAl contains mainly small-sized, loosely arranged, spine-dense neurons, which resemble medium spiny neurons of the striatum, and contain GABA and a variety of neuropeptides, such as corticotropin-releasing hormone, neurotensin, enkephalin, and somatostatin. Cells within the CEAm are also small-sized, but tightly packed, contain sparse to medium spine densities, and contain a variety of neuropeptides and in some situations glutamate. Overall the CEA contains small, tightly packed cell groups (Davis et al., 1994; Krettek & Price, 1978; Maren, 1996; Martina et al., 1999; McDonald, 1998; Petrovich & Swanson, 1997).

From a functional perspective, the BLA is a brain area where CS and US information converge and become associated during fear conditioning (yielding the fear memory). The LA receives auditory (Campeau & Davis, 1995; Doron & Ledoux, 1999; LeDoux et al., 1990a; LeDoux et al., 1991; LeDoux et al., 1984; Romanski & LeDoux, 1992), visual (Rosen et al., 1992; Shi & Davis, 2001), and somatosensory (Shi & Davis, 1999) information about the CS from the thalamic and cortical areas. For auditory information, the medial geniculate nucleus of the thalamus (MGN) is important for transmitting information about the auditory CS to the neurons in the LA. The MGN has excitatory glutamatergic connections with cells in the LA that causes increased cell firing during auditory fear conditioning (Li et al., 1996). However, electron microscopy has also revealed that the MGN has connections with inhibitory GABAergic interneurons within the LA (Li et al., 1996; Woodson et al., 2000). It has been hypothesized that during auditory fear conditioning, the MGN excites GABAegic interneurons in the LA to suppress cell firing is one population of cells, while other glutamatergic projections from

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the MGN excite other cells within the LA.  This type of feed-forward inhibition from GABAergic interneurons within the LA may serve to suppress background neural noise in order to enhance cell firing in the LA to the incoming auditory CS (Li et al., 1996; Woodson et al., 2000).

Information about the context in which conditioning occurs is primarily processed by the hippocampus and then sent to the BLA via projections from ventral CA1 and the subiculum (Kim & Fanselow, 1992; Maren, 1998; Maren et al., 1997; Maren & Fanselow, 1995; Phillips & LeDoux, 1992).  The pathway(s) for conveying information about the aversive US to the BLA is still under investigation, however some suggest that the insular cortex and posterior intralaminar nucleus of the thalamus (PIN) are important for conveying information about the footshock to the amygdala (LeDoux et al., 1985; Shi & Cassell, 1998).  For example, combined lesions of the PIN and caudal insular cortex block acquisition of fear-potentiated startle (Shi & Davis, 1999), however other researchers have shown that combined lesions of the PIN and insular cortex, either prior to or after conditioning do not affect conditioned freezing responses (Brunzell & Kim, 2001; Kim & Jung, 2006).  Brunzell and Kim (2001) suggest that the PIN and insular cortex are a part of a larger distributed pathway conveying US information to the amygdala.  For example, the parabrachial nucleus conveys information about noxious stimuli to the face, limbs, and tail to the amygdala (Basbaum & Woolf, 1999; Bernard & Besson, 1990; Bernard et al., 1989; Bianchi et al., 1998; Fulwiler & Saper, 1984).  In addition, the parabrachial nucleus conveys visceral US information to the amygdala during conditioned taste aversion (Sakai & Yamamoto, 1999).

Within the BLA, neurons from the LA project to the BL (Smith & Pare, 1994). In vivo recordings have revealed that signals from the LA to the BL are slow and subject to attenuation through the administration of an NMDA antagonist or a GABA agonist, which suggests that the LA-BL pathway is involved in synaptic plasticity and signal modulation during associative learning (Wang et al., 2002). LA neurons also have projections to the lateral division of the central nucleus of the amygdala (CEAl), which then in turn has connections with the medial division of the CEA (CEAm) (Jolkkonen & Pitkanen, 1998; Pare et al., 1995; Smith & Pare, 1994). The CEAm also receives input from the BM and BL, and then sends afferent projections to many brainsteam areas that control the expression of fear CRs, such as the periacquiductal gray (freezing behavior), paraventricular nucleus of the hypothalamus and bed nucleus of the stria terminals (glucocorticoid release), parabrachial nucleus (increased respiration), nucleus retiuclaris pontis caudalis (fear-potentiated startle), and the lateral hypothalamus (increases in heart rate and blood pressure) (Davis & Whalen, 2001; De Oca et al., 1998; Fanselow & Gale, 2003; Fendt & Fanselow, 1999; Kapp et al., 1979; LeDoux, 1998; LeDoux, 2000; LeDoux et al., 1988; Maren, 2001b; Pascoe & Kapp, 1985; Schafe et al., 2001). Neurons from the LA also excite inhibitory intercalated cells (ITC), which lie between the BLA and CEA. These ITC cells then project onto a second population of ITC cells and this second population of ITC cells makes direct connections with the CEA, which disinhibits the CEA (Collins & Pare, 1999; LeDoux, 2000; Maren, 2001b; Pare et al., 2004; Pare et al., 2003; Royer et al., 1999; Royer et al., 2000). Hence, direct excitation of the CEm via the BL or indirect disinhibition via the LA-ITC pathway may drive the expression of fear.

Supporting a role for these circuits in fear conditioning, neurotoxic lesions as well as pharmacological inactivation of either the BLA or CEA prevent acquisition and expression of fear memories (Campeau & Davis, 1995b; Cousens & Otto, 1998; Fanselow & Gale, 2003; Gale et al., 2004; Goosens & Maren, 2001, 2003; Helmstetter, 1992; Helmstetter & Bellgowan, 1994; Killcross et al., 1997; Koo et al., 2004; Maren, 1998; Maren, 1999b, 2001a, 2001b; Maren et al., 1996a; Maren et al., 1996b; Muller et al., 1997; Nader et al., 2001; Walker & Davis, 1997; Walker & Davis, 2000; Wilensky et al., 2006; Wilensky et al., 1999; Wilensky et al., 2000; Zimmerman et al., 2007). For example, neurotoxic lesions of the BLA made prior to training impair acquisition of fear conditioning (Cousens & Otto, 1998; Maren et al., 1996a; Sananes & Davis, 1992). Furthermore, neurotoxic lesions of the BLA made either shortly after training (Campeau & Davis, 1995b; Cousens & Otto, 1998; Maren, 1998; Maren, 1999b; Maren et al., 1996a), two weeks following training (Cousens & Otto, 1998; Maren et al., 1996a), or up to one month following training (Lee et al., 1996; Maren et al., 1996a) block the expression of conditioned fear responses, as well as block conditional fear after extensive overtraining (Maren, 1998; Maren, 1999b). Neurotoxic lesions of the CEA also impair acquisition and expression of conditioned fear responses (Campeau & Davis, 1995b; Maren, 1998).

Lesions made in structures afferent to the amygdala impair fear conditioning to specific CS modalities, whereas lesions in efferent structures impair specific behavioral responses. Lesions within the amygdala cause impairments similar to lesions of either afferent or efferent structures (Campeau & Davis, 1995a; Gentile et al., 1986; Goosens & Maren, 2001; Jarrell et al., 1986; LeDoux et al., 1990b; LeDoux et al., 1988; LeDoux et

al., 1986; Romanski & LeDoux, 1992; Wilson & Kapp, 1994).  These findings further support that the amygdala is the critical structure for Pavlovian fear conditioning.

Although studies of the neural basis of fear conditioning have focused on the BLA, several recent studies suggest the CEA may have a role in the acquisition of long-term fear memories (Kapp et al., 1979; Pare et al., 2004; Pascoe & Kapp, 1985; Samson & Pare, 2005; Wilensky et al., 2006; Zimmerman et al., 2007).  For example, rats with BLA lesions will acquire fear if given overtraining (Maren, 1999; Zimmerman et al., 2007).  We have also found that CEA lesions block acquisition and expression of fear memory under these conditions.  Our data also indicate that temporary inactivation of the CEA prevents both acquisition and expression of overtrained fear memories (Gale et al., 2004; Maren, 1998; Maren, 1999b; Zimmerman et al., 2007).  In addition, Wilensky and colleagues have recently shown that temporary inactivation of the CEA produced impairments in acquisition of fear responses (Wilensky et al., 2006).  Infusions of anisomycin, a protein synthesis inhibitor, into the CEA block the acquisition of conditioned taste aversion (Bahar et al., 2003).  Similarly, infusing APV, an NMDA antagonist, into the CEA prevents long-term memory for Pavlovian fear conditioning (Goosens & Maren, 2003).  In fact there are inputs from auditory thalamic nuclei (i.e. medial division of the MGN) that project to the CEAm that may send information about the auditory CS (Ledoux et al., 1987; Linke et al., 2000; Pare et al., 2004; Supple & Kapp, 1989; Turner & Herkenham, 1991) and robust projections from subcortical nociceptive areas to the CEAm that relay information about the US (Alden et al., 1994; Bernard et al., 1993; Bernard et al., 1990, 1992; Pare et al., 2004), making it a candidate structure involved in acquisition of Pavlovian associations.

**What Does the Amygdala Encode?**

During Pavlovian fear conditioning an animal may learn a response that follows a stimulus presentation or they may encode stimulus relationships, which therefore determine the nature of the response (Hull, 1943; Matzel & Shors, 2001; Tolman & Postman, 1954; Watson & Morgan, 1917). As first described by Watson in the early 1900's and later elaborated by Hull (1943) an animal's response to the CS is altered through a reflexive-type process (Watson & Morgan, 1917). Specifically, after conditioning the animal does not consider that meaning or specific properties of the CS, but rather responds to it reflexively like it would to a US (i.e. UR). This type of associative representation is known as stimulus-response associations (S-R) and is a process where the CS directly evokes a CR, without accessing the US. In contrast, Tolman and Postman (1954) claimed that during conditioning animals learn about the relationship between the CS and US, which would allow the association to develop flexible response strategies. This type of associative representation is known as a stimulus-stimulus association (S-S), whereby the CS directly activates the representation of the specific US in order to produce a CR.

To distinguish whether learning is occurring through a S-S or S-R association, Rescorla and colleagues developed a procedure that allowed them to alter the memory representation or "value" of the US after conditioning to probe the associative structure of the animal's response (Rescorla, 1973, 1974). Rats were trained to bar press for a food US. Next the rats were fear conditioned with an auditory CS that co-terminated with a

low-intensity footshock.  Then the rats were presented with either high-intensity

("inflated") footshocks or no shock.  During testing, tone-alone presentations were

superimposed on bar pressing for a food US.  Rats that had received inflated shocks

displayed an increase in bar press suppression when compared to those that did not

undergo US inflation procedures.  Rescorla concluded that because the CRs were

sensitive to US inflation, that CSs generated CRs by activating a memory of the US (S-S

association) (Rescorla, 1974).  If S-R associations had mediated the memory, then the

CRs would be immune to changes in US value.

The neural substrates underlying these different forms of associative

representations have been extensively studied in appetitive paradigms.   For example,

CSs gain motivational significance through their association with a positive reward, such

as food.  In these tasks the amygdala is not necessary for animals to learn that a CS

predicts a food US, however the BLA is necessary for the CS to gain access to the current

value of a specific US with which is has been associated (S-S; Figure 1.2) (Everitt et al.,

1989; Everitt et al., 2003; Everitt et al., 1991; Gallagher et al., 1999; Hatfield et al., 1996;

Holland & Gallagher, 1999, 2004; Killcross et al., 1997; McDonald & White, 1993).

Holland, Gallagher, and colleagues have shown that BLA lesions disrupted

reductions in conditioned responding to a food US after it has been devalued (Gallagher

et al., 1990; Hatfield et al., 1996).  In appetitive paradigms US devaluation is achieved by

either selectively feeding the animal the food US prior to testing until sated or by pairing

the food US with an injection of lithium chloride (LiCl), which induces illness.  BLA

lesions impair reductions in conditioned responding after devaluation of the food US,

however rats with CEA lesions remain sensitive to US devaluation procedures.

Furthermore, BLA lesions do not impair approach CRs to the food cup or conditioned taste aversion, indicating they selectively affected the value of the US (Gallagher et al., 1990; Hatfield et al., 1996; Setlow et al., 2002). Furthermore, inhibiting protein synthesis within the BLA of rats after US devaluation (i.e. satiation) abolished changes in a food reward that were established by incentive learning (Wang et al., 2005). These results indicated that the acquisition and maintenance of reward representations used to guide instrumental performance are dependent on protein synthesis within the amygdala.

In accordance with the devaluation studies above, BLA inactivation has been reported to reduce US inflation in a fear conditioning task (Fanselow & Gale, 2003). In addition, pre-training electrolytic lesions or temporary inactivation of the LA blocks acquisition of conditioned fear responses, as well as, unconditioned fear responses (Blair et al., 2005). These results suggest that the neural activity within the BLA is important for both predicting and perceiving the aversive value of noxious stimuli. This is also consistent with the hypothesis that animals encode associations between the CS and internal representation of the emotional properties of the US (Konorski, 1967).

However, in a conflicting study, rats with BLA lesions were able to remain sensitive to post-training changes in the motivational value of outcomes (i.e. US devaluation) after a sensory preconditioning procedure (Blundell et al., 2003). In this procedure, neutral stimuli (CS1 and CS2) are paired together, after which CS2 is paired with a motivational significant event, such as, an injection of LiCl. During testing, animals show changes in responding to CS1 because of the newly acquired motivational value of CS2 (Rizley & Rescorla, 1972). Blundell and colleagues predicted that rats with BLA lesions would not be impaired in acquiring associations between the sensory

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properties of neutral stimuli (neutral S-S association), which supports their findings that rats with BLA lesions are sensitive to post-training changes in the motivational significance of the CS2 (Blundell et al., 2003). In support of these findings, others have suggested that BLA lesions disrupt the use of CS-evoked cognitive representations of the unique sensory or hedonic properties of motivational significant events, but not the more general reinforcing properties (Balleine et al., 2003).

In non-human primates, BLA inactivation during US devaluation (i.e. selective satiation) blocked the effects of devaluation during testing, whereas inactivation of the BLA following US devaluation did not impair devaluation effects during testing (Wellman et al., 2005). These results suggest that the BLA is necessary for recognizing changes in reinforcer value (i.e. devaluation), but is not necessary for subsequent expression of the effects of devaluation. Neurophysiological recordings in non-human primates have also revealed that the amygdala contains different populations of neurons, in which some respond strongly to a CS associated with an aversive US, whereas others respond more strongly to a CS associated with a reward US. In addition, these populations of neurons are capable of rapidly updating the representation of value upon reversal of contingencies (Belova et al., 2007; Belova et al., 2008; Paton et al., 2006; Salzman et al., 2007). These findings are in agreement with results from instrumental tasks in rodent that also have shown that amygdala neurons were sensitive to reversals in contingencies (Schoenbaum et al., 1999).

In humans, an appetitive olfactory conditioning experiment using US devaluation while measuring neural activity with functional magnetic resonance imaging (fMRI) was designed to investigate the substrates involved in predictive reward value. Subjects were

presented with two arbitrary visual stimuli (CSs), which was each paired with an

appetitive olfactory US.  After devaluation of one US, amygdala responses evoked by the

target CS was decreased following devaluation, while responses to the non-target CS

were maintained (Gottfried et al., 2003).  Like the results of animal studies, this study

suggests that the human amygdala has a role in encoding the current value of reward

representations.

Taken together these results suggest the BLA is important for Pavlovian learning

and in updating associative changes.  As mentioned above, the CEA also has a role in

appetitive and aversive learning (Ponnusamy et al., 2007; Wilensky et al., 2006;

Zimmerman et al., 2007).  Recent work in appetitive studies suggests a role for the CEA

in Pavlovian learning that is quite different than that mediated by the BLA (Blair et al.,

2005; Cardinal et al., 2002; Everitt et al., 2003; Holland & Gallagher, 2004; Holland &

Rescorla, 1975; Parkinson et al., 2000; Pickens & Holland, 2004; Pickens et al., 2003;

Rescorla, 1973, 1974).

One role attributed to the CEA is in directing attention to biologically relevant

stimuli.  The CEA contributes to this process by mediating conditioned orienting

responses during associative learning and enhancing the association between events when

predictions about future events are surprising (Gallagher et al., 1990; Holland, 1984;

Holland & Gallagher, 1999; Hunt & Campbell, 1997; Lubow et al., 1976).  Many studies

suggest that the CEA encodes associations that do not incorporate motivational values

about the US (S-R; Figure 1.2) (Aggleton, 2000; Everitt et al., 2003; Everitt et al., 2001;

Gallagher et al., 1990; Holland, 2004; Holland & Gallagher, 2004; Killcross et al., 1997;

Murray, 2007).  In support of this claim, unlike BLA lesions, CEA lesions do not affect

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sensitivity to the current value of the primary reinforcer (Hatfield et al., 1996), however CEA lesions do disrupt appetitive Pavlovian conditioning (i.e. conditioned locomotor approach, conditioned orienting responses, and conditioned suppression) (Cardinal et al., 2002; Everitt et al., 2001; Gallagher et al., 1990; Hall et al., 2001; Hatfield et al., 1996; Killcross et al., 1997; Parkinson et al., 2000), which may be mediated by S-R or S-S associations that do not depend on the specific reinforcing properties of the US. For example, CRs that are sensitive to CEA lesions are insensitive to devaluation procedures (Setlow et al., 2002). Furthermore, other work has posited that the CEA mediates associations of predictive stimuli with the general affective or emotional properties of biologically significant events (i.e. preparatory conditioning; changes in heart rate, blood pressure, approach, and withdrawal) (Balleine et al., 2003; Balleine & Killcross, 2006; Blundell et al., 2001, 2003; Konorski, 1967).

The type of association that is learned is not entirely confined to be either S-S or S-R. In fact, the type of representation mediating learning can be a function of the amount of training, which allows the representation of the association to be altered during learning (i.e. S-S associations mediate responding early in training, while S-R associations mediate performance in extensively trained animals) (Adams, 1982; Dickinson et al., 1995; Holland, 2004; Holland & Gallagher, 2004). In Pavlovian fear conditioning it is unknown whether the associative structure changes as a function of training. However, BLA lesions disrupt acquisition of conditioned fear responses with limited training, but not overtraining (Maren, 1998; Maren, 1999b; Zimmerman et al., 2007). This suggests that the associative basis of fear conditioning may also change as a function of training. Alternatively, S-S associations (mediated by the BLA) may underlie

fear memory in intact rats, whereas S-R associations (mediated by the CEA) may underlie memory in rats with BLA lesions.

A major factor in determining how the amygdala will contribute to different behavioral processes and responses, beyond fear, is reflected in its connections with other brain structures (Aggleton, 2000). For example, as mentioned above, the amygdala has projections to the brainstem, which control the expression of fear responses, as well as areas of the cortex, which are believed to mediate cognitive aspects of emotional processing, such as the experience of fear (LeDoux, 1996). In addition, amygdala connections with the prefrontal cortex are important for encoding predictive value of cues and the motivational properties of associated outcomes in order to guide goal-directed behavior in other type of learning paradigms, such as appetitive conditioning (Gallagher et al., 1999; Hatfield et al., 1996; Schoenbaum et al., 1998; Schoenbaum et al., 1999). Projections from the BLA to the ventral striatum are important for processing incentive value of the Pavlovian CSs in instrumental learning tasks (Everitt et al., 1991; Groenewegen et al., 1996; Kelley et al., 1982; McDonald & White, 1993). CEA-dependent learning is primarily modulated by its connections with the mesolimbic and nigrostriatal dopaminergic pathways (Han et al., 1997). Furthermore, the CEA has connections with cholinergic cells within the nucleus basalis and substantia innominata that mediate increased attentional processing during learning (Holland & Gallagher, 1993a, 1993b; Holland & Gallagher, 1999). In general, during learning the role of the amygdala is to mediate associative representations between the emotional significance and sensory properties of stimuli, which is used to help guide appropriate behavioral responses to emotional relevant cues. In this sense, the amygdala is a key emotional

interface in the brain that that endows sensory stimuli and the behavioral responses they generate affective valence and value.

## Cellular Mechanisms of Encoding

In addition to mapping the anatomy of fear learning there is also extensive interest in understanding the cellular mechanisms that underlie fear memory storage. One form of synaptic plasticity that may mediate learning and memory is long-term potentiation (LTP). LTP is the long-lasting enhancement of synaptic transmission between two neurons that are simultaneously stimulated; this change lasts over months in vivo, which is a very long time compared to other processes that affect synaptic weight. Therefore, LTP is considered one mechanism that might support long-term memory storage (LeDoux, 2000; Madison et al., 1991; Maren, 2005).

LTP has been extensively studied in the CA1 region of the hippocampus (Bliss & Lomo, 1973; Doyere et al., 1995; Grover, 1998) and involves the interaction between presynaptic glutamate and two classes of postsynaptic receptors, AMPA and NMDA (Abel & Lattal, 2001; Alberini, 2005; Brun et al., 2001; Collinridge et al., 1983; Kelso et al., 1986; Malenka & Nicoll, 1999). First, glutamate released from the presynaptic cell binds to AMPA receptors on the postsynaptic cell, which depolarizes that postsynaptic cell (Mayer & Westbrook, 1984; Nowak et al., 1984). Upon depolarization of the postsynaptic cell, glutamate is able to bind to NMDA, which allows influx of calcium through the NMDA receptor (Jahr & Stevens, 1987). This influx of calcium into the postsynaptic cells improves the cell's sensitivity to glutamate largely by initiating a

cascade of intracellular processes that increase the activity of existing receptors and by increasing the number of AMPA receptors on the postsynaptic cell surface (Abel & Lattal, 2001; Alberini, 2005; Malenka & Nicoll, 1999; Nayak et al., 1998; Rumpel et al., 2005). Many inhibitors of hippocampal LTP also block hippocampal-dependent learning, further supporting LTP as the cellular mechanism for learning and memory (Atkins et al., 1998; Barros et al., 2000; Izquierdo & Medina, 1995; Milner et al., 1998).

Considerable evidence indicates that within the BLA, LTP may be the underlying mechanism associated with acquisition and consolidation of fear (Aroniadou-Adnerjaska et al., 2001; Fanselow & LeDoux, 1999; Maren, 1999a; Maren & Fanselow, 1995, McKernan & Shinnick-Gallagher, 1997). Like the hippocampus, activity in two classes of glutamate receptors, AMPA and NMDA, are important for the induction of some forms of LTP within the amygdala (Bauer & LeDoux, 2004; Goosens & Maren, 2002; Maren, 2001b; McGaugh, 2000). NMDA receptor antagonists such as APV or ifenprodil infused within the BLA not only impair acquisition and expression of fear memory, but also impair the induction of plasticity necessary for consolidation (Bourtchuladze et al., 1994; Campeau et al., 1992; Fanselow & Kim, 1994; Goosens & Maren, 2003, 2004; LeDoux et al., 1990a; Lee & Kim, 1998; Lee et al., 2001; Maren et al., 1996b; Rodrigues et al., 2001; Schafe et al., 1999). Thus, NMDA receptors appear to be necessary for both normal synaptic transmission and the induction of synaptic plasticity within the BLA.

During fear conditioning, the influx of calcium that results from NMDA activation initiates a cascade of intracellular events, such as protein synthesis and gene expression (Abel & Lattal, 2001; LeDoux, 1998). Blocking NMDA receptors or downstream cascades in the BLA impairs long-term memory formation (Bourtchuladze et

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al., 1994; Campeau et al., 1992; Fanselow & Kim, 1994; Goosens & Maren, 2003, 2004; LeDoux et al., 1990a; Lee & Kim, 1998; Lee et al., 2001; Maren et al., 1996b; Maren et al., 2003; Monfils et al., 2007; Rodrigues et al., 2001; Schafe et al., 1999). However, there are some cases in which LTP induction and protein synthesis can occur independently of NMDA receptor function, via voltage-gated calcium channels (Bauer et al., 2002; Chapman & Bellavance, 1992; LeDoux, 2000; Monfils et al., 2007; Weisskopf et al., 1999). In either situation infusions of protein synthesis inhibitors, protein kinase inhibitors, or transcriptional inhibitors into the BLA impairs long-term memory formation supporting that protein synthesis is essential for the induction of LTP (Bailey et al., 1999; Goosens et al., 2000; Lin et al., 2001; Maren et al., 2003; Nader et al., 2000a; Schafe et al., 2000; Schafe & LeDoux, 2000; Walker & Gold, 1994; Wei et al., 2002).

After a memory is consolidated it is stable and long lasting. However, retrieval of the memory, through either recall with a presentation of the CS or even perhaps US revaluation, appears to return it to an active state (Alberini et al., 2006). Inhibition of protein synthesis after memory retrieval produces profound impairments in the retention of the fear memory (Alberini, 2005; Duvarci & Nader, 2004; Miller & Sweatt, 2006; Nader et al., 2000b; Schafe et al., 2001). For example, Nader and colleagues have shown that infusions of anisomycin into the BLA immediately after recall (i.e. exposure to the CS) causes retrograde amnesia regardless if the memory is activated 1 or 14 days after initial consolidation (Nader et al., 2000a). Additional studies also support that protein synthesis is necessary for re-stabilizing fear memories (Alberini, 2005; Alberini et al., 2006; Debiec et al., 2006; Duvarci et al., 2006; Duvarci & Nader, 2004; Nader et al., 2000a; Pedreira & Maldonado, 2003; Pedreira et al., 2002; Przybyslawski & Sara, 1997;

Riccio et al., 2006; Suzuki et al., 2004).  These data suggest that retrieving a fear memory returns it to a labile state, and that *de novo* protein synthesis is required to reconsolidate it into long-term memory (LTM).

In addition to the role of NMDA receptors in the acquisition of fear memory, a recent study indicates that these receptors are also involved in the reconsolidation of fear memories.  Nader and colleagues found that APV, a NMDA antagonist, infused into the BLA prior to recall prevented consolidated memories from returning to a labile state during reactivation, but did not prevent re-stabilization of a memory after reactivation. In addition, NBQX, an AMPA antagonist, did not prevent the induction of labiality indicating that AMPA receptors do not play a role in reconsolidation.  They concluded that NMDA receptor activation is the critical process initiating reconsolidation, but protein synthesis within the BLA is the process that re-stabilizes memory (Ben Mamou et al., 2006).  However, others have shown that NMDA receptor activation is involved in re-stabilization of memory (Lee et al., 2006; Pedreira et al., 2002; Przybyslawski & Sara, 1997; Summers et al., 1997; Suzuki et al., 2004; Torras-Garcia et al., 2005).

Like the BLA, NMDA receptor activity and protein synthesis within the CEA are also involved in the acquisition and consolidation of fear memories (Samson & Pare, 2005; Wilensky et al., 2006; Zimmerman et al., 2007) and consolidation of conditioned taste aversion (Bahar et al., 2004).  Furthermore, conditioning-induced plasticity occurs in the CEA but independent of BLA activity, thus supporting an active role of the CEA in long-term fear storage (McEchron et al., 1995; Samson & Pare, 2005).  Even though the CEA mediates the acquisition of fear memories in the absence of the BLA it is unknown whether the CEA also mediates consolidation of these Pavolvian fear memories.

Furthermore, reconsolidation studies have focused on the role of the BLA, which remains the question of whether the CEA is also necessary for reconsolidation. In fact earlier studies investigating the role of the BLA in reconsolidation have not ruled out the possibility that drug effects were in fact mediated in the CEA. We have shown that the dose of anisomycin ($62.5\mu g$) used in most BLA reconsolidation experiments impairs protein synthesis in structures well outside of the BLA, including the CEA (Maren et al., 2003). Therefore, deficits in conditional responding after infusions of a protein synthesis inhibitor into the amygdala may be due to protein synthesis inhibition in the CEA.

**Specific Aims and Hypotheses**

The primary focus of this dissertation is to investigate how the amygdala represents and encodes memory during fear conditioning. Fanselow and Gale have reported that Pavlovian fear memories are sensitive to inflation procedures, suggesting that these memories are mediated by S-S associations (Fanselow & Gale, 2003). However, it is unknown what associative representation mediates memories in rats that are overtrained. As mentioned previously, work in appetitive conditioning has shown that S-S associations mediate responding early in training, while S-R associations mediate performance in extensively trained animals (Dickinson et al., 1995; Holland, 2004; Holland & Gallagher, 2004). In addition, we wanted to know if the same memory structure acquired by the BLA is the same when fear is learned by the CEA (i.e. in rats with BLA lesions). Both intact rats and rats with BLA lesions were sensitive to US inflation procedures, suggesting that both memories are mediated by S-S association.

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Furthermore, these findings also suggest that the CEA learns fear associations similar to the BLA via S-S associations.

The finding that rats with BLA lesions are sensitive was unexpected, in that appetitive studies have suggested that the amygdala is necessary for not only representing CS-US associations, but also for representing US value (Everitt et al., 1989; Everitt et al., 2003; Everitt et al., 1991; Gallagher et al., 1999; Hatfield et al., 1996; Holland & Gallagher, 1999, 2004; Killcross et al., 1997; McDonald & White, 1993). Through the use of temporary pharmacological inactivation and protein synthesis inhibition within the BLA or CEA, we confirmed the observation in rats with BLA lesions that the amygdala is neither important for representing US value or for maintaining representations of revalued USs.

Since the CEA appears to encode S-S associations like the BLA we investigated whether the cellular mechanisms of memory consolidation and reconsolidation are the same in the CEA as they are in the BLA. Through the use of protein synthesis inhibition within the CEA we found that consolidation was impaired in intact rats and rats with lesions and reconsolidation was impaired in intact rats. These findings suggest that memory encoding by the CEA is mechanistically similar to that in the BLA.
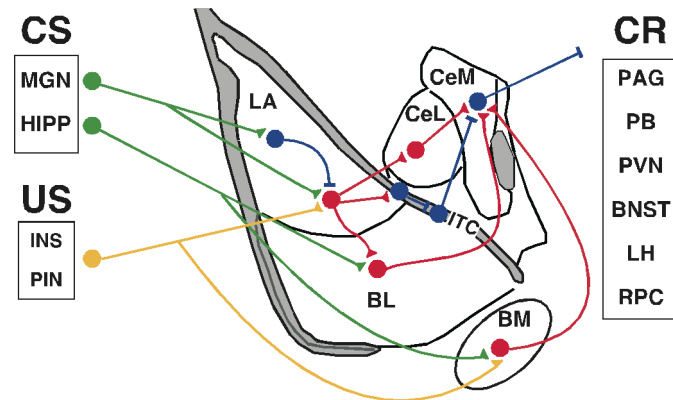
**Figure 1.1.** Neuroanatomy of Pavlovian fear conditioning circuitry. The basolateral amygdala complex (BLA; consisting of the lateral, LA; basolateral, BL; and basomedial, BM; nuclei) is where the CS (green pathway) and US (yellow pathway) information converge and become associated (yielding the fear memory). The medial geniculate nucleus of the thalamus (MGN) has excitatory glutamatergic connections with cells in the LA. However, the MGN also has connections with inhibitory GABAergic interneurons within LA. Information about contextual CSs are primarily processed by the hippocampus (HIPP) and then sent to the BL and BM nuclei. The pathway(s) for conveying information about the aversive US to the LA is still under investigation, however some suggest that the insular cortex (INS) and posterior intralaminar nucleus of the thalamus (PIN) are invovled. Within the BLA, neurons from the LA connect to the BL. LA neurons also have projections to the lateral division of the central nucleus of the amygdala (CEAl), which then in turn has connections with the medial division of the CEA (CEAm). The CEAm also receives input from the BM and BL, and then sends afferent projections to many brainsteam areas that control the expression of fear CRs, such as the periacquiductal gray (PAG; freezing behavior), paraventricular nucleus of the hypothalamus (PVN) and bed nucleus of the stria terminals (BNST; glucocorticoid release), parabrachial nucleus (PB; increased respiration), nucleus retiuclaris pontis caudalis (RPC; fear-potentiated startle), and the lateral hypothalamus (LH; increases in heart rate and blood pressure). In addition, during fear conditioning, neurons from the BLA excite inhibitory intercalated cells (ITC), which lie between the BLA and CEA. These ITC cells then project onto a second population of ITC cells and this second population of ITC cells makes direct connections with the CEA, which disinhibits the CEA. Blue pathway, GABA; red pathway, Glutamatergic. This figure was adapted from Swanson (2004).
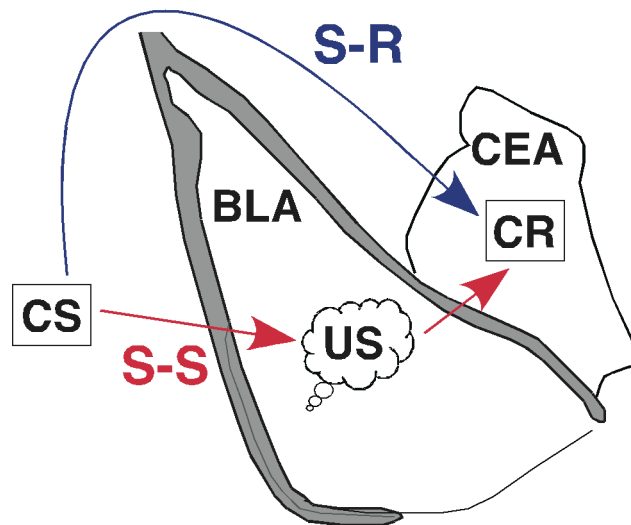
**Figure 1.2.** Associative basis for Pavlovian fear conditioning. The type of associative representation acquired by the BLA may be different than that acquired by the CEA. After conditioning, when the CS is presented it can produce a CR mediated by different associative representations.  BLA-dependent memories access the US representation associated with that CS in order to produce the CR through connections with the CEA (stimulus-stimulus associations; S-S; red pathway).  In contrast, CEA-dependent memories bypass the representation of the US and directly associate the CS with the CR (stimulus-response associations; S-R; blue pathway), however others suggest that the CEA encodes S-S associations like the BLA (not shown). This figure was adapted from Swanson (2004).

## References

Abel, T., & Lattal, K. M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol, 11*(2), 180-187.

Adams, C. D. (1982). Variations in the sensitivity of instrumental responding to reinforcer devaluation. *Quarterly Journal of Experimental Psychology Section B-Comparative and Physiological Psychology, 34*, 77-98.

Adolphs, R., Russell, J. A., & Tranel, D. (1999). A role for the human amygdala in recognizing emotional arousal from unpleasant stimuli. *Psychological Science, 10*(2 %R doi:10.1111/1467-9280.00126), 167-171.

Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. (1994). Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature, 372*(6507), 669-672.

Adolphs, R., Tranel, D., Damasio, H., & Damasio, A. R. (1995). Fear and the human amygdala. *Journal of Neuroscience, 15*(9), 5879-5891.

Aggleton, J. P. (2000). *The amygdala: A functional analysis*. New York: Oxford University Press.

Alberini, C. M. (2005). Mechanisms of memory stabilization: Are consolidation and reconsolidation similar or distinct processes? *Trends Neurosci, 28*(1), 51-56.

Alberini, C. M., Milekic, M. H., & Tronel, S. (2006). Mechanisms of memory stabilization and de-stabilization. *Cell Mol Life Sci, 63*(9), 999-1008.

Alden, M., Besson, J. M., & Bernard, J. F. (1994). Organization of the efferent projections from the pontine parabrachial area to the bed nucleus of the stria terminalis and neighboring regions: A pha-l study in the rat. *J Comp Neurol, 341*(3), 289-314.

Antoniadis, E. A., & McDonald, R. J. (1999). Discriminative fear conditioning to context expressed by multiple measures of fear in the rat. *Behav Brain Res, 101*(1), 1-13.

Aroniadou-Anderjaska, V., Post, R. M., Rogawski, M. A., & Li, H. (2001). Input-specific ltp and depotentiation in the basolateral amygdala. *Neuroreport, 12*(3), 635-640.

Atkins, C. M., Selcher, J. C., Petraitis, J. J., Trzaskos, J. M., & Sweatt, J. D. (1998). The mapk cascade is required for mammalian associative learning. *Nat Neurosci, 1*(7), 602-609.

Bahar, A., Dorfman, N., & Dudai, Y. (2004). Amygdalar circuits required for either consolidation or extinction of taste aversion memory are not required for reconsolidation. *Eur J Neurosci, 19*(4), 1115-1118.

Bahar, A., Samuel, A., Hazvi, S., & Dudai, Y. (2003). The amygdalar circuit that acquires taste aversion memory differs from the circuit that extinguishes it. *Eur J Neurosci, 17*(7), 1527-1530.

Bailey, D. J., Kim, J. J., Sun, W., Thompson, R. F., & Helmstetter, F. J. (1999). Acquisition of fear conditioning in rats requires the synthesis of mrna in the amygdala. *Behavioral Neuroscience, 113*(2), 276-282.

Balleine, B. W., & Killcross, S. (2006). Parallel incentive processing: An integrated view of amygdala function. *Trends Neurosci, 29*(5), 272-279.

Balleine, B. W., Killcross, A. S., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *J. Neurosci., 23*(2), 666-675.

Barros, D. M., Izquierdo, L. A., Mello e Souza, T., Ardenghi, P. G., Pereira, P., Medina, J. H., et al. (2000). Molecular signalling pathways in the cerebral cortex are required for retrieval of one-trial avoidance learning in rats. *Behav Brain Res, 114*(1-2), 183-192.

Basbaum, A. I., & Woolf, C. J. (1999). Pain. *Curr Biol, 9*(12), R429-431.

Bauer, E. P., & LeDoux, J. E. (2004). Heterosynaptic long-term potentiation of inhibitory interneurons in the lateral amygdala. *J Neurosci, 24*(43), 9507-9512.

Bauer, E. P., Schafe, G. E., & LeDoux, J. E. (2002). Nmda receptors and l-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. *J Neurosci, 22*(12), 5239-5249.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science, 269*(5227), 1115-1118.

Belova, M. A., Paton, J. J., Morrison, S. E., & Salzman, C. D. (2007). Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. *Neuron, 55*(6), 970-984.

Belova, M. A., Paton, J. J., & Salzman, C. D. (2008). Moment-to-moment tracking of state value in the amygdala. *J. Neurosci. %R 10.1523/JNEUROSCI.1400-08.2008, 28*(40), 10023-10030.

Ben Mamou, C., Gamache, K., & Nader, K. (2006). Nmda receptors are critical for unleashing consolidated auditory fear memories. *Nat Neurosci, 9*(10), 1237-1239.

Bernard, J. F., Alden, M., & Besson, J. M. (1993). The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex: A phaseolus vulgaris leucoagglutinin (pha-l) study in the rat. *J Comp Neurol, 329*(2), 201-229.

Bernard, J. F., & Besson, J. M. (1990). The spino(trigemino)pontoamygdaloid pathway: Electrophysiological evidence for an involvement in pain processes. *J Neurophysiol, 63*(3), 473-490.

Bernard, J. F., Huang, G. F., & Besson, J. M. (1990). Effect of noxious somesthetic stimulation on the activity of neurons of the nucleus centralis of the amygdala. *Brain Res, 523*(2), 347-350.

Bernard, J. F., Huang, G. F., & Besson, J. M. (1992). Nucleus centralis of the amygdala and the globus pallidus ventralis: Electrophysiological evidence for an involvement in pain processes. *J Neurophysiol, 68*(2), 551-569.

Bernard, J. F., Peschanski, M., & Besson, J. M. (1989). A possible spino (trigemino)-ponto-amygdaloid pathway for pain. *Neurosci Lett, 100*(1-3), 83-88.

Bianchi, R., Corsetti, G., Rodella, L., Tredici, G., & Gioia, M. (1998). Supraspinal connections and termination patterns of the parabrachial complex determined by the biocytin anterograde tract-tracing technique in the rat. *J Anat, 193 ( Pt 3),* 417-430.

Blair, H. T., Sotres-Bayon, F., Moita, M. A. P., & Ledoux, J. E. (2005). The lateral amygdala processes the value of conditioned and unconditioned aversive stimuli. *Neuroscience, 133*(2), 561-569.

Blanchard, D. C., & Blanchard, R. J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *Journal of Comparative and Physiological Psychology, 81*(2), 281-290.

Blanchard, R. J., Blanchard, D. C., Agullana, R., & Weiss, S. M. (1991). Twenty-two khz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiol Behav, 50*(5), 967-972.

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol, 232*(2), 331-356.

Blundell, P., Hall, G., & Killcross, S. (2001). Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. *J. Neurosci., 21*(22), 9018-9026.

Blundell, P., Hall, G., & Killcross, S. (2003). Preserved sensitivity to outcome value after lesions of the basolateral amygdala. *J. Neurosci., 23*(20), 7702-7709.

Borszcz, G. S., & Leaton, R. N. (2003). The effect of amygdala lesions on conditional and unconditional vocalizations in rats. *Neurobiol Learn Mem, 79*(3), 212-225.

Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., & Silva, A. J. (1994). Deficient long-term memory in mice with a targeted mutation of the camp-responsive element-binding protein. *Cell, 79*(1), 59-68.

Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., et al. (1996). Response and habituation of the human amygdala during visual processing of facial expression. *Neuron, 17*(5), 875-887.

Brown, S., & Schafer, E. A. (1888). An investigation into the functions of the occipital and temporal lobes of the monkey's brain. *Philosophical Transactions of the Royal Society of London. B, 179*, 303-327.

Brun, V. H., Ytterbo, K., Morris, R. G., Moser, M. B., & Moser, E. I. (2001). Retrograde amnesia for spatial memory induced by nmda receptor-mediated long-term potentiation. *J Neurosci, 21*(1), 356-362.

Brunzell, D. H., & Kim, J. J. (2001). Fear conditioning to tone, but not to context, is attenuated by lesions of the insular cortex and posterior extension of the intralaminar complex in rats. *Behav Neurosci, 115*(2), 365-375.

Campeau, S., & Davis, M. (1995a). Involvement of subcortical and cortical afferents to the lateral nucleus of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J Neurosci, 15*(3 Pt 2), 2312-2327.

Campeau, S., & Davis, M. (1995b). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J. Neurosci., 15*(3), 2301-2311.

Campeau, S., Miserendino, M. J., & Davis, M. (1992). Intra-amygdala infusion of the n-methyl-d-aspartate receptor antagonist ap5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behav Neurosci, 106*(3), 569-574.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci Biobehav Rev, 26*(3), 321-352.

Chance, W. T., Krynock, G. M., & Rosecrans, J. A. (1978). Antinociception following lesion-induced hyperemotionality and conditioned fear. *Pain, 4*(3), 243-252.

Chapman, P. F., & Bellavance, L. L. (1992). Induction of long-term potentiation in the basolateral amygdala does not depend on nmda receptor activation. *Synapse, 11*(4), 310-318.

Cousens, G., & Otto, T. (1998). Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience, 112*(5), 1092-1103.

Collingridge, G. L., Kehl, S. J., & McLennan, H. (1983). Excitatory amino acids in synaptic transmission in the schaffer collateral-commissural pathway of the rat hippocampus. *J Physiol, 334*, 33-46.

Collins, D. R., & Pare, D. (1999). Reciprocal changes in the firing probability of lateral and central medial amygdala neurons [published erratum appears in j neurosci 1999 apr 1;19(7):2841]. *J Neurosci, 19*(2), 836-844.

Davis, M. (1989). Sensitization of the acoustic startle reflex by footshock. *Behav Neurosci, 103*(3), 495-503.

Davis, M. (2001). Fear-potentiated startle in rats. *Curr Protoc Neurosci, Chapter 8*, Unit 8 11A.

Davis, M., & Whalen, P. J. (2001). The amygdala: Vigilance and emotion. *Mol Psychiatry, 6*(1), 13-34.

Davis, M., Rainnie, D., & Cassell, M. (1994). Neurotransmission in the rat amygdala related to fear and anxiety. *Trends Neurosci, 17*(5), 208-214.

De Oca, B. M., DeCola, J. P., Maren, S., & Fanselow, M. S. (1998). Distinct regions of the periaqueductal gray are involved in the acquisition and expression of defensive responses. *J Neurosci, 18*(9), 3426-3432.

Debiec, J., Doyere, V., Nader, K., & Ledoux, J. E. (2006). Directly reactivated, but not indirectly reactivated, memories undergo reconsolidation in the amygdala. *Proc Natl Acad Sci U S A, 103*(9), 3428-3433.

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., & Boakes, R. A. (1995). Motivational control after extended instrumental training. *Animal Learning & Behavior, 23*, 197-206.

Doron, N. N., & Ledoux, J. E. (1999). Organization of projections to the lateral amygdala from auditory and visual areas of the thalamus in the rat. *J Comp Neurol, 412*(3), 383-409.

Doyere, V., Redini-Del Negro, C., Dutrieux, G., Le Floch, G., Davis, S., & Laroche, S. (1995). Potentiation or depression of synaptic efficacy in the dentate gyrus is determined by the relationship between the conditioned and unconditioned stimulus in a classical conditioning paradigm in rats. *Behav Brain Res, 70*(1), 15-29.

Duvarci, S., Mamou, C. B., & Nader, K. (2006). Extinction is not a sufficient condition to prevent fear memories from undergoing reconsolidation in the basolateral amygdala. *Eur J Neurosci, 24*(1), 249-260.

Duvarci, S., & Nader, K. (2004). Characterization of fear memory reconsolidation. *J Neurosci, 24*(42), 9269-9275.

Everitt, B. J., Cador, M., & Robbins, T. W. (1989). Interactions between the amygdala and ventral striatum in stimulus-reward associations: Studies using a second-order schedule of sexual reinforcement. *Neuroscience, 30*(1), 63-75.

Everitt, B. J., Cardinal, R. N., Parkinson, J. A., & Robbins, T. W. (2003). Appetitive behavior: Impact of amygdala-dependent mechanisms of emotional learning. *Ann N Y Acad Sci, 985*, 233-250.

Everitt, B. J., Dickinson, A., & Robbins, T. W. (2001). The neuropsychological basis of addictive behaviour. *Brain Res Brain Res Rev, 36*(2-3), 129-138.

Everitt, B. J., Morris, K. A., O'Brien, A., & Robbins, T. W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: Further evidence of limbic-striatal interactions underlying reward-related processes. *Neuroscience, 42*(1), 1-18.

Fanselow, M. S. (1980). Conditioned and unconditional components of post-shock freezing. *Pavlov J Biol Sci, 15*(4), 177-182.

Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin and Review, 1*(4), 429-438.

Fanselow, M. S., & Bolles, R. C. (1979). Naloxone and shock-elicited freezing in the rat. *J Comp Physiol Psychol, 93*(4), 736-744.

Fanselow, M. S., & Gale, G. D. (2003). The amygdala, fear, and memory. *Ann N Y Acad Sci, 985*, 125-134.

Fanselow, M. S., & Kim, J. J. (1994). Acquisition of contextual pavlovian fear conditioning is blocked by application of an nmda receptor antagonist d,l-2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behavioral Neuroscience, 108*(1), 210-212.

Fanselow, M. S., & LeDoux, J. E. (1999). Why we think plasticity underlying pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron, 23*(2), 229-232.

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews, 23*, 743-760.

Fulwiler, C. E., & Saper, C. B. (1984). Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. *Brain Res, 319*(3), 229-259.

Gale, G. D., Anagnostaras, S. G., Godsil, B. P., Mitchell, S., Nozawa, T., Sage, J. R., et al. (2004). Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. *J Neurosci, 24*(15), 3810-3815.

Gallagher, M., Graham, P. W., & Holland, P. C. (1990). The amygdala central nucleus and appetitive pavlovian conditioning: Lesions impair one class of conditioned behavior. *Journal of Neuroscience, 10*(6), 1906-1911.

Gallagher, M., McMahan, R. W., & Schoenbaum, G. (1999). Orbitofrontal cortex and representation of incentive value in associative learning. *J. Neurosci., 19*(15), 6610-6614.

Gentile, C. G., Jarrell, T. W., Teich, A., McCabe, P. M., & Schneiderman, N. (1986). The role of amygdaloid central nucleus in the retention of differential pavlovian conditioning of bradycardia in rabbits. *Behav Brain Res, 20*(3), 263-273.

Goosens, K. A., Holt, W., & Maren, S. (2000). A role for amygdaloid pka and pkc in the acquisition of long-term conditional fear memories in rats. *Behavioural Brain Research, 114*(1-2), 145-152.

Goosens, K. A., & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn Mem, 8*(3), 148-155.

Goosens, K. A., & Maren, S. (2002). Long-term potentiation as a substrate for memory: Evidence from studies of amygdaloid plasticity and pavlovian fear conditioning. *Hippocampus, 12*(5), 592-599.

Goosens, K. A., & Maren, S. (2003). Pretraining nmda receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear. *Behav Neurosci, 117*(4), 738-750.

Goosens, K. A., & Maren, S. (2004). Nmda receptors are essential for the acquisition, but not expression, of conditional fear and associative spike firing in the lateral amygdala. *Eur J Neurosci, 20*(2), 537-548.

Gottfried, J. A., O'Doherty, J., & Dolan, R. J. (2003). Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science, 301*(5636), 1104-1107.

Groenewegen, H. J., Wright, C. I., & Beijer, A. V. (1996). The nucleus accumbens: Gateway for limbic structures to reach the motor system? *Prog Brain Res, 107*, 485-511.

Grover, L. M. (1998). Evidence for postsynaptic induction and expression of nmda receptor independent ltp. *J Neurophysiol, 79*(3), 1167-1182.

Hall, J., Parkinson, J. A., Connor, T. M., Dickinson, A., & Everitt, B. J. (2001). Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating pavlovian influences on instrumental behaviour. *Eur J Neurosci, 13*(10), 1984-1992.

Han, J. S., McMahan, R. W., Holland, P., & Gallagher, M. (1997). The role of an amygdalo-nigrostriatal pathway in associative learning. *J Neurosci, 17*(10), 3913-3919.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with pavlovian second-order conditioning and reinforcer devaluation effects. *Journal of Neuroscience, 16*(16), 5256-5265.

Helmstetter, F. J. (1992). The amygdala is essential for the expression of conditional hypoalgesia. *Behavioral Neuroscience, 106*(3), 518-528.

Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience, 108*(5), 1005-1009.

Hoge, C. W., Castro, C. A., Messer, S. C., McGurk, D., Cotting, D. I., & Koffman, R. L. (2004). Combat duty in iraq and afghanistan, mental health problems, and barriers to care. *N Engl J Med, 351*(1), 13-22.

Holland, P. C. (1984). The origins of pavlovian conditioned behavior. In G. Bower (Ed.), *The psychology of learning and motivation* (Vol. 18, pp. 129-173): Prentice-Hall.

Holland, P. C. (2004). Relations between pavlovian-instrumental transfer and reinforcer devaluation. *Journal of Experimental Psychology:  Animal Behavior Processes, 30*, 104-117.

Holland, P. C., & Gallagher, M. (1993a). Amygdala central nucleus lesions disrupt increments, but not decrements, in conditioned stimulus processing. *Behav Neurosci, 107*(2), 246-253.

Holland, P. C., & Gallagher, M. (1993b). Effects of amygdala central nucleus lesions on blocking and unblocking. *Behav Neurosci, 107*(2), 235-245.

Holland, P. C., & Gallagher, M. (1999). Amygdala circuitry in attentional and representational processes. *Trends in Cognitive Sciences, 3*(2), 65-73.

Holland, P. C., & Gallagher, M. (2004). Amygdala-frontal interactions and reward expectancy. *Current Opinion in Neurobiology, 14*(2), 148-155.

Holland, P. C., & Rescorla, R. A. (1975). The effect of two ways of devaluing the unconditioned stimulus after first- and second-order appetitive conditioning. *J Exp Psychol Anim Behav Process, 1*(4), 355-363.

Hull, C. L. (1943). *Principles of behavior: An introduction to behavior theory*. New York: D. Appleton-Century Co.

Hunt, P. S., & Campbell, B. A. (1997). Autonomic and behavioral correlates of appetitive conditioning in rats. *Behav Neurosci, 111*(3), 494-502.

Izquierdo, I., & Medina, J. H. (1995). Correlation between the pharmacology of long-term potentiation and the pharmacology of memory. *Neurobiol Learn Mem, 63*(1), 19-32.

Jahr, C. E., & Stevens, C. F. (1987). Glutamate activates multiple single channel conductances in hippocampal neurons. *Nature, 325*(6104), 522-525.

Jarrell, T. W., Gentile, C. G., McCabe, P. M., & Schneiderman, N. (1986). The role of the medial geniculate region in differential pavlovian conditioning of bradycardia in rabbits. *Brain Res, 374*(1), 126-136.

Jolkkonen, E., & Pitkanen, A. (1998). Intrinsic connections of the rat amygdaloid complex: Projections originating in the central nucleus. *J Comp Neurol, 395*(1), 53-72.

Kapp, B. S., Frysinger, R. C., Gallagher, M., & Haselton, J. R. (1979). Amygdala central nucleus lesions: Effect on heart rate conditioning in the rabbit. *Physiol Behav, 23*(6), 1109-1117.

Kelley, A. E., Domesick, V. B., & Nauta, W. J. (1982). The amygdalostriatal projection in the rat--an anatomical study by anterograde and retrograde tracing methods. *Neuroscience, 7*(3), 615-630.

Kelso, S. R., Ganong, A. H., & Brown, T. H. (1986). Hebbian synapses in hippocampus. *Proc Natl Acad Sci U S A, 83*(14), 5326-5330.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature (London), 388*(6640), 377-380.

Kim, J. J., & Fanselow, M. S. (1992). Modality-specific retrograde amnesia of fear. *Science, 256*(5057), 675-677.

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in pavlovian fear conditioning: A critical review. *Neuroscience & Biobehavioral Reviews, 30*(2), 188-202.

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

Konorski, J. (1967). *Integrative activity of the brain*. Chicago: University of Chicago Press.

Koo, J. W., Han, J. S., & Kim, J. J. (2004). Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. *J Neurosci, 24*(35), 7654-7662.

Krettek, J. E., & Price, J. L. (1978). A description of the amygdaloid complex in the rat and cat with observations on intra-amygdaloid axonal connections. *J Comp Neurol, 178*(2), 255-280.

LaBar, K. S., Gatenby, J. C., Gore, J. C., LeDoux, J. E., & Phelps, E. A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: A mixed-trial fmri study. *Neuron, 20*(5), 937-945.

Le Doux, J. E. (1996). *The emotional brain: The mysterious underpinnings of emotional life* (1st ed.). New York: Touchstone.

LeDoux, J. (1998). Fear and the brain: Where have we been, and where are we going? *Biol Psychiatry, 44*(12), 1229-1238.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990a). The lateral amygdaloid nucleus: Sensory interface of the amygdala in fear conditioning. *Journal of Neuroscience, 10*(4), 1062-1069.

LeDoux, J. E., Farb, C., & Ruggiero, D. A. (1990b). Topographic organization of neurons in the acoustic thalamus that project to the amygdala. *J Neurosci, 10*(4), 1043-1054.

LeDoux, J. E., Farb, C. R., & Romanski, L. M. (1991). Overlapping projections to the amygdala and striatum from auditory processing areas of the thalamus and cortex. *Neurosci Lett, 134*(1), 139-144.

LeDoux, J. E., Iwata, J., Cicchetti, P., & Reis, D. J. (1988). Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear. *J Neurosci, 8*(7), 2517-2529.

LeDoux, J. E., Iwata, J., Pearl, D., & Reis, D. J. (1986). Disruption of auditory but not visual learning by destruction of intrinsic neurons in the rat medial geniculate body. *Brain Res, 371*(2), 395-399.

Ledoux, J. E., Ruggiero, D. A., Forest, R., Stornetta, R., & Reis, D. J. (1987). Topographic organization of convergent projections to the thalamus from the inferior colliculus and spinal cord in the rat. *J Comp Neurol, 264*(1), 123-146.

LeDoux, J. E., Ruggiero, D. A., & Reis, D. J. (1985). Projections to the subcortical forebrain from anatomically defined regions of the medial geniculate body in the rat. *J Comp Neurol, 242*(2), 182-213.

LeDoux, J. E., Sakaguchi, A., & Reis, D. J. (1984). Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. *J Neurosci, 4*(3), 683-698.

Lee, H., & Kim, J. J. (1998). Amygdalar nmda receptors are critical for new fear learning in previously fear-conditioned rats. *Journal of Neuroscience, 18*(20), 8444-8454.

Lee, H. J., Choi, J. S., Brown, T. H., & Kim, J. J. (2001). Amygdalar nmda receptors are critical for the expression of multiple conditioned fear responses. *Journal of Neuroscience, 21*(11), 4116-4124.

Lee, J. L., Milton, A. L., & Everitt, B. J. (2006). Reconsolidation and extinction of conditioned fear: Inhibition and potentiation. *J Neurosci, 26*(39), 10051-10056.

Lee, Y., Walker, D., & Davis, M. (1996). Lack of a temporal gradient of retrograde amnesia following nmda-induced lesions of the basolateral amygdala assessed with the fear-potentiated startle paradigm. *Behavioral Neuroscience, 110*(4), 836-839.

Li, X. F., Armony, J. L., & LeDoux, J. E. (1996). Gabaa and gabab receptors differentially regulate synaptic transmission in the auditory thalamo-amygdala

pathway: An in vivo microiontophoretic study and a model. *Synapse, 24*(2), 115-124.

Lin, C. H., Yeh, S. H., Lin, C. H., Lu, K. T., Leu, T. H., Chang, W. C., et al. (2001). A role for the pi-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. *Neuron, 31*(5), 841-851.

Linke, R., Braune, G., & Schwegler, H. (2000). Differential projection of the posterior paralaminar thalamic nuclei to the amygdaloid complex in the rat. *Exp Brain Res, 134*(4), 520-532.

Lubow, R. E., Schnur, P., & Rifkin, B. (1976). Latent inhibition and conditioned attention theory. *J. Exp. Psychol. Anim. Behav. Process., 2*, 163-174.

Madison, D. V., Malenka, R. C., & Nicoll, R. A. (1991). Mechanisms underlying long-term potentiation of synaptic transmission. *Annu Rev Neurosci, 14*, 379-397.

Mahanty, N. K., & Sah, P. (1998). Calcium-permeable ampa receptors mediate long-term potentiation in interneurons in the amygdala. *Nature, 394*(6694), 683-687.

Malenka, R. C., & Nicoll, R. A. (1999). Long-term potentiation--a decade of progress? *Science, 285*(5435), 1870-1874.

Maren, S. (1996). Synaptic transmission and plasticity in the amygdala. An emerging physiology of fear conditioning circuits. *Mol Neurobiol, 13*(1), 1-22.

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *Journal of Neuroscience, 18*(8), 3088-3097.

Maren, S. (1999a). Long-term potentiation in the amygdala: A mechanism for emotional learning and memory. *Trends in Neurosciences, 22*(12), 561-567.

Maren, S. (1999b). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J. Neurosci., 19*(19), 8696-8703.

Maren, S. (2001a). Is there savings for pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? *Neurobiology of Learning and Memory, 76*(3), 268-283.

Maren, S. (2001b). Neurobiology of pavlovian fear conditioning. *Annual Review of Neuroscience, 24*(1), 897-931.

Maren, S. (2005). Synaptic mechanisms of associative memory in the amygdala. *Neuron, 47*(6), 783-786.

Maren, S., Aharonov, G., & Fanselow, M. S. (1996a). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: Absence of a temporal gradient. *Behavioral Neuroscience, 110*(4), 718-726.

Maren, S., Aharonov, G., & Fanselow, M. S. (1997). Neurotoxic lesions of the dorsal hippocampus and pavlovian fear conditioning in rats. *Behav Brain Res, 88*(2), 261-274.

Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996b). N-methyl-d-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience, 110*(6), 1365-1374.

Maren, S., & Fanselow, M. S. (1995). Synaptic plasticity in the basolateral amygdala induced by hippocampal formation stimulation in vivo. *Journal of Neuroscience, 15*(11), 7548-7564.

Maren, S., Ferrario, C. R., Corcoran, K. A., Desmond, T. J., & Frey, K. A. (2003). Protein synthesis in the amygdala, but not the auditory thalamus, is required for consolidation of pavlovian fear conditioning in rats. *European Journal of Neuroscience, 18*(11), 3080-3088.

Martina, M., Royer, S., & Pare, D. (1999). Physiological properties of central medial and central lateral amygdala neurons. *J Neurophysiol, 82*(4), 1843-1854.

Matzel, L. D., & Shors, T. J. (2001). Long-term potentiation and associative learning: Can the mechanism subserve the process? In C. Holscher (Ed.), *Neuronal mechanisms of memory formation* (pp. 294-324). New York: Cambridge University Press.

Mayer, M. L., & Westbrook, G. L. (1985). The action of n-methyl-d-aspartic acid on mouse spinal neurones in culture. *J Physiol, 361*, 65-90.

McDonald, A. J. (1998). Cortical pathways to the mammalian amygdala. *Prog Neurobiol, 55*(3), 257-332.

McDonald, R. J., & White, N. M. (1993). A triple dissociation of memory systems: Hippocampus, amygdala, and dorsal striatum. *Behav Neurosci, 107*(1), 3-22.

McEchron, M. D., McCabe, P. M., Green, E. J., Llabre, M. M., & Schneiderman, N. (1995). Simultaneous single unit recording in the medial nucleus of the medial geniculate nucleus and amygdaloid central nucleus throughout habituation, acquisition, and extinction of the rabbit's classically conditioned heart rate. *Brain Res, 682*(1-2), 157-166.

McGaugh, J. L. (2000). Memory--a century of consolidation. *Science, 287*(5451), 248-251.

McKernan, M. G., & Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature, 390*(6660), 607-611.

Miller, C. A., & Sweatt, J. D. (2006). Amnesia or retrieval deficit? Implications of a molecular approach to the question of reconsolidation. *Learn Mem, 13*(5), 498-505.

Milner, B., Squire, L. R., & Kandel, E. R. (1998). Cognitive neuroscience and the study of memory. *Neuron, 20*(3), 445-468.

Mineka, S., & Ohman, A. (2002). Phobias and preparedness: The selective, automatic, and encapsulated nature of fear. *Biol Psychiatry, 52*(10), 927-937.

Monfils, M. H., Cowansage, K. K., & LeDoux, J. E. (2007). Brain-derived neurotrophic factor: Linking fear learning to memory consolidation. *Mol Pharmacol, 72*(2), 235-237.

Morris, J. S., Frith, C. D., Perrett, D. I., Rowland, D., Young, A. W., Calder, A. J., et al. (1996). A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature, 383*(6603), 812-815.

Morris, J. S., Ohman, A., & Dolan, R. J. (1998). Conscious and unconscious emotional learning in the human amygdala. *Nature, 393*(6684), 467-470.

Muller, J., Corodimas, K. P., Fridel, Z., & LeDoux, J. E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behavioral Neuroscience, 111*(4), 683-691.

Murray, E. A. (2007). The amygdala, reward and emotion. *Trends Cogn Sci, 11*(11), 489-497.

Nader, K., Majidishad, P., Amorapanth, P., & LeDoux, J. E. (2001). Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. *Learn Mem, 8*(3), 156-163.

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000a). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*(6797), 722-726.

Nader, K., Schafe, G. E., & LeDoux, J. E. (2000b). The labile nature of consolidation theory. *Nat Rev Neurosci, 1*(3), 216-219.

Nayak, A., Zastrow, D. J., Lickteig, R., Zahniser, N. R., & Browning, M. D. (1998). Maintenance of late-phase ltp is accompanied by pka-dependent increase in ampa receptor synthesis. *Nature, 394*(6694), 680-683.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., & Prochiantz, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature, 307*(5950), 462-465.

Olsson, A., & Phelps, E. A. (2004). Learned fear of "Unseen" Faces after pavlovian, observational, and instructed fear. *Psychol Sci, 15*(12), 822-828.

Pare, D., Royer, S., Smith, Y., & Lang, E. J. (2003). Contextual inhibitory gating of impulse traffic in the intra-amygdaloid network. *Ann N Y Acad Sci, 985*, 78-91.

Pare, D., Quirk, G. J., & Ledoux, J. E. (2004). New vistas on amygdala networks in conditioned fear. *J Neurophysiol, 92*(1), 1-9.

Pare, D., Smith, Y., & Pare, J. F. (1995). Intra-amygdaloid projections of the basolateral and basomedial nuclei in the cat: Phaseolus vulgaris-leucoagglutinin anterograde tracing at the light and electron microscopic level. *Neuroscience, 69*(2), 567-583.

Parkinson, J. A., Robbins, T. W., & Everitt, B. J. (2000). Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. *European Journal of Neuroscience, 12*(1), 405-413.

Pascoe, J. P., & Kapp, B. S. (1985). Electrophysiological characteristics of amygdaloid central nucleus neurons during pavlovian fear conditioning in the rabbit. *Behavioural Brain Research, 16*(2-3), 117-133.

Paton, J. J., Belova, M. A., Morrison, S. E., & Salzman, C. D. (2006). The primate amygdala represents the positive and negative value of visual stimuli during learning. *Nature, 439*(7078), 865-870.

Pedreira, M. E., & Maldonado, H. (2003). Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron, 38*(6), 863-869.

Pedreira, M. E., Perez-Cuesta, L. M., & Maldonado, H. (2002). Reactivation and reconsolidation of long-term memory in the crab chasmagnathus: Protein synthesis requirement and mediation by nmda-type glutamatergic receptors. *J Neurosci, 22*(18), 8305-8311.

Petrovich, G. D., & Swanson, L. W. (1997). Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit. *Brain Res, 763*(2), 247-254.

Phillips, M. L., Young, A. W., Scott, S. K., Calder, A. J., Andrew, C., Giampietro, V., et al. (1998). Neural responses to facial and vocal expressions of fear and disgust. *Proc Biol Sci, 265*(1408), 1809-1817.

Phillips, M. L., Young, A. W., Senior, C., Brammer, M., Andrew, C., Calder, A. J., et al. (1997). A specific neural substrate for perceiving facial expressions of disgust. *Nature, 389*(6650), 495-498.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci, 106*(2), 274-285.

Pickens, C. L., & Holland, P. C. (2004). Conditioning and cognition. *Neuroscience & Biobehavioral Reviews, 28*(7), 651-661.

Pickens, C. L., Saddoris, M. P., Setlow, B., Gallagher, M., Holland, P. C., & Schoenbaum, G. (2003). Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. *J. Neurosci., 23*(35), 11078-11084.

Ponnusamy, R., Poulos, A. M., & Fanselow, M. S. (2007). Amygdala-dependent and amygdala-independent pathways for contextual fear conditioning. *Neuroscience, 147*(4), 919-927.

Przybyslawski, J., & Sara, S. J. (1997). Reconsolidation of memory after its reactivation. *Behav Brain Res, 84*(1-2), 241-246.

Rainnie, D. G., Asprodini, E. K., & Shinnick-Gallagher, P. (1993). Intracellular recordings from morphologically identified neurons of the basolateral amygdala. *J Neurophysiol, 69*(4), 1350-1362.

Rescorla, R. A. (1973). Effect of us habituation following conditioning. *Journal of Comparative & Physiological Psychology, 82*(1), 137-143.

Rescorla, R. A. (1974). Effect of inflation of the unconditioned stimulus value following conditioning. *Journal of Comparative & Physiological Psychology, 86*, 101-106.

Riccio, D. C., Millin, P. M., & Bogart, A. R. (2006). Reconsolidation: A brief history, a retrieval view, and some recent issues. *Learn Mem, 13*(5), 536-544.

Richards, C. (2007). Why I fight for peace. Retrieved November 3rd, 2008, from http://www.robkall.com/articles/life_a_allen_l__070521_poems_from_a__p_t_s_.htm

Rizley, R. C., & Rescorla, R. A. (1972). Associations in second-order conditioning and sensory preconditioning. *J Comp Physiol Psychol, 81*(1), 1-11.

Rodrigues, S. M., Schafe, G. E., & LeDoux, J. E. (2001). Intra-amygdala blockade of the nr2b subunit of the nmda receptor disrupts the acquisition but not the expression of fear conditioning. *Journal of Neuroscience, 21*(17), 6889-6896.

Romanski, L. M., & LeDoux, J. E. (1992). Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. *J Neurosci, 12*(11), 4501-4509.

Rosen, J. B., Hitchcock, J. M., Miserendino, M. J., Falls, W. A., Campeau, S., & Davis, M. (1992). Lesions of the perirhinal cortex but not of the frontal, medial prefrontal, visual, or insular cortex block fear-potentiated startle using a visual conditioned stimulus. *J Neurosci, 12*(12), 4624-4633.

Rosen, J. B., & Schulkin, J. (1998). From normal fear to pathological anxiety. *Psychol Rev, 105*(2), 325-350.

Royer, S., Martina, M., & Pare, D. (1999). An inhibitory interface gates impulse traffic between the input and output stations of the amygdala. *J Neurosci, 19*(23), 10575-10583.

Rumpel, S., LeDoux, J., Zador, A., & Malinow, R. (2005). Postsynaptic receptor trafficking underlying a form of associative learning. *Science, 308*(5718), 83-88.

Sakai, N., & Yamamoto, T. (1999). Possible routes of visceral information in the rat brain in formation of conditioned taste aversion. *Neurosci Res, 35*(1), 53-61.

Salzman, C. D., Paton, J. J., Belova, M. A., & Morrison, S. E. (2007). Flexible neural representations of value in the primate brain. *Ann N Y Acad Sci, 1121*, 336-354.

Samson, R. D., & Pare, D. (2005). Activity-dependent synaptic plasticity in the central nucleus of the amygdala. *J. Neurosci., 25*(7), 1847-1855.

Sananes, C. B., & Davis, M. (1992). N-methyl-d-aspartate lesions of the lateral and basolateral nuclei of the amygdala block fear-potentiated startle and shock sensitization of startle. *Behav Neurosci, 106*(1), 72-80.

Schafe, G. E., Atkins, C. M., Swank, M. W., Bauer, E. P., Sweatt, J. D., & LeDoux, J. E. (2000). Activation of erk/map kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning. *Journal of Neuroscience, 20*(21), 8177-8187.

Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase a in the amygdala. *Journal of Neuroscience, 20*(18), RC96.

Schafe, G. E., Nadel, N. V., Sullivan, G. M., Harris, A., & LeDoux, J. E. (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, pka, and map kinase. *Learn Mem, 6*(2), 97-110.

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of pavlovian fear conditioning: A cellular and molecular perspective. *Trends in Neurosciences, 24*(9), 540-546.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1998). Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. *Nat Neurosci, 1*(2), 155-159.

Schoenbaum, G., Chiba, A. A., & Gallagher, M. (1999). Neural encoding in orbitofrontal cortex and basolateral amygdala during olfactory discrimination learning. *J Neurosci, 19*(5), 1876-1884.

Setlow, B., Gallagher, M., & Holland, P. C. (2002). The basolateral complex of the amygdala is necessary for acquisition but not expression of cs motivational value in appetitive pavlovian second-order conditioning. *European Journal of Neuroscience, 15*(11), 1841-1853.

Shi, C., & Davis, M. (1999). Pain pathways involved in fear conditioning measured with fear-potentiated startle: Lesion studies. *J Neurosci, 19*(1), 420-430.

Shi, C., & Davis, M. (2001). Visual pathways involved in fear conditioning measured with fear-potentiated startle: Behavioral and anatomic studies. *J Neurosci, 21*(24), 9844-9855.

Shi, C. J., & Cassell, M. D. (1998). Cascade projections from somatosensory cortex to the rat basolateral amygdala via the parietal insular cortex. *J Comp Neurol, 399*(4), 469-491.

Smith, Y., & Pare, D. (1994). Intra-amygdaloid projections of the lateral nucleus in the cat: Pha-l anterograde labeling combined with postembedding gaba and glutamate immunocytochemistry. *J Comp Neurol, 342*(2), 232-248.

Sullivan, G. M., Apergis, J., Bush, D. E., Johnson, L. R., Hou, M., & Ledoux, J. E. (2004). Lesions in the bed nucleus of the stria terminalis disrupt corticosterone and freezing responses elicited by a contextual but not by a specific cue-conditioned fear stimulus. *Neuroscience, 128*(1), 7-14.

Summers, M. J., Crowe, S. F., & Ng, K. T. (1997). Administration of dl-2-amino-5-phosphonovaleric acid (ap5) induces transient inhibition of reminder-activated memory retrieval in day-old chicks. *Brain Res Cogn Brain Res, 5*(4), 311-321.

Supple, W. F., Jr., & Kapp, B. S. (1989). Response characteristics of neurons in the medial component of the medial geniculate nucleus during pavlovian differential fear conditioning in rabbits. *Behav Neurosci, 103*(6), 1276-1286.

Suzuki, A., Josselyn, S. A., Frankland, P. W., Masushige, S., Silva, A. J., & Kida, S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci, 24*(20), 4787-4795.

Swanson, L. W. (2004). *Brain maps: Structure of the rat brain* (Third ed.). San Diego: Academic Press.

Tolman, E. C., & Postman, L. (1954). Learning. *Annu Rev Psychol, 5*, 27-56.

Torras-Garcia, M., Lelong, J., Tronel, S., & Sara, S. J. (2005). Reconsolidation after remembering an odor-reward association requires nmda receptors. *Learn Mem, 12*(1), 18-22.

Turner, B. H., & Herkenham, M. (1991). Thalamoamygdaloid projections in the rat: A test of the amygdala's role in sensory processing. *J Comp Neurol, 313*(2), 295-325.

Walker, D. L., & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J. Neurosci., 17*(23), 9375-9383.

Walker, D. L., & Davis, M. (2000). Involvement of nmda receptors within the amygdala in short- versus long-term memory for fear conditioning as assessed with fear-potentiated startle. *Behavioral Neuroscience, 114*(6), 1019-1033.

Walker, D. L., & Gold, P. E. (1994). Intra-amygdala kinase inhibitors disrupt retention of a learned avoidance response in rats. *Neurosci Lett, 176*(2), 255-258.

Wang, C., Kang-Park, M. H., Wilson, W. A., & Moore, S. D. (2002). Properties of the pathways from the lateral amygdal nucleus to basolateral nucleus and amygdalostriatal transition area. *J Neurophysiol, 87*(5), 2593-2601.

Wang, S. H., Ostlund, S. B., Nader, K., & Balleine, B. W. (2005). Consolidation and reconsolidation of incentive learning in the amygdala. *J Neurosci, 25*(4), 830-835.

Washburn, M. S., & Moises, H. C. (1992). Electrophysiological and morphological properties of rat basolateral amygdaloid neurons in vitro. *J Neurosci, 12*(10), 4066-4079.

Watson, J. B., & Morgan, J. J. B. (1917). Emotional reactions and psychological experimentation. *The American Journal of Psychology, 28*(2), 163-174.

Wei, F., Qiu, C. S., Liauw, J., Robinson, D. A., Ho, N., Chatila, T., et al. (2002). Calcium-calmodulin-dependent protein kinase iv is required for fear memory. *Nature Neuroscience, 5*(6), 573-579.

Weiskrantz, L. (1956). Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *J Comp Physiol Psychol, 49*(4), 381-391.

Weisskopf, M. G., Bauer, E. P., & LeDoux, J. E. (1999). L-type voltage-gated calcium channels mediate nmda-independent associative long-term potentiation at thalamic input synapses to the amygdala. *J Neurosci, 19*(23), 10512-10519.

Wellman, L. L., Gale, K., & Malkova, L. (2005). Gabaa-mediated inhibition of basolateral amygdala blocks reward devaluation in macaques. *J Neurosci, 25*(18), 4577-4586.

Wilensky, A. E., Schafe, G. E., Kristensen, M. P., & LeDoux, J. E. (2006). Rethinking the fear circuit: The central nucleus of the amygdala is required for the acquisition, consolidation, and expression of pavlovian fear conditioning. *J. Neurosci., 26*(48), 12387-12396.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (1999). Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. *J. Neurosci., 19*(24), 48RC-.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (2000). Functional inactivation of amygdala nuclei during acquisition of pavlovian fear conditioning. *Society for Neuroscience Abstracts, 30*, 465.469.

Wilson, A., & Kapp, B. S. (1994). Effect of lesions of the ventrolateral periaqueductal gray on the pavlovian conditioned heart rate response in the rabbit. *Behav Neural Biol, 62*(1), 73-76.

Wolpe, J. (1981). The dichotomy between classical conditioned and cognitively learned anxiety. *J Behav Ther Exp Psychiatry, 12*(1), 35-42.

Womble, M. D., & Moises, H. C. (1992). Muscarinic inhibition of m-current and a potassium leak conductance in neurones of the rat basolateral amygdala. *J Physiol, 457*, 93-114.

Woodson, W., Farb, C. R., & Ledoux, J. E. (2000). Afferents from the auditory thalamus synapse on inhibitory interneurons in the lateral nucleus of the amygdala. *Synapse, 38*(2), 124-137.

Zald, D. H., Lee, J. T., Fluegel, K. W., & Pardo, J. V. (1998). Aversive gustatory stimulation activates limbic circuits in humans. *Brain, 121 ( Pt 6)*, 1143-1154.

Zald, D. H., & Pardo, J. V. (1997). Emotion, olfaction, and the human amygdala: Amygdala activation during aversive olfactory stimulation. *Proc Natl Acad Sci U S A, 94*(8), 4119-4124.

Zimmerman, J. M., Rabinak, C. A., McLachlan, I. G., & Maren, S. (2007). The central nucleus of the amygdala is essential for acquiring and expressing conditional fear after overtraining. *Learn Mem, 14*(9), 634-64.

# CHAPTER II

## ASSOCIATIVE STRUCTURE OF FEAR MEMORY AFTER BASOLATERAL AMYGDALA LESIONS IN RATS

Pavlovian fear conditioning is a behavioral model used to investigate the neurobiology underlying the development and maintenance of fear learning and memory (Bouton et al., 2001; Grillon et al., 1996; Kim & Jung, 2006; LeDoux, 1998; LeDoux, 2000; Maren, 2001b; Maren, 2005).  In this paradigm an innocuous conditioned stimulus (CS), such as a tone, is paired with an aversive unconditioned stimulus (US), such as a footshock.  After one or more pairings the rat learns that the CS predicts the US.  As a consequence, CS presentations alone elicit a conditioned fear response (CR), which includes increases in heart rate, arterial blood pressure, hypoalgesia, potentiated acoustic startle, stress hormone release, and freezing (somatomotor immobility).

Many years of work have identified the critical brain structures involved in the formation, consolidation, and retrieval of fear memories (Davis & Whalen, 2001; Fendt & Fanselow, 1999; LeDoux, 2000; Maren, 2001b; Maren & Quirk, 2004).  Among them, the amygdala is a candidate region in which fear memories are encoded and stored. Within the amygdala there are two sub-regions that contribute to fear learning and the expression of learned fear responses.  The basolateral complex of the amygdala (BLA;

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consisting of the lateral, basolateral, and basomedial nuclei) is where CS and US information converge and become associated (yielding the fear memory), and the central nucleus of the amygdala (CEA) translates this information into behavioral fear response (Davis & Whalen, 2001; Fanselow & Gale, 2003; Fendt & Fanselow, 1999; LeDoux, 1998; LeDoux, 2000; Maren, 2001b; Schafe et al., 2001).  In support of this view, many studies have shown that either neurotoxic lesions or pharmacological inactivation of the BLA or CEA prevent the acquisition and/or expression of fear memories (Campeau & Davis, 1995; Cousens & Otto, 1998; Fanselow & Gale, 2003; Gale et al., 2004; Goosens & Maren, 2001, 2003; Helmstetter, 1992; Helmstetter & Bellgowan, 1994; Killcross et al., 1997; Koo et al., 2004; Maren, 1998; Maren, 1999, 2001a, 2001b; Maren et al., 1996a; Maren et al., 1996b; Muller et al., 1997; Nader et al., 2001; Walker & Davis, 1997; Wilensky et al., 2006; Wilensky et al., 1999; Wilensky et al., 2000; Zimmerman et al., 2007).

However, rats with pre-training BLA lesions can acquire fear CRs if given sufficient training (Maren, 1999; Zimmerman et al., 2007).  This suggests that another brain area is involved in forming fear associations in the absence of the BLA (Gale et al., 2004; Maren, 1998; Maren, 1999; Zimmerman et al., 2007).  Recent studies implicate the CEA in the acquisition and consolidation of fear memories (Goosens & Maren, 2003; Pare et al., 2004; Samson & Pare, 2005; Wilensky et al., 2006; Zimmerman et al., 2007). For example, Wilensky and colleagues (2006) have shown that temporary inactivation of the CEA impairs the acquisition of fear responses.  In addition, we have recently reported that neurotoxic CEA lesions completely eliminate both the acquisition and expression of conditioned freezing even in rats that have been overtrained (Zimmerman et al., 2007).

Temporary inactivation of the CEA also prevents both the acquisition and expression of overtrained fear memories (Zimmerman et al., 2007). This suggests that the CEA may mediate fear memory in the absence of the BLA.

Interestingly, recent work in appetitive conditioning paradigms also suggests a role for the CEA in Pavlovian learning. In these paradigms, it has been proposed that the type of association mediated by the CEA might be quite different than that mediated by the BLA (Blair et al., 2005; Cardinal et al., 2002; Everitt et al., 2003; Holland & Gallagher, 2004; Holland & Rescorla, 1975; Parkinson et al., 2000; Pickens & Holland, 2004; Pickens et al., 2003; Rescorla, 1973, 1974). Everitt and colleagues suggest that while the BLA may represent associations between the CS and US (stimulus-stimulus associations; S-S) and code US value, CEA neurons may drive Pavlovian CRs through direct associations with the behavioral response (stimulus-response associations; S-R) (Everitt et al., 2003; Killcross et al., 1997). In addition, these associations are a function of the amount of training. In instrumental conditioning, for example, S-S associations mediate responding early in training, while S-R associations mediate performance in extensively trained animals (Dickinson et al., 1995; Holland, 2004; Holland & Gallagher, 2004). It is unknown whether the associative structure of Pavlovian fear memories changes as a function of training. However, BLA lesions disrupt acquisition of conditioned fear responses with limited training, but not overtraining (Maren, 1998; Maren, 1999; Zimmerman et al., 2007). This suggests that the associative basis of fear conditioning may also change as a function of training. Alternatively, it is possible that the associative basis of conditioned fear in rats with BLA lesions is different from that in intact rats. More specifically, S-S associations (mediated by the BLA) may underlie fear

memory in intact rats, whereas S-R associations (mediated by the CEA) may underlie memory in rats with BLA lesions. The following experiments addressed these possibilities by using a US inflation procedure (Rescorla, 1974) to probe the associative structure of fear memory in intact rats and rats with BLA lesions after overtraining.

## General Methods

*Subjects*

The subjects were 192 adult male Long-Evans rats (60-90 days old; 200-224 grams; Blue Spruce) obtained from a commercial supplier (Harlan Sprague-Dawley, Indianapolis, IN). Upon arrival all rats were individually housed in conventional Plexiglas hanging cages and kept on a 14 hr light/10 hr dark cycle (lights on at 7:00am) with free access to food and tap water. To acclimate the rats to the experimenter they were handled daily (10-15 sec per rat) for 5 days following their arrival. All experimental procedures were conducted in accordance with the approved guidelines as stated by the University of Michigan Committee on Use and Care of Animals (UCUCA).

*Behavioral Apparatus*

All sessions were conducted in eight identical rodent conditioning chambers (30 x 24 x 21 cm; MED Associates, St. Albans, VT). The chambers were positioned inside sound-attenuating cabinets located in an isolated room. Each chamber was constructed of aluminum (2 side walls) and Plexiglas (rear wall, ceiling, and hinged front door); the floor consisted of 19 stainless-steel rods, (4 mm diameter) spaced apart 1.5 cm (center to center). The grid floor was connected to a shock source and solid-state grid scrambler (MED Associates), which delivered the footshock US. Mounted on one wall of the

chamber was a speaker to provide a distinct auditory CS and on the opposite wall was a 15-W house light; a fan provided background noise (65dB).

Three distinct contexts were created by manipulating multiple visual, olfactory and tactile cues: 1) Context A: 1% acetic acid odor in the chamber, houselights and room lights on, fans on in the cabinets, cabinet doors open, and grid floors; 2) Context B: 1% ammonium hydroxide odor in the chamber, red lights on in the room, houselights off, fans off in the cabinets, cabinet doors closed, and Plexiglas floors; 3) Context C: 70% ethanol odor in the chamber, house lights on, room lights off, fans off in the cabinets, cabinet doors open, and grid floors.

Each chamber rested on a load-cell platform, which was used to record chamber displacement in response to each rat's motor activity. The output from each load-cell was amplified to a level previously established to detect freezing responses. For each chamber, the load-cell amplifier output was digitized at 5 Hz (300 observations per minutes per rat) and acquired online using Threshold Activity software (MED Associates). Locomotor activity was quantified by the raw load cell values (range = 0 - 100) and freezing behavior was quantified by calculating the number of load cell values below the freezing threshold (threshold = 10). However, to prevent the inclusion of momentary bouts of inactivity as freezing, (i.e., < 1 sec) freezing was only scored after five or more contiguous observations below the freezing threshold (for details see Maren, 1998; Maren, 1999, 2001a). Freezing observations during each session were transformed into a percentage of total observations. In Experiments 2 & 3 sensitivity to the footshock US was measured by comparing the average locomotor activity over the 2-sec period

prior to the first footshock presentation and the average locomotor activity during the first presentation of the footshock (2 sec).

*Data Analysis*

Freezing data were converted to a percentage of total observations, which is a probability estimate that is amenable to analysis with parametric statistics. These values were analyzed using analysis of variance (ANOVA) and post hoc comparisons using Fishers LSD tests were performed after a significant overall $F$ ratio was obtained. All data are represented as means ± SEMs.

**Experiment 1**

In order to characterize fear memory in rats with BLA lesions, an overtraining procedure must be used. However, overtraining itself may alter the associative basis of fear memory. Indeed, the associative structure of instrumental learning is a function of training insofar as S-S associations control performance early in learning and S-R associations dominates performance of well-trained responses (Dickinson et al., 1995; Holland, 2004). Therefore, the purpose of Experiment 1 was to determine whether overtraining itself alters the associative basis of Pavlovian fear conditioning, which normally depends on S-S associations (Rescorla, 1974).

*Method*

*Subjects and design.* Thirty-two rats were randomly assigned to one of three training groups. One group received 75 paired tone-shock trials (P75), while another group received 75 unsignaled shocks (U75); the third group did not receive training (NS). After overtraining rats in each group received a US inflation session (INF), in which

several high-intensity shocks were delivered in a novel context. The U75-INF and NS-INF groups served as controls to assess the contribution of sensitization to conditioned responding to the CS. In addition, another group of rats that received 75 conditioning trials, but no inflation (P75-NoINF), served as a control for the magnitude of inflation in the P75-INF group. Conditioned freezing was measured during all phases of training to index fear to the conditioning context and the auditory CS. This yielded the following groups: P75-INF ($n = 8$), P75-NoINF ($n = 8$), U75-INF ($n=8$), and NS-INF ($n = 8$).

*Conditioning, inflation and test procedure.* Fear conditioning was conducted using an overtraining procedure. Rats were transported from their home cages in squads of eight and placed in the conditioning chambers (Context A). Chamber position and experimental group were counterbalanced for each squad. Rats in the paired overtraining group (P75) received 75 paired presentations of a tone (10 seconds, 2kHz, 85dB) that co-terminated with a footshock (1.0mA, 2 seconds) beginning 3 minutes after being placed in the chambers. There was a 60 second intertrial interval (ITI) and the animals remained in the boxes 60 seconds after the last footshock presentation. Rats in the unsignaled overtraining group (U75) received a similar procedure except that they were given 75 unsignaled presentations of the same footshock. Rats in the no-training group were placed in the conditioning chambers for the same amount of time as the training groups, but did not receive tone or shock presentations. Twenty-four hours after conditioning all rats were placed in another, novel environment (Context C) for US inflation. The inflation session consisted of exposure to 5 high-intensity footshocks (3.0mA, 2 seconds) 3 minutes after placement in the chambers. There was a 60-sec ITI, and the animals remained in the boxes 60 seconds after the last footshock. Rats in the P75-NoINF group

were placed in the chamber for the same duration as the rats in the inflation groups but did not receive footshocks. Forty-eight hours after conditioning, all rats were placed back into Context A for 10 minutes to assess contextual fear. Twenty-four hours after the context test, fear to the tone was tested by placing the rats into a third novel context (Context B) and presenting 30 tone alone presentations (10 seconds, 2kHz, 85dB, 60 sec ITI) 3 minutes after placement into the chambers. Freezing behavior was measured throughout all experimental sessions.

### *Results and Discussion*

*Behavior.* An ANOVA of the average post-shock freezing during the conditioning session revealed a significant main effect of group [$F_{(2,28)} = 32.2$; $p < 0.0001$] (Figure 2.1). Not surprisingly, both groups of rats that received 75 footshocks (P75 and U75) acquired high levels of freezing, while the no-training group remained low. Rats in the no-training group exhibited some immobility late in the session that was not related to fear, but rather to quiescence late in the session. This significant difference in freezing between the groups that had received training versus the no-training group was confirmed by further post hoc comparisons ($p < 0.0001$).

Data from the inflation session are shown in Figure 2.2. An ANOVA calculated for the average freezing during the post-shock ITI periods of the inflation session (minutes 4-8) revealed a significant main effect of group [$F_{(3,27)} = 9.0$; $p < 0.0003$]. Post-hoc comparisons revealed that all rats receiving inflated shocks (P75-INF, NS-INF, and U75-INF) exhibited more freezing than rats not receiving shock (P75-NoINF) ($p < 0.0001$ for comparison to the P75-INF group; $p < 0.0002$ for comparison to the U75-INF

group; and p < 0.01 for comparison to the NS-INF group). There was no significant difference between any of the inflation groups.

Conditioned freezing during the context test is displayed in Figure 2.3a. During the context test, rats in the P75-INF and U75-INF groups exhibited significantly more freezing than rats in the NS-INF and P75-NoINF groups. This observation was confirmed in an ANOVA that revealed a significant main effect of group [$F_{(3,27)}$ = 24.7; p < 0.0001]. Post hoc comparisons revealed that the groups that had received overtraining and inflation (P75-INF and U75-INF) displayed elevated levels of freezing that were significantly different from the other groups (NS-INF and P75-NoINF) (Figure 2.3a). Although both the NS-INF and P75-NoINF groups froze significantly less than the groups that had received overtraining and inflation, the NS-INF group displayed higher levels of freezing than the P75-NoINF group (p = 0.01). Elevated levels of freezing behavior in the NS-INF group is likely due to the generalization of fear from the inflation context to the test context. Overall, US inflation increased contextual fear in animals that had previously received 75 footshocks in that context, whether they were signaled or not. This was not simply the result of sensitization by intense footshocks, insofar as the NS-INF rats exhibited relatively low levels of freezing.

An ANOVA performed for the tone freezing data (Figure 2.3b) revealed that there was a significant main effect of group [$F_{(3,27)}$ = 4.8; p < 0.01] and post-hoc tests revealed significantly higher levels of freezing in the P75-INF group when compared to all other groups throughout the entire session (p < 0.02 for comparison to the P75-NoINF group; p < 0.005 for comparison to the U75-INF group; and p < 0.002 for comparison to the NS-INF group). There was no significant difference between any of the other groups (P75-

NoINF, U75-INF, and NS-INF).  In contrast to the context test, only rats that received

inflation after 75 tone-shock trials exhibited elevated freezing during the tone test (Figure

2.3b).  These results indicate that elevated freezing in the P75-INF group was due to US

revaluation rather than non-associative shock sensitization, because neither 75

conditioning shocks nor inflation shocks alone were sufficient to elevate freezing to the

tone CS.  During both the context and tone tests, the non-inflated group displayed low

levels of freezing, which may be due to generalized extinction from exposure to similar

chambers during the inflation session.  Nonetheless, freezing in the inflation groups

following overtraining was augmented by the inflation procedure, and this is due to an

associative increase in fear.  Overall these data indicate that overtrained fear memories

are sensitive to US inflation, suggesting that S-S associations mediate fear memory even

after extended training.

**Experiment 2**

Experiment 1 reveals that S-S associations contribute to the expression of fear in

overtrained rats.  It has been argued in previous work that BLA damage impairs both the

encoding of S-S associations and interferes with US revaluation (Blundell et al., 2001;

Hatfield et al., 1996; Holland & Gallagher, 2004; Killcross et al., 1997; Pickens et al.,

2003).  Because rats with BLA damage acquire conditioned fear after overtraining

(Maren, 1999; Zimmerman et al., 2007), it is therefore possible that S-R associations

mediate this memory.  If so, we hypothesized that the fear memory in rats with BLA

lesions may be insensitive to US revaluation procedures.  Experiment 2 used the inflation

procedure to examine this possibility in rats in which neurotoxic BLA lesions were made

prior to overtraining.

*Method*

   *Subjects and design.* The subjects were 64 rats housed and handled as described

in Experiment 1.   Prior to overtraining they were divided into two equal groups: one

group that received bilateral neurotoxic lesions in the basolateral complex of the

amygdala (BLA) and a second group that underwent sham surgery (SHAM).  Following

overtraining each surgery group was further divided into two groups: one that received

US inflation (INF) after overtraining or a group that did not undergo US inflation

(NoINF).

   *Surgery.* One week prior to training, each rat was anesthetized with an

intraperitoneal (i.p.) injection of a Nembutal (sodium pentobarbital; 65 mg/kg body

weight) and atropine methyl nitrate (0.4 mg/kg body weight) cocktail.  Ocular lubricant

was used to moisten the eyes.  The scalp was shaved, cleaned with antiseptic (Betadine)

and the rat was mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga,

CA).  After the scalp was incised and retracted, the skull was positioned so that bregma

and lambda were in the same horizontal plane.  Small burr holes were drilled bilaterally

in the skull to allow placement of 28-gauge injectors in the BLA (3.3 mm posterior to

bregma and 5.0 mm lateral to the midline).  Injectors were attached to polyethylene

tubing and connected to 10 μl syringes mounted on an infusion pump (Harvard

Apparatus, South Natick, MA).  *N*-methyl-D-aspartate (NMDA; 20 mg/mL dissolved in

100 mM PBS, pH 7.4; Sigma, St. Louis, MO) was infused (0.1 μl/min) at two sites for

each BLA lesion: 8.0 mm ventral to the brain surface (0.2 μl) and 7.5 mm ventral to the

brain surface (0.1 µl) ventral to the brain surface.  Five minutes were allowed for

diffusion of the drug into the target structure before the injectors were removed.  SHAM

rats received a similar surgery except that the injectors were not lowered into the brain.

After surgery the incision was closed with stainless steel wound clips and the rats were

kept on a heating pad until they recovered from anesthesia before returning to their home

cages.  The rats were allowed one week of recovery prior to overtraining.

*Conditioning, inflation and test procedure.*  One week after surgery, all groups

received overtraining, US inflation, and retention testing as described in Experiment 1.

*Histology.*  After behavioral testing, rats were euthanized with an overdose of

sodium pentobarbital (i.p. 100 mg/kg) and were transcardially perfused with

physiological saline followed by 10 % formalin.  Brains were removed and post-fixed in

10% formalin followed by 10% formalin/30% sucrose solution until sectioning.  Coronal

brain sections (45 µm) were cut on a cryostat and wet-mounted with 70% ethanol on

glass microscope slides.  Once dry, the sections were stained with 0.25% thionin to

visualize neuronal cell bodies and identify lesion sites.


***Results and Discussion***

*Histology.*  Eight rats were excluded from the analyses because their lesions were

either larger than intended, misplaced, or unilateral.  This yielded the following groups

designated by lesion type and inflation condition:  BLA-INF ($n = 12$), BLA-NoINF ($n$

=11), SHAM-INF ($n = 16$) and SHAM-NoINF ($n = 16$).  Successful lesions were

generally confined to the targeted structure, although some rats in the BLA group had

damage to the rostral endopiriform nucleus and caudate putamen (Figure 2.4). NMDA infusions into the BLA spared the central nucleus of the amygdala.

*Behavior.* A two-way ANOVA of the average post-shock freezing during the overtraining session revealed a significant main effect of lesion [$F_{(1, 53)} = 7.7$; $p < 0.01$], a main effect of time (5 minute blocks) [$F_{(15, 795)} = 92.5$; $p < 0.0001$], and an interaction between lesion and time [$F_{(15, 795)} = 5.5$; $p < 0.0001$] (Figure 2.5a). These results indicate that freezing levels differed among the groups across the session. Rats with BLA lesions displayed freezing levels that were slightly below those in the SHAM group. However, a post hoc comparison of average freezing during the last 10 trials of the overtraining session revealed that rats with BLA lesions acquired a level of freezing comparable to that of SHAM rats ($p = 0.05$; Figure 2.5b). In addition, BLA lesions did not affect sensitivity to the footshock US [$F_{(1, 53)} = 0.69$; $p = 0.41$], which suggests that BLA lesions impaired conditional freezing compared to controls during the beginning of the overtraining session (data not shown).

Post-shock freezing during the inflation session is shown in Figure 2.6. A two-way ANOVA calculated for the average freezing during minutes 4-8 of the session revealed a significant main effect of inflation condition [$F_{(1, 51)} = 72.3$; $p < 0.0001$], and an interaction between lesion and inflation condition [$F_{(1, 51)} = 8.4$; $p < 0.01$]. It is apparent that rats in the SHAM-INF group displayed more freezing behavior relative to their no-inflation controls than those in the BLA-INF group ($p < 0.005$ for comparison with BLA-INF and $p < 0.001$ for all other comparisons). Rats in the BLA-INF group displayed lower levels of freezing than rats in the SHAM-INF group. Importantly, BLA

lesions did not alter sensitivity to the intense footshocks used during the inflation session [$F_{(1, 26)} = 2.56$; $p = 0.12$] (data not shown).

Conditioned freezing during the context is displayed in Figure 2.7a. Rats in both the SHAM-INF and BLA-INF groups exhibited more freezing than non-inflated controls, and this effect appear more robust in the SHAM rats. These impressions were confirmed by a two-way ANOVA that revealed a significant main effect of lesion [$F_{(1,51)} = 17.6$; $p < 0.0001$], a main effect of inflation condition [$F_{(1,51)} = 136.6$; $p < 0.0001$], and an interaction between lesion and inflation condition [$F_{(1,51)} = 18.5$; $p < 0.0001$]. Although rats with BLA lesions exhibited less inflation than SHAM controls, rats in the BLA-INF group did display significantly higher levels of freezing than those in the BLA-NoINF group ($p < 0.0001$).

A two-way ANOVA of the tone test data (Figure 2.7b) revealed a significant main effect of lesion [$F_{(1,51)} = 16.7$; $p < 0.0005$] and a main effect of inflation condition [$F_{(1,51)} = 19.4$; $p < 0.0001$], however the interaction between lesion and inflation condition was not significant [$F_{(1,51)} = 2.4$; $p = 0.125$]. These data indicate that although BLA lesions generally blunted freezing, rats in both conditions exhibited inflation. Indeed, planned comparisons revealed that the BLA-INF group exhibited higher levels of freezing than the BLA-NoINF group [$t_{(21)} = 2.3$; $p < 0.05$]. These data reveal that rats with BLA lesions exhibit inflation despite the fact that amygdala damage produces a general reduction in freezing. In addition, these data suggest that S-S associations underlie overtrained fear in both intact rats and rats with BLA lesions.

**Experiment 3**

Experiment 2 indicates that S-S associations mediate overtrained fear memories acquired by rats with BLA lesions. Nonetheless, the inflation effect was attenuated in rats with BLA lesion (at least during the context test). Amygdala damage may have produced a general deficit in freezing that could have obscured US inflation. Moreover, the BLA itself has been implicated in US revaluation (Blundell et al., 2001; Everitt et al., 2003; Hatfield et al., 1996; Holland, 2004; Killcross et al., 1997; Pickens et al., 2003), and BLA lesions may have therefore disrupted US inflation independently of the nature of the association underlying fear memory. To address this issue, Experiment 3 used temporary pharmacological inactivation of the BLA during overtraining to mimic the conditions of acquiring fear in the absence of the BLA. Rats then received the inflation procedure with a functional BLA. Hence, any deficits in US inflation in rats trained under BLA inactivation cannot be attributed to either lesion-induced performance deficits on test or impaired US revaluation.

*Method*

*Subjects and design.* The subjects were 32 rats housed and handled as described in Experiment 1. The rats were divided into two equal groups: one group that received pre-training bilateral infusions of muscimol (MUS), a $GABA_A$ agonist, and a second group that received bilateral infusions of a vehicle control (VEH; artificial cerebral spinal fluid) prior to overtraining. Muscimol has long-lasting effects that have been shown to impair fear acquisition during overtraining when infused into the central nucleus of the amygdala (Zimmerman et al., 2007). After overtraining, each drug group was further divided into two groups: one that received the US inflation procedure (INF) and a group that did not undergo US inflation (NoINF).

*Surgery.*  One week prior to training, the rats were anesthetized and prepared for surgery as described in Experiment 2.  Small burr holes were drilled bilaterally in the skull to allow for the placement of 26-gauge guide cannulae (Plastics One, Roanoke, VA) in the BLA (3.3 mm posterior to bregma, 5.0 mm lateral to the midline, and 6.5 mm ventral to the brain surface), along with holes for 3 small jeweler's screws.  Dental acrylic was applied to the cannulae, screws, and skull surface to hold the guide cannulae in place.  After surgery, 33-gauge dummy cannulae (16 mm; Plastics One) were inserted into the guide cannulae and the rats were allowed to recover as described in Experiment 2.  Dummy cannulae were replaced daily during the week of recovery.

*Procedure.*  Prior to overtraining, rats were transported to the infusion room in squads of eight from their home cages in white 5-gallon buckets.  Hamilton syringes (10 μl; Harvard Apparatus) were mounted in two infusion pumps (10 syringes/pump; Harvard Apparatus) and connected to 33-gauge internal cannula (1.0 mm longer than the implanted guide cannulae) with polyethylene tubing (A-M Systems).  Dummy cannulae were removed from each rat and internal cannulae were inserted into each guide cannula.  Either muscimol (1 mg/mL dissolved in ACSF, pH 7.4; Sigma) or vehicle (same volume and rate) was infused bilaterally into the BLA (0.25 μl/side; 0.1 μl/min).  One minute was allowed for diffusion of the drug into the target structure before the injectors were removed.  Dummy cannulae were inserted into the guide cannulae once the injectors were removed and the rats were immediately taken to the conditioning chambers for overtraining.  All groups received overtraining, US inflation, and retention testing as described in Experiment 1.

*Histology.* Histology was conducted as described in Experiment 2.

*Muscimol-TMR-X Intracranial Microinfusion*.  After behavioral testing, six rats that had previously received muscimol infusions prior to overtraining were given bilateral infusions of fluorescent muscimol (muscimol-TMR-X conjugate; Invitrogen, Carlsbad, CA) to map the spread of muscimol infused into the BLA (Allen et al., 2008).  Prior to the infusion, the rats were anesthetized with Nembutal (i.p. injection; 65 mg/kg body weight).  Muscimol-TMR-X (1 mg/mL dissolved in 0.01M PBS) was infused bilaterally into the BLA (0.25μl/side; 0.1 μl/min).  One minute was allowed for drug diffusion into the target structure before the injectors were removed.  Rats were sacrificed 80 minutes after the infusion (comparable to the duration of the overtraining session).  The rats were transcardially perfused with physiological saline followed by 4% paraformaldehyde.  Brains were post-fixed in 4% paraformaldehyde/30% sucrose solution until sectioning.  Coronal brain slices (45 μm) were cut on a cryostat in the dark and wet-mounted with 70% ethanol on glass microscope slides.  Sections were re-hydrated with 0.01M PBS and pictures were taken under a light field as a reference for cytoarchitecture and under a 543/569 nm fluorescent filter to visualize muscimol-TMR-X spread.  Sections were later stained with 0.25% thionin to identify cannulae track placements.

### Results and Discussion

*Histology*.  Four rats were excluded from the analyses because their cannulae placements were not targeted at the BLA (Figure 2.8a).  This yielded the following groups designated by drug type and inflation condition: MUS-INF ($n = 6$), MUS-NoINF ($n = 7$), VEH-INF ($n = 7$), and VEH-NoINF ($n = 8$).  Terminal injections of fluorescent

muscimol revealed that muscimol infusions were generally confined to the BLA, although some rats had spread to the rostral endopiriform nucleus (Figure 2.8b).

*Behavior.* As shown in Figure 2.9, muscimol slowed, but did not prevent, the acquisition of conditioned freezing during the overtraining session. An ANOVA of the average post-shock freezing during the conditioning session revealed a significant main effect of time (5 minute blocks) [$F_{(15, 390)}$ = 31.6; p < 0.0001], and an interaction between drug and time [$F_{(15, 390)}$ = 5.9; p < 0.0001] (Figure 2.9). The effect of muscimol on freezing during overtraining was similar to that of BLA damage (see Figure 2.5). Muscimol in the BLA did not affect sensitivity to the footshock US [$F_{(1, 26)}$ = 0.09; p = 0.76] (data not shown).

Post-shock freezing during the inflation session is shown in Figure 2.10. It is apparent that rats receiving inflation shocks displayed increased freezing relative to their no-inflation controls (p < 0.0001 for comparison between INF and NoINF and p = 0.576 for comparison between MUS and VEH). Prior drug experience did not have a significant effect on freezing levels during inflation. These observations were confirmed by a two-way ANOVA on average freezing over minutes 4-8 that revealed a significant main effect of inflation condition [$F_{(1,24)}$ = 48.9; p < 0.0001].

Conditioned freezing during the context test is displayed in Figure 2.11a. Rats in the VEH-INF and MUS-INF groups exhibited significantly more freezing than rats in the non-inflated controls. This observation was confirmed by a two-way ANOVA that revealed a significant main effect of inflation condition [$F_{(1,24)}$ = 81.7; p < 0.0001], however there was no significant interaction between the inflation condition and drug [$F_{(1,24)}$ = 0.07; p = 0.8]. These data indicate that although muscimol blunted freezing

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during overtraining, rats in both conditions exhibited similar degrees of inflation when inflation and testing occurred with a functional amygdala.

A two-way ANOVA of the tone test data (Figure 2.11b) revealed a significant main effect of drug type [$F_{(1,24)} = 6.4$; $p < 0.02$] and a main effect of inflation condition [$F_{(1,24)} = 14.8$; $p < 0.0001$], but the interaction between the inflation condition and drug type was not significant [$F_{(1,24)} = 0.5$; $p = 0.475$]. Both inflation groups (MUS-INF and VEH-INF) displayed significantly higher levels of freezing when compared to the non-inflated groups (MUS-NoINF and VEH-NoINF; $p < 0.05$ for all comparisons). Although both non-inflated groups displayed lower levels of freezing than the inflated groups, the VEH-NoINF group displayed significantly higher levels of freezing than the MUS-NoINF group ($p < 0.05$). Previous reports suggest that remote memories acquired by rats with BLA inactivation are weaker than BLA-dependent memories, which could explain the difference in freezing between the non-inflated groups (Poulos et al., 2006). Overall these data suggest that S-S associations mediate overtrained fear memories in rats with temporary BLA lesions and that the BLA is not necessary for US revaluation.

**General Discussion**

The present experiments used a post-conditioning manipulation of US value (an inflation procedure) to assess the associative structure of overtrained fear in both intact rats and rats with amygdala lesions. We found that despite being overtrained, the fear memory of intact rats is sensitive to the inflation procedure, which suggests it is mediated by an S-S association (Experiment 1). Moreover, neurotoxic lesions of the BLA (Experiment 2) or temporary inactivation of the BLA during overtraining (Experiment 3)

did not prevent inflation of fear memory.  These results reveal that S-S associations

mediate conditional fear not only in intact rats, but also in rats with BLA lesions.  These

data suggest that brain structures, such as the CEA, that mediate fear in the absence of the

BLA encode CS-US associations during fear conditioning.

Unlike previous studies in instrumental conditioning tasks, we found no evidence

that the associative structure of fear conditioning changes as a function of training.  That

is, early in training instrumental responding relies upon action-outcome (A-O)

representations, but late in training these responses come to depend on S-R associations

(Dickinson et al., 1995; Holland, 2004).  The emergence of S-R associations in

instrumental conditioning drives the transition of instrumental responding from goal-

directed actions to outcome-independent habits.  In our experiments, overtrained fear

responses remain sensitive to inflation procedures, suggesting continued involvement of

S-S associations in their expression.  Pavlovian fear responses are notoriously insensitive

to instrumental contingencies (Bolles et al., 1974), and appear to require US

representations for expression in behavior even after overtraining.

An important aim of these experiments was to determine whether rats with BLA

lesions that acquire fear during overtraining use the same underlying associative structure

as intact rats.  We found that rats with BLA lesions exhibited US inflation, suggesting

that S-S associations underline fear in both intact rats and rats with BLA lesions.

However, the inflation effect was blunted in rats with BLA lesions; amygdala damage

may have produced a general deficit in freezing that could have obscured US inflation.

To address this issue, we used temporary pharmacological inactivation of the BLA during

overtraining to mimic the conditions of acquiring fear in the absence of the BLA.  Hence,

any deficits in US inflation in rats trained under BLA inactivation could not be attributed to either lesion-induced performance deficits on test or impaired performance. Fear memories acquired in the absence of the BLA during overtraining were still sensitive to inflation, which suggests that these memories are mediated by S-S associations.

Recent evidence indicates that the CEA mediates conditional fear in rats with BLA lesions (Ponnusamy et al., 2007; Wilensky et al., 2006; Zimmerman et al., 2007). The present experiments suggest that the CEA may encode such memories in the form of S-S associations. This contradicts work indicating that the CEA may mediate Pavlovian S-R associations. For example, CEA lesions disrupt conditioned suppression, conditioned orienting, and conditioned locomotor approach, all of which are thought to depend on S-R associations (Gallagher et al., 1990; Hall et al., 2001; Hatfield et al., 1996; Killcross et al., 1997; Parkinson et al., 2000). In these cases, associations formed by the CEA are sensorimotor associations that do not incorporate US value, which supports the idea that the CEA mediates learning through S-R associations under some conditions (Cardinal et al., 2002). However, other work has posited that the CEA does form associations with the US, although with motivational properties of the US which are apparently insensitive to US revaluation procedures (Balleine et al., 2003; Balleine & Killcross, 2006; Blundell et al., 2001, 2003; Konorski, 1967). The present data indicate that the CEA represents properties of the US that are sensitive to inflation.

In contrast to previous work in appetitive paradigms, the present data indicate that the BLA is not essential for US revaluation (Balleine et al., 2003; Blundell et al., 2001; Everitt et al., 2003; Hatfield et al., 1996; Holland, 2004; Holland & Gallagher, 2004; Killcross et al., 1997; Pickens et al., 2003). Surprisingly, we found that rats with BLA

lesions are sensitive to inflation procedures, suggesting that the BLA is not necessary for

coding the value of aversive USs (but see Fanselow & Gale, 2003). There are many

differences between the appetitive paradigms and the present study that could account for

differences in whether the BLA is involved in US revaluation. For example, the nature of

the USs (food versus shock) and the motivational systems they engage are different in the

two paradigms. In addition, devaluation procedures in appetitive conditioning typically

rely upon an instrumental component. For example, US devaluation through selective

satiety depends on the animal approaching a food pellet and consuming it until the animal

is sated. Perhaps BLA dysfunction only impairs "instrumental" devaluation. Another

major difference is the direction in which the US is revalued. In appetitive studies the US

experiences a decrease in value, whereas in our experiments the US experiences an

increase in value. Studies of attention have revealed differential involvement of neural

structures that depends on whether there are increases or decreases in attention (Baxter et

al., 1997; Holland & Gallagher, 1999). For example, the hippocampus mediates

decrements and not increases in attention (Baxter et al., 1999; Holland & Gallagher,

1993). Similar to the role of the hippocampus in attention, the BLA may be differentially

responsible for US revaluation depending on which direction the US is revalued.

Together, these experiments provide important information regarding the

neuroanatomical substrates involved in maintaining and updating the representation of

aversive stimuli. Our experiments suggest that the CEA may encode fear associations in

a manner similar to that observed in the BLA. Indeed common cellular mechanisms

appear to underlie fear conditioning in both structures (Goosens & Maren, 2003;

Wilensky et al., 2006; Zimmerman et al., 2007). Elucidating the mechanisms by which

the amygdala encodes fear memory is critical for developing effective treatments for

anxiety disorders, including Post-Traumatic Stress Disorder (PTSD) (Davey, 1989;

Hosoba et al., 2001; Unger et al., 2003; White & Davey, 1989).
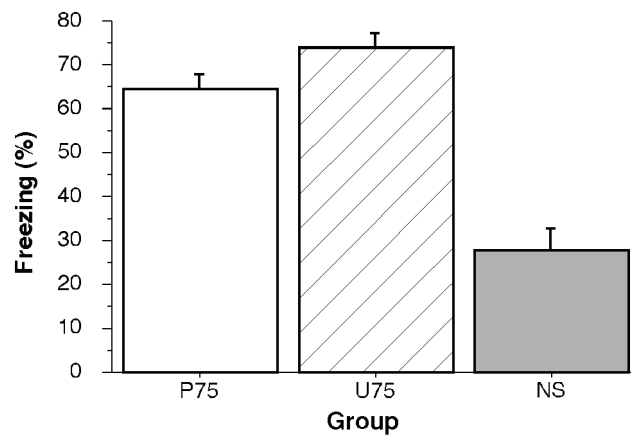
**Figure 2.1.** Post-shock freezing during the overtraining session (Experiment 1). Mean percentage of freezing (± SEM) during the 75-trial session in Context A are displayed for each of the three groups.
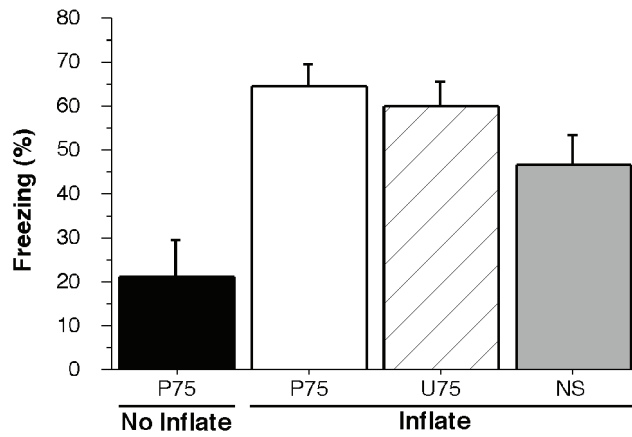
**Figure 2.2.** Post-shock freezing during the inflation session (Experiment 1). Mean percentage of freezing (± SEM) during the five 60 sec ITI periods between the inflation shocks. Data are shown for NS-INF (gray bar), U75-INF (hatched bar), P75-INF (white bar) and P75-NoINF (black bar).

**Figure 2.3.** Conditioned freezing during the context and tone tests (Experiment 1). *A,* Mean percentage of freezing (± SEM) across the 10-minute context test. *B,* Mean percentage of freezing (± SEM) during the 10-trial tone test. Data are an average of freezing during the ITI periods. Data are shown for NS-INF (gray bar), U75-INF (hatched bar), P75-INF (white bar) and P75-NoINF (black bar).
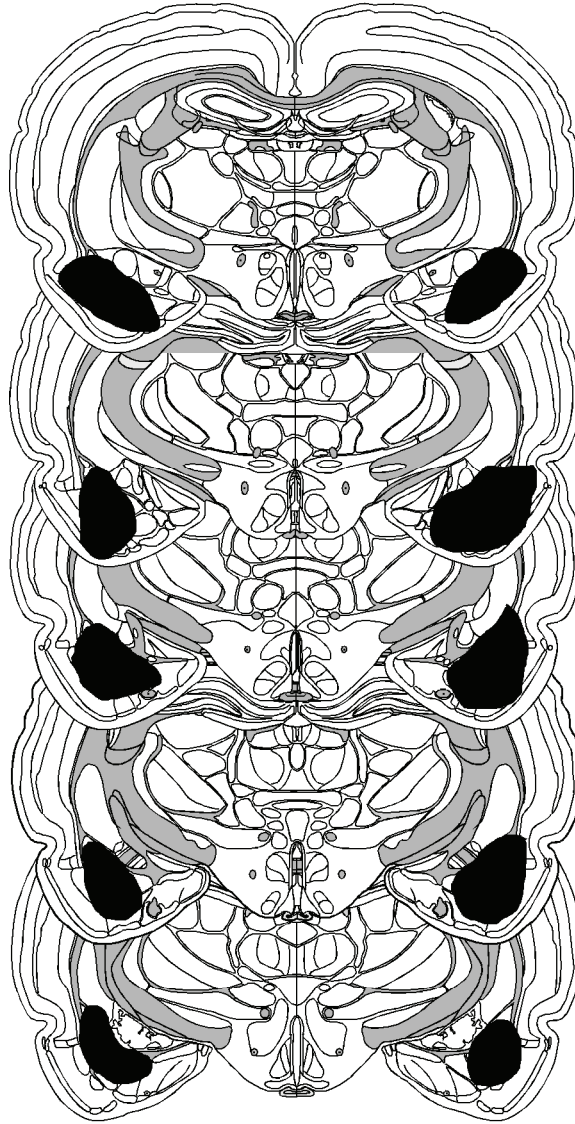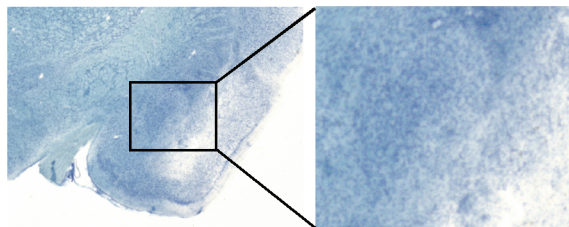
**Figure 2.4.** *A,* Schematic representation of the extent of pre-training NMDA lesions in the BLA for Experiment 2. Coronal brain images were adapted from (Swanson, 1992). *B,* Representative thionin-stained section from rats that received lesions of the BLA.
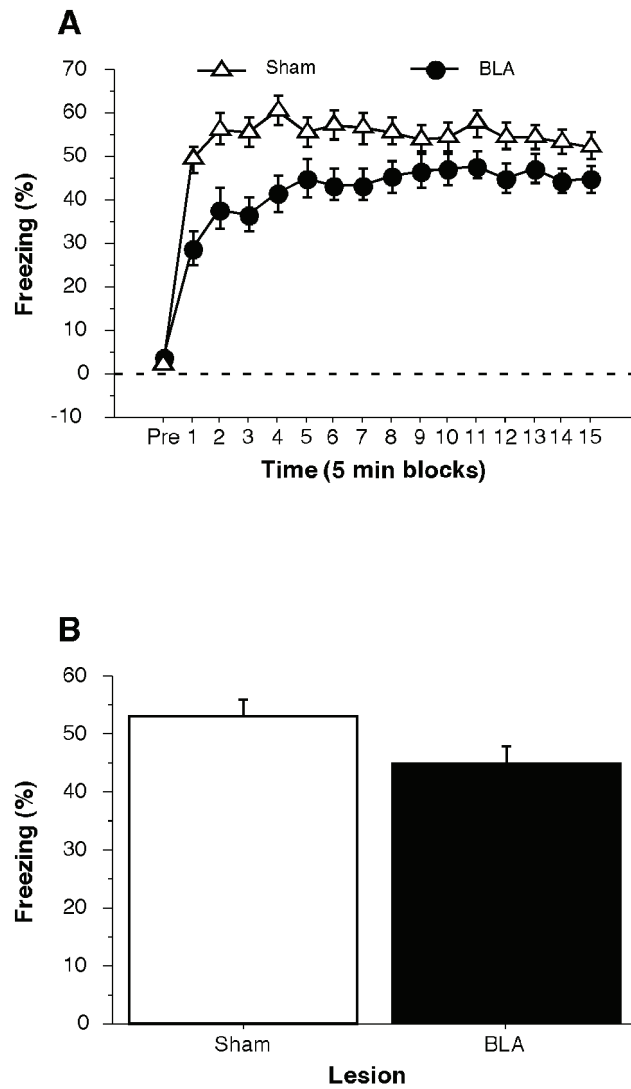
**Figure 2.5.** Post-shock freezing in rats with pre-training amygdala lesions during the paired overtraining session (Experiment 2). *A,* Mean percentage of freezing (± SEM) during the 75-trial session. Data are shown for rats with BLA lesions (closed circles) and SHAM rats (open triangles). *B,* Mean percentage of freezing (± SEM) during the last 10 conditioning trials in rats with BLA lesions (black bar) and SHAM rats (white bar).
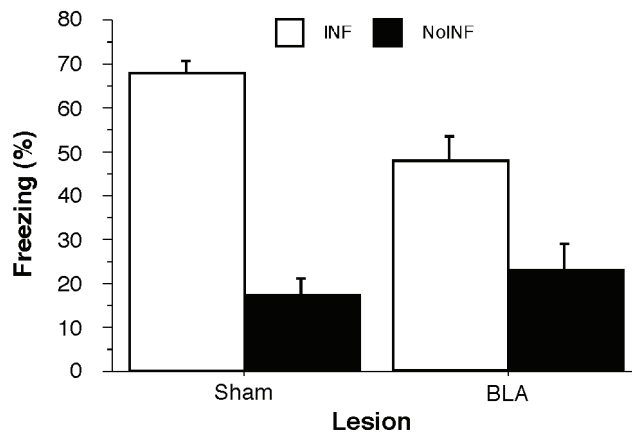
**Figure 2.6.** Post-shock freezing in rats with pre-training amygdala lesions during the inflation session (Experiment 2). Mean percentage of freezing (± SEM) during the post-shock ITIs for the INF groups (white bar) and the NoINF groups (black bar) within each lesion type.
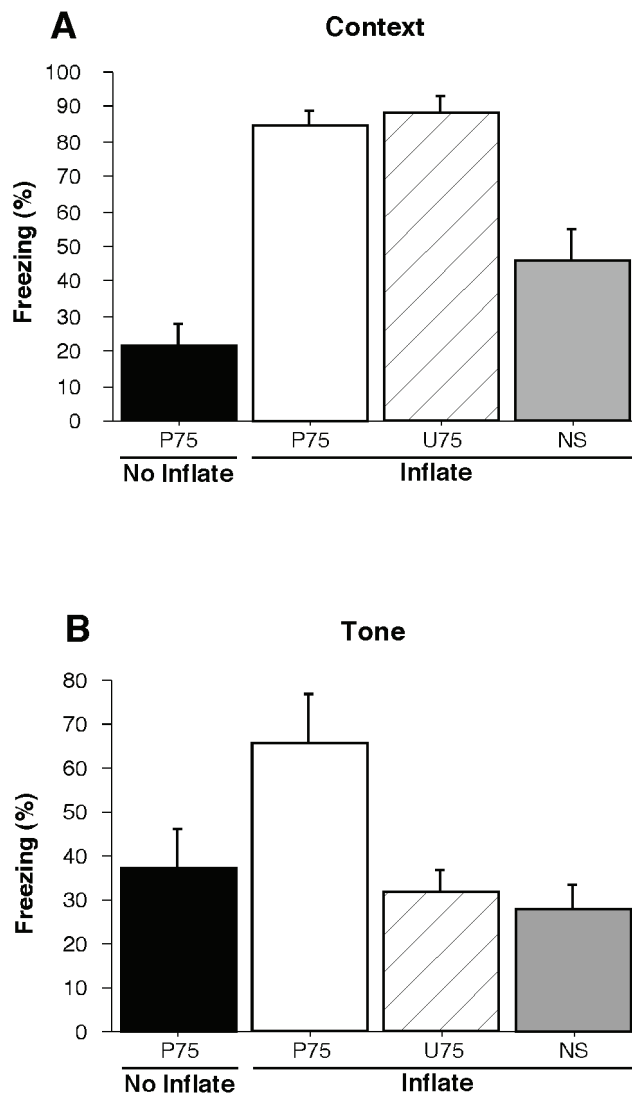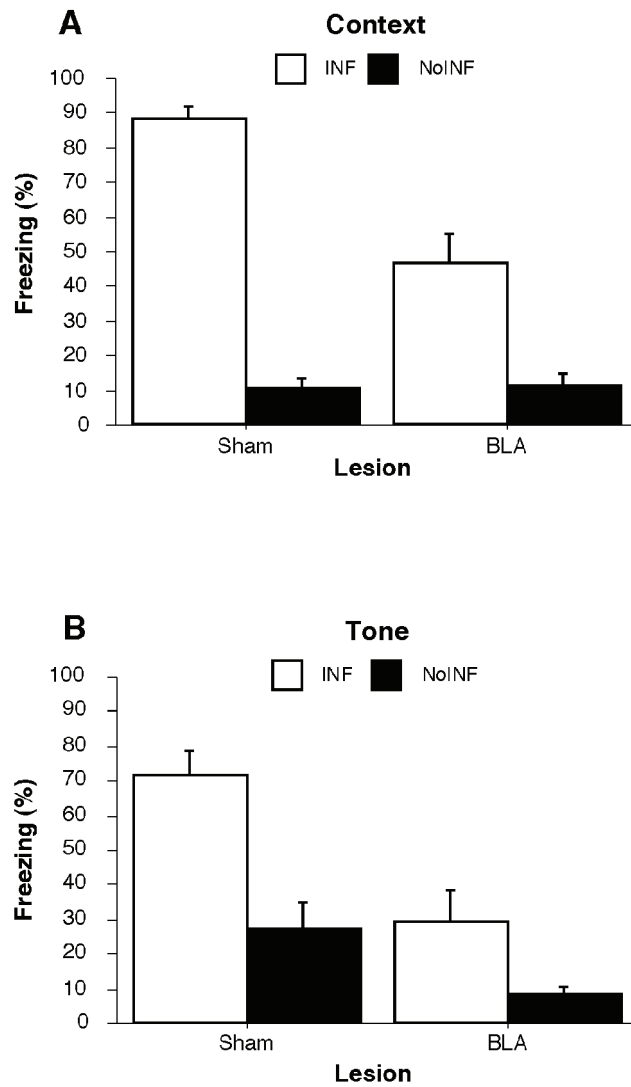
**Figure 2.7.** Conditioned freezing during the context and tone tests (Experiment 2). *A,* Mean percentage of freezing (± SEM) during the 10-minute context test. *B,* Mean percentage of freezing (± SEM) during the 10-trial tone test for the INF groups (white bars) and for the NoINF groups (black bar) within each lesion type.

**B**

**Muscimol-TMR-X**

**Thionin**
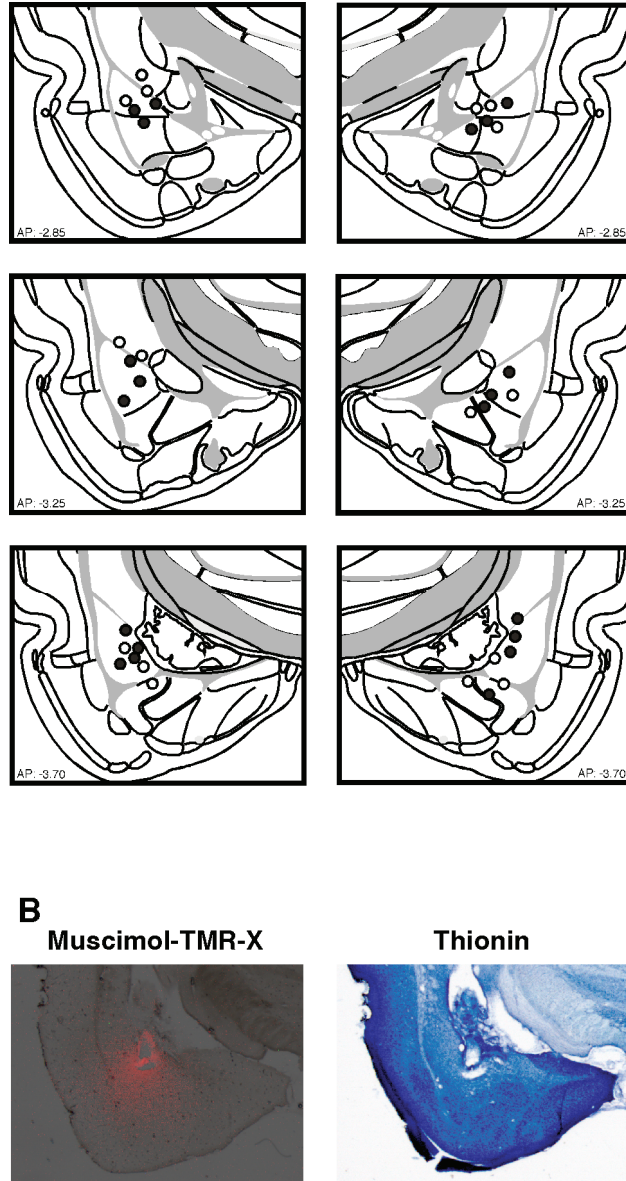
**Figure 2.8.** *A,* Schematic representation of the locations of included cannula placements for the infusion of MUS (closed circles) or VEH (open circles) in the BLA for Experiment 3. A magnification of the amgydala is shown adjacent to the coronal brain sections. Coronal brain images were adapted from (Swanson, 1992). *B,* Representative images of muscimol-TMR-X spread 80 minutes following drug infusion (left) and a corresponding thionin-stained section (right).
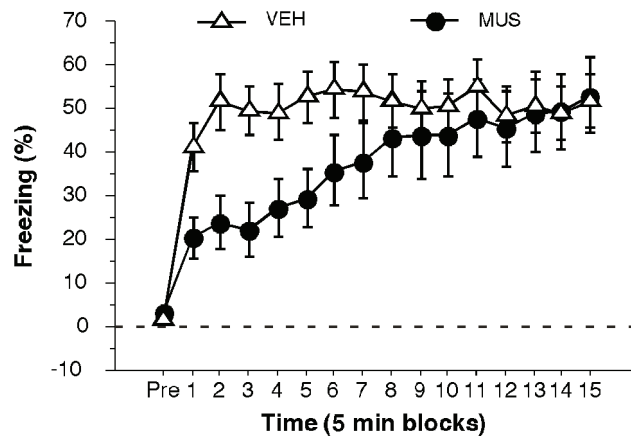
**Figure 2.9.** Post-shock freezing in rats with pre-training muscimol infusions during the paired overtraining session (Experiment 3). *A,* Mean percentage of freezing (± SEM) during the 75-trial session. Data are shown for rats that received MUS (closed circles) and rats that received VEH (open triangles).
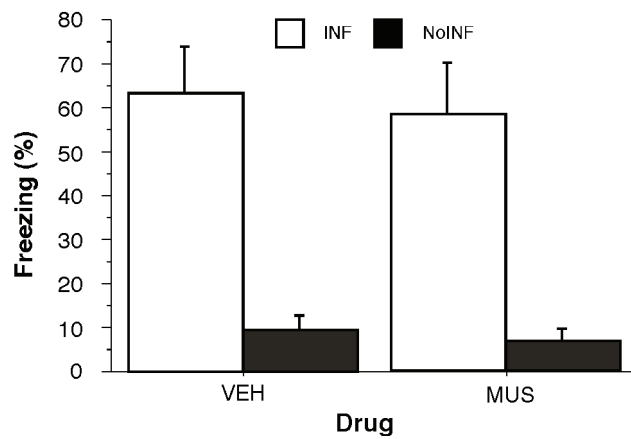
**Figure 2.10.** Post-shock freezing during the inflation session (Experiment 3). Mean percentage of freezing (± SEM) during the post-shock ITIs for the INF groups (white bar) and the NoINF groups (black bar) within each drug group.

**A  Context**

**B  Tone**
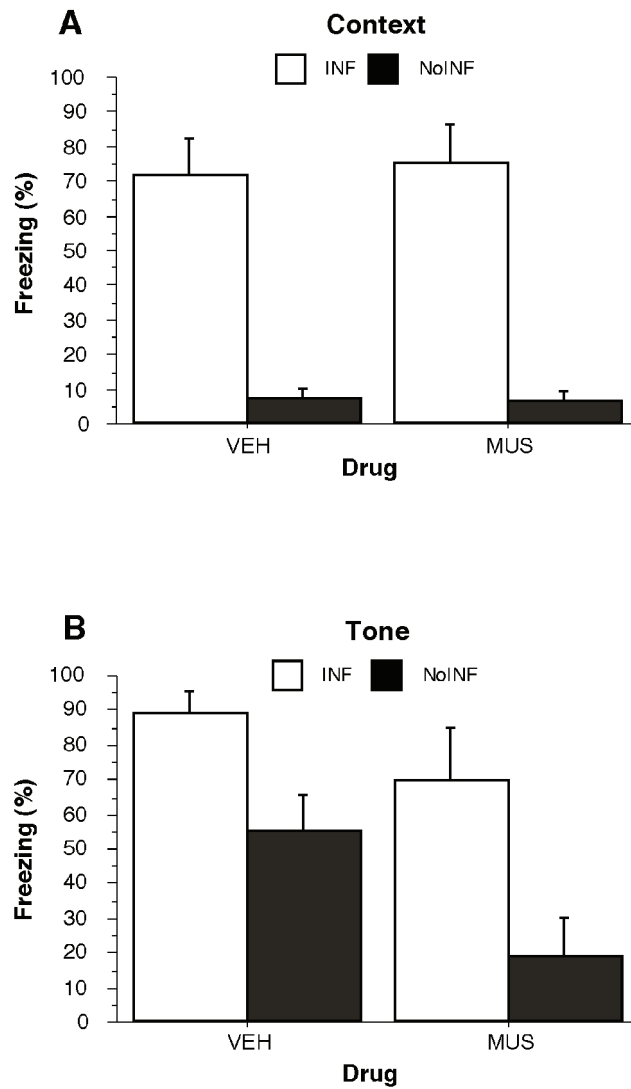
**Figure 2.11.** Conditioned freezing during the context and tone tests (Experiment 3). *A,* Mean percentage of freezing (± SEM) during the 10-minute context test. *B,* Mean percentage of freezing (± SEM) during the 5-trial tone test for the INF groups (white bars) and for the NoINF groups (black bar) within each drug group.

# References

Allen, T. A., Narayanan, N. S., Kholodar-Smith, D. B., Zhao, Y., Laubach, M., & Brown, T. H. (2008). Imaging the spread of reversible brain inactivations using fluorescent muscimol. *J Neurosci Methods*.

Balleine, B. W., Killcross, A. S., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *J. Neurosci., 23*(2), 666-675.

Balleine, B. W., & Killcross, S. (2006). Parallel incentive processing: An integrated view of amygdala function. *Trends Neurosci, 29*(5), 272-279.

Baxter, M. G., Bucci, D. J., Holland, P. C., & Gallagher, M. (1999). Impairments in conditioned stimulus processing and conditioned responding after combined selective removal of hippocampal and neocortical cholinergic input. *Behav Neurosci, 113*(3), 486-495.

Baxter, M. G., Holland, P. C., & Gallagher, M. (1997). Disruption of decrements in conditioned stimulus processing by selective removal of hippocampal cholinergic input. *J Neurosci, 17*(13), 5230-5236.

Blair, H. T., Sotres-Bayon, F., Moita, M. A. P., & Ledoux, J. E. (2005). The lateral amygdala processes the value of conditioned and unconditioned aversive stimuli. *Neuroscience, 133*(2), 561-569.

Blundell, P., Hall, G., & Killcross, S. (2001). Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. *J. Neurosci., 21*(22), 9018-9026.

Blundell, P., Hall, G., & Killcross, S. (2003). Preserved sensitivity to outcome value after lesions of the basolateral amygdala. *J. Neurosci., 23*(20), 7702-7709.

Bolles, R. C., Riley, A. L., Cantor, M. B., & Duncan, P. M. (1974). The rat's failure to anticipate regularly scheduled daily shock. *Behav Biol, 11*(3), 365-372.

Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A modern learning theory perspective on the etiology of panic disorder. *Psychol Rev, 108*(1), 4-32.

Campeau, S., & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J. Neurosci., 15*(3), 2301-2311.

Cardinal, R. N., Parkinson, J. A., Hall, J., & Everitt, B. J. (2002). Emotion and motivation: The role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci Biobehav Rev, 26*(3), 321-352.

Cousens, G., & Otto, T. (1998). Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience, 112*(5), 1092-1103.

Davey, G. C. L. (1989). Ucs revaluation and conditioning models of acquired fears. *Behaviour Research and Therapy, 27*(5), 521-528.

Davis, M., & Whalen, P. J. (2001). The amygdala: Vigilance and emotion. *Mol Psychiatry, 6*(1), 13-34.

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., & Boakes, R. A. (1995). Motivational control after extended instrumental training. *Animal Learning & Behavior, 23*, 197-206.

Everitt, B. J., Cardinal, R. N., Parkinson, J. A., & Robbins, T. W. (2003). Appetitive behavior: Impact of amygdala-dependent mechanisms of emotional learning. *Ann N Y Acad Sci, 985*, 233-250.

Fanselow, M. S., & Gale, G. D. (2003). The amygdala, fear, and memory. *Ann N Y Acad Sci, 985*, 125-134.

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews, 23*, 743-760.

Gale, G. D., Anagnostaras, S. G., Godsil, B. P., Mitchell, S., Nozawa, T., Sage, J. R., et al. (2004). Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. *J Neurosci, 24*(15), 3810-3815.

Gallagher, M., Graham, P. W., & Holland, P. C. (1990). The amygdala central nucleus and appetitive pavlovian conditioning: Lesions impair one class of conditioned behavior. *Journal of Neuroscience, 10*(6), 1906-1911.

Goosens, K. A., & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn Mem, 8*(3), 148-155.

Goosens, K. A., & Maren, S. (2003). Pretraining nmda receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear. *Behav Neurosci, 117*(4), 738-750.

Grillon, C., Southwick, S. M., & Charney, D. S. (1996). The psychobiological basis of posttraumatic stress disorder. *Molecular Psychiatry, 1*(4), 278-297.

Hall, J., Parkinson, J. A., Connor, T. M., Dickinson, A., & Everitt, B. J. (2001). Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating pavlovian influences on instrumental behaviour. *Eur J Neurosci, 13*(10), 1984-1992.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with pavlovian second-order conditioning and reinforcer devaluation effects. *Journal of Neuroscience, 16*(16), 5256-5265.

Helmstetter, F. J. (1992). The amygdala is essential for the expression of conditional hypoalgesia. *Behavioral Neuroscience, 106*(3), 518-528.

Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience, 108*(5), 1005-1009.

Holland, P. C. (2004). Relations between pavlovian-instrumental transfer and reinforcer devaluation. *Journal of Experimental Psychology:  Animal Behavior Processes, 30*, 104-117.

Holland, P. C., & Gallagher, M. (1993). Amygdala central nucleus lesions disrupt increments, but not decrements, in conditioned stimulus processing. *Behav Neurosci, 107*(2), 246-253.

Holland, P. C., & Gallagher, M. (1999). Amygdala circuitry in attentional and representational processes. *Trends in Cognitive Sciences, 3*(2), 65-73.

Holland, P. C., & Gallagher, M. (2004). Amygdala-frontal interactions and reward expectancy. *Current Opinion in Neurobiology, 14*(2), 148-155.

Holland, P. C., & Rescorla, R. A. (1975). The effect of two ways of devaluing the unconditioned stimulus after first- and second-order appetitive conditioning. *J Exp Psychol Anim Behav Process, 1*(4), 355-363.

Hosoba, T., Iwanaga, M., & Seiwa, H. (2001). The effect of ucs inflation and deflation procedures on `fear' conditioning. *Behaviour Research and Therapy, 39*(4), 465-475.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature (London), 388*(6640), 377-380.

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in pavlovian fear conditioning: A critical review. *Neuroscience & Biobehavioral Reviews, 30*(2), 188-202.

Konorski, J. (1967). *Integrative activity of the brain*. Chicago: University of Chicago Press.

Koo, J. W., Han, J. S., & Kim, J. J. (2004). Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. *J Neurosci, 24*(35), 7654-7662.

LeDoux, J. (1998). Fear and the brain: Where have we been, and where are we going? *Biol Psychiatry, 44*(12), 1229-1238.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184.

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *Journal of Neuroscience, 18*(8), 3088-3097.

Maren, S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J. Neurosci., 19*(19), 8696-8703.

Maren, S. (2001a). Is there savings for pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? *Neurobiology of Learning and Memory, 76*(3), 268-283.

Maren, S. (2001b). Neurobiology of pavlovian fear conditioning. *Annual Review of Neuroscience, 24*(1), 897-931.

Maren, S. (2005). Building and burying fear memories in the brain. *Neuroscientist, 11*(1), 89-99.

Maren, S., Aharonov, G., & Fanselow, M. S. (1996a). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: Absence of a temporal gradient. *Behavioral Neuroscience, 110*(4), 718-726.

Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996b). N-methyl-d-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience, 110*(6), 1365-1374.

Maren, S., & Quirk, G. J. (2004). Neuronal signalling of fear memory. *Nat Rev Neurosci, 5*(11), 844-852.

Muller, J., Corodimas, K. P., Fridel, Z., & LeDoux, J. E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behavioral Neuroscience, 111*(4), 683-691.

Nader, K., Majidishad, P., Amorapanth, P., & LeDoux, J. E. (2001). Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. *Learn Mem, 8*(3), 156-163.

Pare, D., Quirk, G. J., & Ledoux, J. E. (2004). New vistas on amygdala networks in conditioned fear. *J Neurophysiol, 92*(1), 1-9.

Parkinson, J. A., Robbins, T. W., & Everitt, B. J. (2000). Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. *European Journal of Neuroscience, 12*(1), 405-413.

Pickens, C. L., & Holland, P. C. (2004). Conditioning and cognition. *Neuroscience & Biobehavioral Reviews, 28*(7), 651-661.

Pickens, C. L., Saddoris, M. P., Setlow, B., Gallagher, M., Holland, P. C., & Schoenbaum, G. (2003). Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. *J. Neurosci., 23*(35), 11078-11084.

Ponnusamy, R., Poulos, A. M., & Fanselow, M. S. (2007). Amygdala-dependent and amygdala-independent pathways for contextual fear conditioning. *Neuroscience, 147*(4), 919-927.

Poulos, A. M., Sterlace, M., & Fanselow, M. S. (2006). *Deficits in remote but not recent long-term memory in the absence of the fta: A retrieval or storage failure.* Paper presented at the Society for Neuroscience, Atlanta, GA.

Rescorla, R. A. (1973). Effect of us habituation following conditioning. *Journal of Comparative & Physiological Psychology, 82*(1), 137-143.

Rescorla, R. A. (1974). Effect of inflation of the unconditioned stimulus value following conditioning. *Journal of Comparative & Physiological Psychology, 86*, 101-106.

Samson, R. D., & Pare, D. (2005). Activity-dependent synaptic plasticity in the central nucleus of the amygdala. *J. Neurosci., 25*(7), 1847-1855.

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of pavlovian fear conditioning: A cellular and molecular perspective. *Trends in Neurosciences, 24*(9), 540-546.

Swanson, L. W. (1992). *Brain maps: Structure of the rat brain.* New York: Elsevier.

Unger, W., Evans, I. M., Rourke, P., & Levis, D. J. (2003). The s-s construct of expectancy versus the s-r construct of fear: Which motivates the acquisition of avoidance behavior? *J Gen Psychol, 130*(2), 131-147.

Walker, D. L., & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J. Neurosci., 17*(23), 9375-9383.

White, K., & Davey, G. C. L. (1989). Sensory preconditioning and ucs inflation in human `fear' conditioning. *Behaviour Research and Therapy, 27*(2), 161-166.

Wilensky, A. E., Schafe, G. E., Kristensen, M. P., & LeDoux, J. E. (2006). Rethinking the fear circuit: The central nucleus of the amygdala is required for the acquisition, consolidation, and expression of pavlovian fear conditioning. *J. Neurosci., 26*(48), 12387-12396.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (1999). Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. *J. Neurosci., 19*(24), 48RC-.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (2000). Functional inactivation of amygdala nuclei during acquisition of pavlovian fear conditioning. *Society for Neuroscience Abstracts, 30*, 465.469.

Zimmerman, J. M., Rabinak, C. A., McLachlan, I. G., & Maren, S. (2007). The central nucleus of the amygdala is essential for acquiring and expressing conditional fear after overtraining. *Learn Mem, 14*(9), 634-644.

**CHAPTER III**

**THE BASOLATERAL AMYGDALA IS NOT NECESSARY FOR US INFLATION AFTER FEAR CONDITIONING IN RATS**

Pavlovian fear conditioning is a behavioral model used to investigate the neurobiology underlying the development and maintenance of fear learning and memory (Bouton et al., 2001; Grillon et al., 1996; Kim & Jung, 2006; LeDoux, 1998; LeDoux, 2000; Maren, 2001b; Maren, 2005). In this model an innocuous conditioned stimulus (CS), such as a tone, is paired with an aversive unconditioned stimulus (US), such as a footshock. After one or more pairings the rat learns that the CS predicts the US. As a consequence, CS presentations alone elicits a conditioned fear response (CR), which includes increases in heart rate, arterial blood pressure, hypoalgesia, potentiated acoustic startle, stress hormone release, and freezing (somatomotor immobility).

The amygdala has been identified as one of the major regions in which fear memories are encoded and stored. Within the amygdala there are two sub-regions that contribute to fear learning and the expression of learned fear responses. The basolateral complex of the amygdala (BLA; consisting of the lateral, basolateral, and basomedial nuclei) is where CS and US information converge and become associated (yielding the fear memory). The second sub-region is the central nucleus of the amygdala (CEA),

which translates associative information into behavioral fear responses, but like the BLA, is also necessary for fear acquisition (Davis & Whalen, 2001; Fanselow & Gale, 2003; Fendt & Fanselow, 1999; LeDoux, 1998; LeDoux, 2000; Maren, 2001b; Schafe et al., 2001; Wilensky et al., 2006; Zimmerman et al., 2007). In support of this many studies have shown that either permanent or temporary lesions of the BLA or CEA prevent the acquisition and/or expression of fear memories (Campeau & Davis, 1995; Cousens & Otto, 1998; Fanselow & Gale, 2003; Gale et al., 2004; Goosens & Maren, 2001, 2003; Helmstetter, 1992; Helmstetter & Bellgowan, 1994; Killcross et al., 1997; Koo et al., 2004; Maren, 1998; Maren, 1999, 2001a, 2001b; Maren et al., 1996a; Maren et al., 1996b; Muller et al., 1997; Nader et al., 2001; Walker & Davis, 1997; Wilensky et al., 2006; Wilensky et al., 1999; Wilensky et al., 2000; Zimmerman et al., 2007).

Interestingly, recent work in appetitive conditioning paradigms also suggests that the BLA also has a role in representing US value. That is, BLA inactivation or lesions reduces the decrement in conditioned responding after devaluation of a food US (Balleine et al., 2003; Blundell et al., 2001; Everitt et al., 2003; Hatfield et al., 1996; Holland, 2004; Killcross et al., 1997; Pickens et al., 2003). However, in contrast to these devaluation studies, in Chapter II we have found that rats with BLA lesions exhibit US inflation after an overtraining procedure, suggesting that the BLA may not be necessary for coding the value of the aversive USs. In a post-fear conditioning US inflation procedure Fanselow and Gale (2003) found that muscimol when infused into the BLA prior to the inflation session blocked enhancement of fear. Similar to the devaluation studies above, they concluded that the BLA is essential for inflation of fear. Therefore, the following experiments were designed to further probe the role of the BLA in coding

US value and maintaining updated representations of USs of BLA-dependent memories in intact rats (Maren, 1999).

## General Methods

*Subjects*

The subjects were 208 adult male Long-Evans rats (60-90 days old; 200-224 grams; Blue Spruce) obtained from a commercial supplier (Harlan Sprague-Dawley, Indianapolis, IN).  Upon arrival all rats were individually housed in conventional Plexiglas hanging cages and kept on a 14 hr light/10 hr dark cycle (lights on at 7:00am) with free access to food and tap water.  To acclimate the rats to the experimenter they were handled daily (10-15 sec per rat) for 5 days following their arrival.  All experimental procedures were conducted in accordance with the approved guidelines as stated by the University of Michigan Committee on Use and Care of Animals (UCUCA).

*Behavioral Apparatus*

All sessions were conducted in eight identical rodent conditioning chambers (30 x 24 x 21 cm; MED Associates, St. Albans, VT).  The chambers were positioned inside sound-attenuating cabinets located in an isolated room.  Each chamber was constructed of aluminum (2 side walls) and Plexiglas (rear wall, ceiling, and hinged front door); the floor consisted of 19 stainless-steel rods, (4 mm diameter) spaced apart 1.5 cm (center to center).  The grid floor was connected to a shock source and solid-state grid scrambler (MED Associates), which delivered the footshock US.  Mounted on one wall of the chamber was a speaker to provide a distinct auditory CS and on the opposite wall was a 15-W house light; a fan provided background noise (65dB).

Three distinct contexts were created by manipulating multiple visual, olfactory and tactile cues: 1) Context A: 1% acetic acid odor in the chamber, houselights and room lights on, fans on in the cabinets, cabinet doors open, and grid floors; 2) Context B: 1% ammonium hydroxide odor in the chamber, red lights on in the room, houselights off, fans off in the cabinets, cabinet doors closed, and Plexiglas floors; 3) Context C: 70% ethanol odor in the chamber, house lights on, room lights off, fans off in the cabinets, cabinet doors open, and grid floors.

Each chamber rested on a load-cell platform, which was used to record chamber displacement in response to each rat's motor activity. The output from each load-cell was amplified to a level previously established to detect freezing responses. For each chamber, the load-cell amplifier output was digitized at 5 Hz (300 observations per minutes per rat) and acquired online using Threshold Activity software (MED Associates). Locomotor activity was quantified by the raw load cell values (range = 0 - 100) and freezing behavior was quantified by calculating the number of load cell values below the freezing threshold (threshold = 10). However, to prevent the inclusion of momentary bouts of inactivity as freezing, (i.e., < 1 sec) freezing was only scored after five or more contiguous observations below the freezing threshold (for details see Maren, 1998; Maren, 1999, 2001a). Freezing observations during each session were transformed into a percentage of total observations. In Experiments 1 & 2 sensitivity to the footshock US was measured by comparing the average locomotor activity over the 2-sec period prior to the first footshock presentation and the average locomotor activity during the first presentation of the footshock (2 sec).

*Data Analysis*

Freezing data were converted to a percentage of total observations, which is a probability estimate that is amenable to analysis with parametric statistics. These values were analyzed using analysis of variance (ANOVA) and post hoc comparisons using Fishers LSD tests were performed after a significant overall $F$ ratio was obtained. All data are represented as means $\pm$ SEMs.

## Experiment 1

In Chapter II we have found that overtrained fear memories learned by rats with BLA lesions are still sensitive to inflation, which suggests that the BLA is not necessary for coding US value. However, permanent lesions of the BLA may have allowed other neural systems to compensate and mediate the inflation effect (Maren, 1999). To address this issue, Experiment 1 used temporary pharmacological inactivation of the BLA during the inflation procedure to assess the necessity of the BLA for revaluing shock USs after overtraining. Overtraining was used in this experiment to directly compare the results with those obtained with BLA lesions (Chapter II); Experiment 2 explores the consequences of BLA involvement on inflation after limited training.

*Method*

*Subjects and design.* Prior to inflation, 32 rats were first divided into two equal groups: one group that received bilateral infusions of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX; Sigma, St. Louis, MO), an AMPA receptor antagonist and a second group that received bilateral infusions of a vehicle control (VEH; ACSF; Sigma). NBQX is a short-acting drug with effects lasting up to about 30 minutes, which is ideal for the duration of the inflation procedure (Gill et al., 1992; Lees, 2000).

Then each drug group was further divided into two groups: one that received the US inflation procedure (INF) and a group that did not undergo US inflation (NoINF).

*Surgery.* One week prior to training and after having been handled for 1 week each rat was anesthetized with an intraperitoneal (i.p.) injection of a Nembutal (sodium pentobarbital; 65mg/kg body weight) and atropine methyl nitrate (0.4mg/kg body weight) cocktail. Ocular lubricant was used to moisten the eyes. The scalp was shaved, cleaned with antiseptic (Betadine) and the rat was mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). After the scalp was incised and retracted, the skull was positioned so that bregma and lambda were in the same horizontal plane. Small burr holes were drilled bilaterally in the skull to allow for the placement of 26-gauge guide cannulae (Plastics One, Roanoke, VA) in the BLA (3.3 mm posterior to bregma, 5.0 mm lateral to the midline, and 6.5 mm ventral to the brain surface), along with holes for 3 small jeweler's screws. Dental acrylic was applied to the cannulae, screws, and skull surface to hold the guide cannulae in place. After surgery, 33-gauge dummy cannulae (16 mm; Plastics One) were inserted into the guide cannulae and the rats were kept on a heating pad until they recovered from anesthesia before returning to their home cages. Dummy cannulae were replaced daily during the week of recovery.

*Conditioning, inflation and test procedure.* Fear conditioning was conducted using an overtraining procedure. Rats were transported from their home cages in squads of eight and placed in the conditioning chambers (Context A). Chamber position and experimental group were counterbalanced for each squad. Rats received 75 paired presentations of a tone (10 seconds, 2kHz, 85dB) that co-terminated with a footshock (1.0mA, 2 seconds) beginning 3 minutes after being placed in the chambers. There was a

60 second intertrial interval (ITI) and the animals remained in the boxes 60 seconds after

the last footshock presentation. Twenty-four hours after overtraining and prior to the

inflation procedure, rats were transported to the infusion room in squads of eight from

their home cages in white 5-gallon buckets.  Hamilton syringes (10μl; Harvard

Apparatus) were mounted in two infusion pumps (10 syringes/pump; Harvard Apparatus)

and connected to 33-gauge internal cannula (1.0 mm longer than the implanted guide

cannulae) with polyethylene tubing (A-M Systems).  Dummy cannulae were removed

from each rat and internal cannulae were inserted into each guide cannula. Either NBQX

(12mg/mL dissolved in ACSF, pH 7.4; Sigma) or ACSF (same volume and rate) was

infused bilaterally into the BLA (0.5μl/side; 0.1 μl/min).  One minute was allowed for

diffusion of the drug into the target structure before the injectors were removed.  Dummy

cannulae were inserted into the guide cannulae once the injectors were removed and the

rats were immediately taken to the conditioning chambers for the inflation procedure.  All

rats were placed in another, novel environment (Context C) for US inflation.  The

inflation session consisted of exposure to 5 high-intensity footshocks (3.0mA, 2 seconds)

beginning 3 minutes after being placed in the chambers.  There was a 60 second ITI, and

the animals remained in the boxes 60 seconds after the last footshock. Rats in the NoINF

group were placed in the chamber for the same duration as the rats in the inflation groups

but did not receive footshocks.  Forty-eight hours after conditioning, all rats were placed

back into Context A for 10 minutes to assess contextual fear.  Twenty-four hours after the

context test, fear to the tone was tested by placing the rats into a third novel context

(Context B) and presenting 30 tone alone presentations (10 seconds, 2kHz, 85dB, 60 sec

ITI) beginning 3 minutes after being placed into the chambers. Freezing behavior was measured throughout all experimental sessions.

*Histology.* After behavioral testing, rats were euthanized with an overdose of sodium pentobarbital (i.p. 100 mg/kg) and were transcardially perfused with physiological saline followed by 10 % formalin.  Brains were removed and post-fixed in 10% formalin followed by 10% formalin/30% sucrose solution until sectioning.  Coronal brain sections (45 μm) were cut on a cryostat and wet-mounted with 70% ethanol on glass microscope slides.  Once dry, the sections were stained with 0.25% thionin to visualize neuronal cell bodies and identify lesion sites.

### Results and Discussion

*Histology.*  Nine rats were excluded from the analyses because their cannulae placements missed the BLA (Figure 3.1).  This yielded the following groups designated by drug type during inflation (NBQX; vehicle, VEH) and inflation condition (inflation, INF; no inflation, NoINF): NBQX-INF ($n = 6$), NBQX-NoINF ($n = 6$), VEH-INF ($n = 6$), and VEH-NoINF ($n = 5$).

*Behavior.*  An ANOVA of the average post-shock freezing during the conditioning session revealed a significant main effect of time (15 trial blocks) [$F_{(1, 19)} = 188.3$; $p < 0.0001$], (Figure 3.2a), but no main effect of drug group or inflation condition. These results indicated that all groups displayed similar levels of freezing during overtraining.

Conditional freezing during the inflation session is shown in Figure 3.2b.  It is apparent that rats receiving inflation shocks displayed increased freezing relative to the

no-inflation controls ($p < 0.0001$ for comparison between INF and NoINF). These

observations were confirmed by a two-way ANOVA on the average post-shock freezing

during inflation that revealed a significant main effect of inflation condition [$F_{(1, 19)}$ =

32.0; $p < 0.0001$], however there was no effect of drug [$F_{(1, 19)}$ = 2.1; $p = 0.16$] or an

interaction between drug and inflation condition during the inflation session [$F_{(1, 19)}$ = 3.0;

$p = 0.10$]. However, a one-way ANOVA on the average post-shock freezing during the

inflation session revealed a significant main effect of group [$F_{(3, 19)}$ = 12.5; $p < 0.0001$], a

significant main effect of time [$F_{(5, 95)}$ = 9.3; $p < 0.0001$], and a significant interaction

between group and time during the inflation session [$F_{(15, 95)}$ = 3.7; $p < 0.0001$]. The

VEH-INF group displayed higher levels of freezing than any of the other groups ($p <$

0.03 for all comparisons). Most importantly, the VEH-INF group displayed significantly

higher levels of freezing than the NBQX-INF group ($p < 0.03$), which suggests that

NBQX inactivation impaired conditional freezing compared to controls. In addition,

NBQX in the BLA did not affect shock reactivity to the first inflation footshock [$F_{(1, 10)}$ =

1.5; $p = 0.25$] (data not shown).

　　　　Conditioned freezing during the context test is displayed in Figure 3.2c. Rats in

both the VEH-INF and NBQX-INF groups exhibited more freezing than non-inflated

controls. These observations were confirmed by a two-way ANOVA that revealed a

significant main effect of inflation condition [$F_{(1, 19)}$ = 20.0; $p = 0.0003$], however there

was no significant effect of drug type [$F_{(1, 19)}$ = 1.2; $p = 0.29$] or an interaction between

the inflation condition and drug type [$F_{(1, 19)}$ = 2.1; $p = 0.16$]. Both inflation groups

(NBQX-INF and VEH-INF) displayed significantly higher levels of freezing when

compared to the non-inflated groups (NBQX-NoINF and VEH-NoINF; $p < 0.04$ for all

comparisons).  These data indicate that rats under BLA inactivation during inflation were still sensitive to inflation, at a level comparable to rats with a functional BLA ($p = 0.0003$ for comparison between INF and NoINF and $p = 0.19$ for comparison between NBQX and VEH).

Similar results were found during the tone test (Figure 3.2d).  A two-way ANOVA revealed a significant main effect of inflation condition [$F_{(1, 42)} = 18.0$; $p = 0.0001$], however there was no significant effect of drug type [$F_{(1, 42)} = 0.2$; $p = 0.66$] or an interaction between the inflation condition and drug type [$F_{(1, 42)} = 0.5$; $p = 0.48$]. Both inflation groups (NBQX-INF and VEH-INF) displayed significantly higher levels of freezing when compared to the non-inflated groups (NBQX-NoINF and VEH-NoINF; $p < 0.01$ for all comparisons).  Like the context test, these data suggest that drug inactivation during inflation did not impair sensitivity to inflation ($p < 0.0001$ for comparison between INF and NoINF and $p = 0.55$ for comparison between NBQX and VEH).  Overall overtrained fear memories in intact rats appear to remain sensitive to US inflation, even in the absence of BLA during inflation, suggesting that, the BLA does not mediate changes in US value.

## Experiment 2

Experiment 1 suggests that the BLA is not necessary for US revaluation of overtrained fear memories.  However, these fear associations may have been resilient to manipulations of the amygdala as a consequence of the overtraining procedure.  For instance, Nader and colleagues find that reconsolidation of weak memories is disrupted with protein synthesis inhibitors in the BLA, but strong, overtrained memories are

immune to disruption (for review see Wang et al., 2005).  Hence, it is possible that

revaluation of limited trained fear memories would be more sensitive than overtrained

memories to disruption by BLA inactivation. To address this issue, Experiment 2

examined the consequence of pharmacological inactivation of the BLA on the inflation of

fear memories acquired after limited training.

*Method*

 *Subjects and design.*  Prior to inflation, 64 rats were first divided into two equal

groups: one group that received bilateral infusions of NBQX and a second group that

received bilateral infusions of a vehicle control (VEH; 0.1M PBS; Sigma).  Then each

drug group was further divided into two groups: one that received the US inflation

procedure (INF) and a group that did not undergo US inflation (NoINF).

 *Surgery.*  One week prior to training and after having been handled for 1 week the

rats were anesthetized and prepared for surgery as described in Experiment 1.  Half of the

rats received bilateral cannulae targeting the BLA were implanted as described in

Experiment 1 and the other half received bilateral cannulae implantations targeting the

CEA (2.5 mm posterior to bregma, 4.3 mm lateral to the midline, and 6.9 mm ventral to

the brain surface).

 *Conditioning, inflation, and test procedure.*  Fear conditioning was conducted

using a limited training procedure. Rats were transported from their home cages in squads

of eight and placed in the conditioning chambers (Context A).  Chamber position and

experimental group were counterbalanced for each squad.  Rats received 10 paired

presentations of a tone (10 seconds, 2kHz, 85dB) that co-terminated with a footshock

(1.0mA, 2 seconds) beginning 9 minutes after being placed in the chambers.  There was a

60 second intertrial interval (ITI) and the animals remained in the boxes 60 seconds after the last footshock presentation. Twenty-four hours after conditioning and prior to the inflation procedure, rats were transported to the infusion room in squads of eight from their home cages in white 5-gallon buckets.  Hamilton syringes (10µl; Harvard Apparatus) were mounted in two infusion pumps (10 syringes/pump; Harvard Apparatus) and connected to 33-gauge internal cannula (1.0 mm longer than the implanted guide cannulae) with polyethylene tubing (A-M Systems).  Dummy cannulae were removed from each rat and internal cannulae were inserted into each guide cannula. Either NBQX (12mg/mL dissolved in 0.1M PBS, pH 7.4; Sigma) or 0.1M PBS (same volume and rate) was infused bilaterally into the BLA or CEA (0.25µl/side; 0.25 µl/min).  One minute was allowed for diffusion of the drug into the target structure before the injectors were removed.  Dummy cannulae were inserted into the guide cannulae once the injectors were removed and 15 minutes after the drug infusion the rats were taken to the conditioning chambers for the inflation procedure.  All rats were placed in another, novel environment (Context C) for US inflation.  The inflation session consisted of exposure to 5 high-intensity footshocks (3.0mA, 2 seconds) beginning 6 minutes after being placed in the chambers.  There was a 60 second ITI, and the animals remained in the boxes 60 seconds after the last footshock. Rats in the NoINF group were placed in the chamber for the same duration as the rats in the inflation groups but did not receive footshocks.  Forty-eight hours after conditioning, fear to the tone was tested by placing the rats into a third novel context (Context B) and presenting 30 tone alone presentations (10 seconds, 2kHz, 85dB, 60 sec ITI) beginning 6 minutes after being placed into the chambers. Freezing behavior was measured throughout all experimental sessions.

*Histology.* Histology was conducted as described in Experiment 1.

### Results and Discussion

*Histology.* Six rats were excluded from analyses because the cannulae placements missed the BLA or CEA (Figure 3.3). This yielded the following groups designated by drug type (NBQX in the BLA, BLA; NBQX in the CEA, CEA; vehicle (collapsed across BLA and CEA), VEH) and inflation condition (inflation, INF; no inflation, NoINF): BLA-INF ($n = 8$), BLA-NoINF ($n = 8$), CEA-INF ($n = 8$), CEA-NoINF ($n = 8$), VEH-INF ($n = 14$), and VEH-NoINF ($n = 12$).

*Behavior.* An ANOVA of the average post-shock freezing during the conditioning session revealed a significant main effect of time (2 trial blocks) [$F_{(5, 180)} = 57.9$; $p < 0.0001$], (Figure 3.4), but no main effect of drug group or inflation condition. These results indicated that all groups displayed similar levels of freezing during training.

Conditional freezing during the inflation session is show in Figure 3.5. In the VEH groups it is apparent that rats receiving inflation shocks (VEH-INF) displayed increased freezing relative to the no-inflation controls (VEH-NoINF) ($p < 0.01$ for comparison between INF and NoINF) (Figure 3.5a). In contrast, NBQX infusions into the BLA or CEA reduced freezing during the inflation sessions. There was no effect of inflation in separate ANOVAs conducted on the session for the BLA [$F_{(1, 12)} = 1.4$; $p = 0.25$] (Figure 3.5b) or CEA [$F_{(1, 10)} = 0.7$; $p = 0.43$] (Figure 3.5c). Overall, NBQX infused into the amygdala blocked conditioned freezing to the inflation shocks, however it did not affect shock reactivity to the first inflation footshock [$F_{(2, 20)} = 1.622$; $p = 0.2225$] (data not shown).

Conditioned freezing during the tone test is displayed in Figure 3.6. Rats in the VEH-INF, BLA-INF and CEA-INF groups exhibited more freezing than non-inflated controls. These observations were confirmed by a two-way ANOVA that revealed a significant main effect of inflation condition [$F_{(1, 35)}$ = 74.8; $p < 0.0001$], however there was no significant effect of drug type [$F_{(2, 35)}$ = 0.03; $p = 0.97$] or an interaction between the inflation condition and drug type [$F_{(2, 35)}$ = 1.2; $p = 0.3$]. All inflation groups (BLA-INF, CEA-INF, and VEH-INF) displayed significantly higher levels of freezing when compared to the non-inflated groups (BLA-NoINF, CEA-NoINF, and VEH-NoINF; $p < 0.0002$ for all comparisons). These data indicate that rats under amygdala inactivation during inflation were still sensitive to inflation, at a level comparable to rats with a functional amygdala ($p < 0.0001$ for comparison between INF and NoINF and $p = 0.99$ for comparison between BLA and CEA; $p = 0.27$ for comparison between BLA and VEH; $p = 0.29$ for comparison between CEA and VEH). Overall, like the results from Experiment 1, the BLA is not necessary for representing changes in US value during an inflation procedure after limited fear conditioning.

**Experiment 3**

Although Experiment 1 and 2 indicated that BLA neurotransmission during the inflation procedure is not necessary for US revaluation of fear memories, it is still possible that synaptic plasticity within the BLA plays a role in consolidating or maintaining representations of revalued USs. In Experiment 3 anisomycin, a protein synthesis inhibitor, was infused into the BLA immediately after the inflation procedure to assess the role of the BLA in maintaining a representation of revalued USs.

*Methods*

*Subjects and design.* Prior to inflation 32 rats were divided into two equal groups: one that received exposure to US inflation (INF) and a group that did not undergo US inflation (NoINF). Immediately following the inflation session the groups were further divided into two groups: one group that received bilateral infusions of anisomycin (ANI), a protein synthesis inhibitor, and a second group that received bilateral infusions of a vehicle control (VEH; ACSF).

*Surgery.* One week prior to training and after having been handled for 1 week the rats were anesthetized and prepared for surgery as described in Experiment 1. Bilateral cannulae targeting the BLA were implanted also as described in Experiment 1.

*Conditioning, inflation, and test procedure.* All groups received overtraining, US inflation, and retention testing as described in Experiment 1. Immediately following the inflation session intracranial microinfusions were conducted as described in Experiment 1. Either anisomycin (125mg/mL dissolved in ACSF, pH 7.4; Sigma) or ACSF (same volume and rate) was infused bilaterally into the BLA (0.25µl/side; 0.1 µl/min). One minute was allowed for diffusion of the drug into the target structure before the injectors were removed. Dummy cannulae were inserted into the guide cannulae once the injectors were removed and the rats were transported back to their home cages.

*Histology.* Histology was conducted as described in Experiment 1.

**Results and Discussion**

*Histology.* One rat was excluded from analyses because the cannulae placements missed the BLA (Figure 3.7). This yielded the following groups designated by drug type

after inflation (anisomycin, ANI; vehicle, VEH) and inflation condition (inflation, INF; no inflation, NoINF): ANI-INF (*n* = 8), ANI-NoINF (*n* = 8), VEH-INF (*n* = 8), and VEH-NoINF (*n* = 7).

*Behavior.* All groups displayed similar levels of freezing during overtraining. This observation was confirmed by an ANOVA of the average freezing during the conditioning session that revealed a significant main effect of time (15 trial blocks) [$F_{(5, 125)}$ = 70.7; $p < 0.0001$] (Figure 3.8a).

Rats receiving inflation shocks displayed increased freezing relative to the no-inflation controls ($p < 0.0001$ for comparison between INF and NoINF and $p = 0.52$ for comparison between ANI and VEH). These observations were confirmed by a two-way ANOVA on average freezing during the inflation session that revealed a significant main effect of inflation condition [$F_{(1, 25)}$ = 33.7; $p < 0.0001$] (Figure 3.8b).

Conditioned freezing during the context is displayed in Figure 3.8c. For the context test, rats in both the VEH-INF and ANI-INF groups exhibited more freezing than the non-inflated controls ($p < 0.002$ for all comparisons). This observation was confirmed by a two-way ANOVA that revealed a significant main effect of inflation condition [$F_{(1, 25)}$ = 34.0; $p < 0.0001$], however there was no significant effect of drug type [$F_{(1, 25)}$ = 0.6; $p = 0.43$] or an interaction between the inflation condition and drug type [$F_{(1, 25)}$ = 0.4; $p = 0.52$].

Similar results were found during the tone test (Figure 3.8d). A two-way ANOVA revealed a significant main effect of inflation condition [$F_{(1, 25)}$ = 4.2; $p = 0.05$], however the was not a significant effect of drug type [$F_{(1, 25)}$ = 0.3; $p = 0.60$] or an interaction between the inflation condition and drug type [$F_{(1, 25)}$ = 0.2; $p = 0.65$]. These

data indicate that rats in both drug conditions exhibited inflation ($p = 0.05$ for comparison between INF and NoINF and $p = 0.56$ for comparison between ANI and VEH). Together these results indicate that protein synthesis within the BLA is not necessary for maintaining a representation of revalued USs in rats that have been overtrained.

## Experiment 4

Experiment 3 revealed that protein synthesis within the BLA is not necessary for maintaining revalued US representations after inflation. However, like Experiment 1, these memories may have been resilient to manipulations of the amygdala as a consequence of the overtraining procedure as Nader and colleagues have suggested (Wang et al., 2005). Experiment 4 examined the consequence of protein synthesis inhibition in the BLA on the revaluation of fear memories acquired after limited training.

*Methods*

*Subjects and design.* Prior to inflation 80 rats were divided into two equal groups: one that received exposure to US inflation (INF) and a group that did not undergo US inflation (NoINF). Immediately following the inflation session the groups were further divided into two groups: one group that received bilateral infusions of anisomycin (ANI), a protein synthesis inhibitor, and a second group that received bilateral infusions of a vehicle control (VEH; ACSF).

*Surgery.* One week prior to training and after having been handled for 1 week the rats were anesthetized and prepared for surgery as described in Experiment 1. Bilateral cannulae targeting the BLA or CEA were implanted as described in Experiment 1 and Experiment 2.

*Conditioning, inflation, and test procedure.*  Fear conditioning was conducted

using a limited training procedure. Rats were transported from their home cages in squads

of eight and placed in the conditioning chambers (Context A).  Chamber position and

experimental group were counterbalanced for each squad.  Rats received 5 paired

presentations of a tone (10 seconds, 2kHz, 85dB) that co-terminated with a footshock

(1.0mA, 2 seconds) beginning 3 minutes after being placed in the chambers.  There was a

60 second intertrial interval (ITI) and the animals remained in the boxes 60 seconds after

the last footshock presentation. All groups received US inflation and retention testing as

described in Experiment 1.  Immediately following the inflation session intracranial

microinfusions were conducted as described in Experiment 1.  Either anisomycin

(125mg/mL dissolved in ACSF, pH 7.4; Sigma) or ACSF (same volume and rate) was

infused bilaterally into the BLA (0.25μl/side; 0.1 μl/min).  One minute was allowed for

diffusion of the drug into the target structure before the injectors were removed.  Dummy

cannulae were inserted into the guide cannulae once the injectors were removed and the

rats were transported back to their home cages.

*Histology.* Histology was conducted as described in Experiment 1.

**Results and Discussion**

*Histology.*  Four rats were excluded from analyses because the cannulae

placements missed the BLA (Figure 3.9).  This yielded the following groups designated

by drug type (anisomycin in the BLA, BLA; anisomycin in the CEA, CEA; vehicle

(collapsed across BLA and CEA), VEH) after inflation and inflation condition (inflation,

INF; no inflation, NoINF): BLA-INF ($n = 12$), BLA-NoINF ($n = 10$), CEA-INF ($n = 12$), CEA-NoINF ($n = 11$), VEH-INF ($n = 15$), and VEH-NoINF ($n = 16$).

*Behavior.* All groups displayed similar levels of freezing during training. This observation was confirmed by an ANOVA of the average freezing during the conditioning session that revealed a significant main effect of time [$F_{(5, 135)} = 26.0$; $p < 0.0001$] (Figure 3.10a).

Rats receiving inflation shocks displayed increased freezing relative to the no-inflation controls ($p < 0.0001$ for comparison between INF and NoINF and $p = 0.10$ for comparison between BLA and CEA; $p = 0.32$ for comparison between BLA and VEH; $p = 0.57$ for comparison between CEA and VEH). These observations were confirmed by a two-way ANOVA on average freezing during the inflation session that revealed a significant main effect of inflation condition [$F_{(1, 27)} = 39.2$; $p < 0.0001$] (Figure 3.10b).

Conditioned freezing during the context is displayed in Figure 3.10c. Rats in the VEH-INF, BLA-INF, and CEA-INF groups exhibited more freezing than the non-inflated controls ($p < 0.02$ for all comparisons except CEA-INF and VEH-NoINF ($p = 0.80$)). This observation was confirmed by a two-way ANOVA that revealed a significant main effect of inflation condition [$F_{(1, 27)} = 34.5$; $p < 0.0001$] and a significant main effect of drug type [$F_{(1, 27)} = 5.8$; $p = 0.008$], however there was no significant interaction between the inflation condition and drug type [$F_{(2, 27)} = 1.8$; $p = 0.18$]. Anisomycin infusions into the CEA following inflation procedures reduced the level of conditioned freezing to the tone as compared to the BLA-INF and VEH-NoINF groups ($p < 0.002$ for all comparisons). However the CEA-INF group still displayed significantly higher levels of freezing when compared to the CEA-NoINF group ($p < 0.005$), thus indicating that

anisomycin in the CEA did not prevent the representation of a revalued US from being maintained.

Similar results were found during the tone test (Figure 3.10d). A two-way ANOVA revealed a significant main effect of inflation condition [$F_{(1, 60)}$ = 17.101; p = 0.0001] and a significant main effect of drug type [$F_{(2, 60)}$ = 9.618; p = 0.0002], however there was no significant interaction between the inflation condition and drug type [$F_{(2, 60)}$ = 1.669; p = 0.1971]. These data indicate that rats in both drug conditions exhibited inflation when compared to the no-inflation controls (p < 0.0001 for comparison between INF and NoINF), however the BLA-NoINF group displayed high levels of freezing that was not significantly different from any of the inflation groups. Even so, the BLA-INF group displayed elevated levels of freezing comparable to the VEH-INF and CEA-INF groups, and was significantly higher than either the CEA-NoINF and VEH-NoINF groups. Together these results indicate that protein synthesis within the amygdala is not necessary for maintaining a representation of revalued USs after either overtraining or limited training.

**General Discussion**

The present experiments used temporary pharmacological inactivation or protein synthesis inhibition within the BLA during a post-conditioning manipulation of US value (an inflation procedure) to assess the contribution of the BLA to US inflation. We found that inactivation did not impair the inflation of conditioned freezing (Experiments 1 and 2). This suggests that BLA neurotransmission is not necessary for coding changes in US value. In addition, inhibition of protein synthesis in the BLA after the inflation session

did not prevent inflation of either overtrained or limited trained fear memories (Experiment 3 and 4). Theses results reveal that protein synthesis-dependent mechanisms within the BLA are not necessary for consolidating or maintaining representations of revalued USs.

The present findings are in agreement with results from previous studies in our lab that found that rats with BLA lesions exhibit normal inflation (Chapter II). These data contradict Fanselow and Gale (2003) in which they reported that disrupting BLA neurotransmission during the inflation session blocked enhancements in conditioned freezing. The exact reasons why these results are contradictory are not clear, however it may be due to potential differences within the experimental protocol.

Previous studies in appetitive paradigms indicate that the BLA is essential for US revaluation (Balleine et al., 2003; Blundell et al., 2001; Everitt et al., 2003; Hatfield et al., 1996; Holland, 2004; Holland & Gallagher, 2004; Killcross et al., 1997; Pickens et al., 2003). There are many differences between appetitive and aversive conditioning that could account for these differences in whether the BLA is involved in US revaluation, which also hold true for the current study. Devaluation procedures in appetitive conditioning typically rely upon an instrumental component. In food devaluation the animal voluntarily approaches a food reward and consumes it. The food US is then devalued by either following consumption with an injection of LiCl or allowing the animal to consume more food pellets until it is sated. Thus, on subsequent presentations of the food approach towards and consummation of the food pellet is decreased. Perhaps BLA dysfunction only impairs "instrumental" devaluation. Another difference includes the direction in with the US is revalued. In appetitive studies the US experiences a

decrease in value, whereas in our experiments the US experiences an increase in value. Studies of attention have revealed differential involvement of neural structures that depends on whether there are increases or decreases in attention (Baxter et al., 1999; Holland & Gallagher, 1993). For example, the CEA mediates increasing attentional performance when there is a shift from a predictive relationship between stimuli to a surprising relationship, but not when the predictive relationship is consistent. Rats with CEA lesions do not show increased attentional performance and in fact display deficits in learning (Holland & Gallagher, 1999). The hippocampus also mediates decrements and not increases in attention (Baxter et al., 1999; Holland & Gallagher, 1993) and like the CEA and hippocampus in attention, the BLA may be differentially involved in US revaluation depending on the direction the US is revalued. Human neuroimaging studies have shown that responding in the amygdala is altered after US devaluation in an appetitive olfactory conditioning paradigm, whereas amygdala responses are unaltered after US inflation in an aversive olfactory conditioning paradigm (Gottfried & Dolan, 2004; Gottfried et al., 2003). These authors provide similar explanations for the differential amygdala involvement in US revaluation as we mention above (Gottfried & Dolan, 2004).

Together, these experiments provide important information regarding the neural substrates involved in updating and maintaining the representation of aversive stimuli. Our experiments suggest that the amygdala is not involved in US revaluation of aversive memories, however the neural substrate involved in this process remains unknown. Cortical structures, such as the orbitofrontal cortex (OFC), may be involved in US revaluation. Evidence from appetitive studies suggest that the OFC plays a role in

maintaining associative representations and/or coding and updating US value (Holland & Gallagher, 2004; Pickens et al., 2005; Pickens et al., 2003).  For example, rats with OFC lesions made prior to devaluation of a food US are not sensitive to subsequent food devaluation (Pickens et al., 2005; Pickens et al., 2003).  Experiments will need to be conducted to determine whether the OFC or another cortical structure plays a role in coding US value and/or maintenance of updated aversive USs in Pavlovian fear conditioning.  Understanding the neural structures of US revaluation through manipulation of US representations is critical for developing effective treatments for anxiety disorders, including Post-Traumatic Stress Disorder (PTSD) (Davey, 1989; Hosoba et al., 2001; Unger et al., 2003; White & Davey, 1989).

Furthermore, the CEA, like the BLA, not only appears to encode S-S memories, but is also not necessary for US inflation, at least of limited trained fear memories (Experiments 2 & 4).  These experiments suggest that the BLA and CEA acquire fear associations in very similar manners, however we don't know if the mechanisms of memory storage (e.g. consolidation) observed in another S-S system (i.e. the BLA) also operates in the CEA to store fear memories.  That is, are the cellular mechanisms of memory consolidation and reconsolidation the same in the CEA as they are in the BLA?  Chapter IV investigates whether the CEA encodes fear associations into long-term memory storage by the same mechanisms as the BLA.

**Figure 3.1.** Schematic representation of the locations of included cannula placements for the infusion of NBQX (closed circles) or VEH (open circles) in the BLA for Experiment 1. Coronal brain images were adapted from (Swanson, 2004).

**Figure 3.2.** Conditioned freezing during the overtraining session, inflation session, context, and tone tests (left to right, respectively) (Experiment 1). *A,* Mean percentage of freezing (± SEM) during the 75-minute overtraining session (15-trial blocks). *B,* Mean percentage of freezing (± SEM) during the 5-trial inflation session. For both line graphs groups are denoted as follows: NBQX-INF group (open circles), the VEH-INF group (open squares), the NBQX-NoINF groups (closed circles), and the VEH-NoINF (closed squares). *C,* Mean percentage of freezing (± SEM) across the 10-minute context test. *D,* Mean percentage of freezing (± SEM) during the 2-trial tone test. Data are an average of freezing during the ITI periods. In both bar graphs data are shown for INF groups (white bar) and the NoINF groups (black bar) within each drug type.
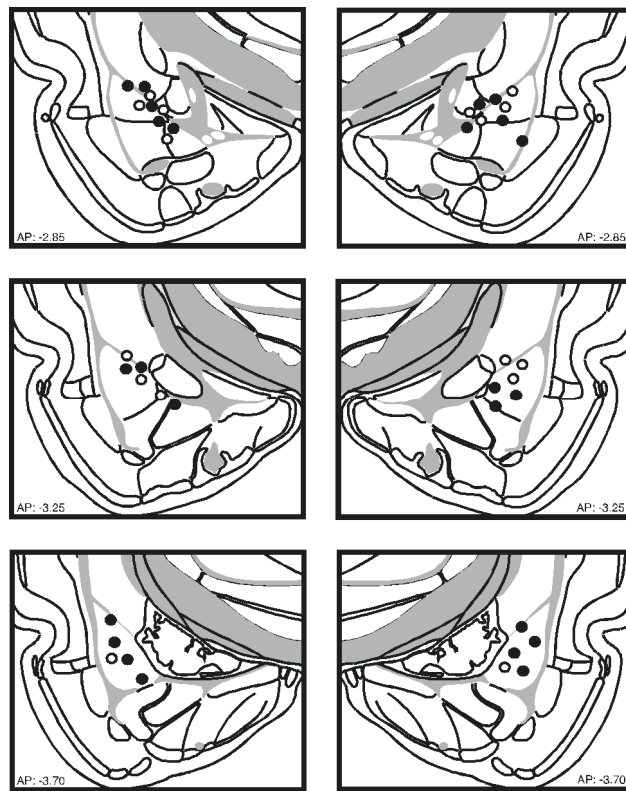
**Figure 3.3.** Schematic representation of the locations of included cannula placements for the infusion of NBQX (closed) or VEH (open) in the BLA (circles) or CEA (squares) for Experiment 2. Coronal brain images were adapted from (Swanson, 2004).

**Figure 3.4.** Conditioned freezing during the training session (Experiment 2). Mean percentage of freezing (± SEM) during the 10-trial (2-trial blocks) training session are shown for BLA-INF (open circle), CEA-INF (open square), VEH-INF (open triangle), BLA-NoINF (closed circle), CEA-NoINF (closed square), and VEH-NoINF (closed triangle).
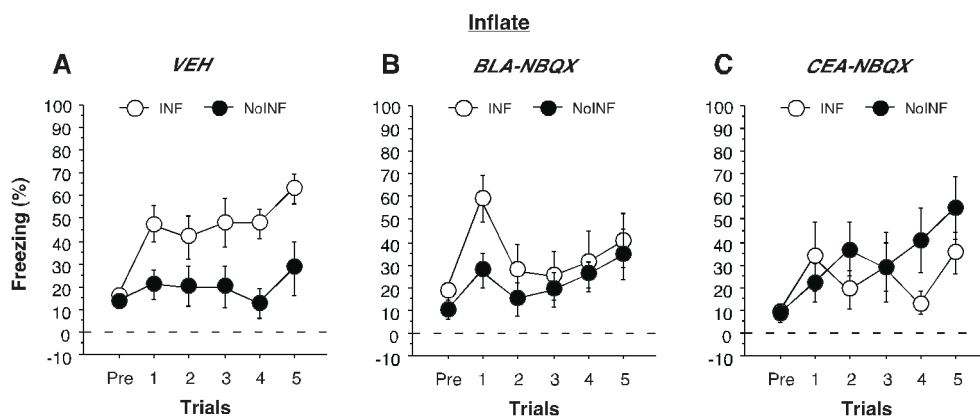
**Figure 3.5.** Conditioned freezing during the inflation session (Experiment 2). Mean percentage of freezing (± SEM) during the 5-trial inflation session for INF (open circle) and NoINF (closed circles) for *A,* rats that received vehicle infusions (collapsed across brain areas), *B,* NBQX infused into the BLA, or *C,* NBQX infused into the CEA. Data are an average of freezing during the ITI periods.
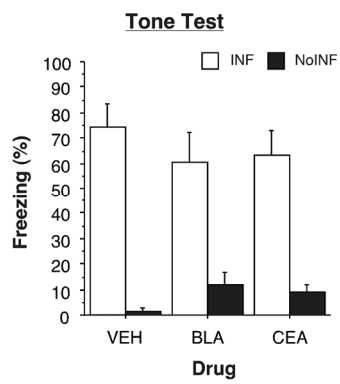
**Figure 3.6.** Conditioned freezing during the tone test (Experiment 2). Mean percentage of freezing (± SEM) during the 2-trial tone test for the INF groups (white bars) and for the NoINF groups (black bar) within each drug group.
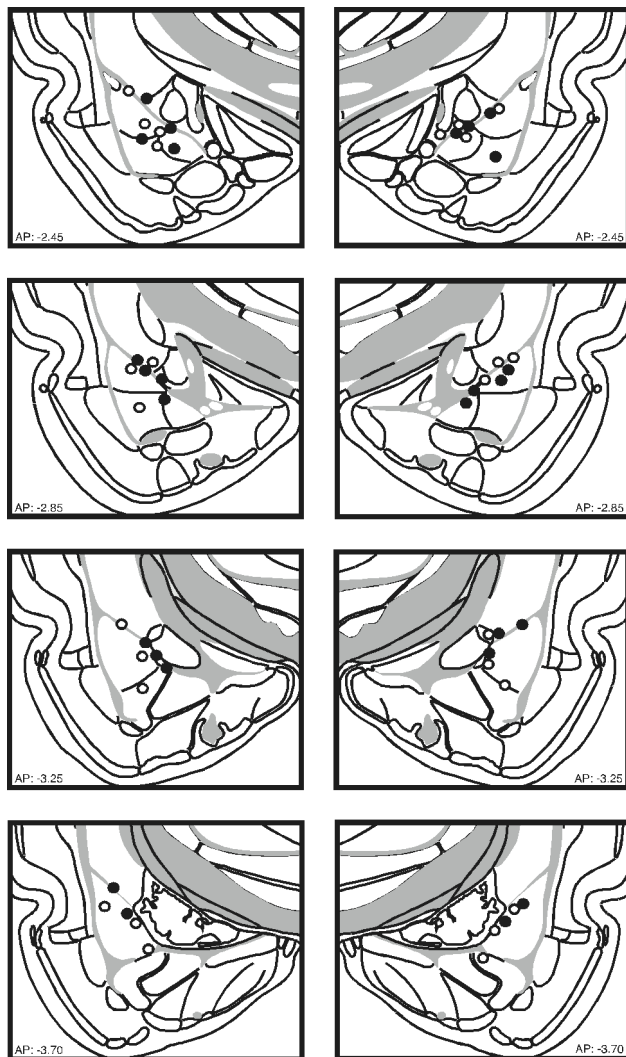
**Figure 3.7.** Schematic representation of the locations of included cannula placements for the infusion of ANI (closed circles) or VEH (open circles) in the BLA for Experiment 3. Coronal brain images were adapted from (Swanson, 2004).
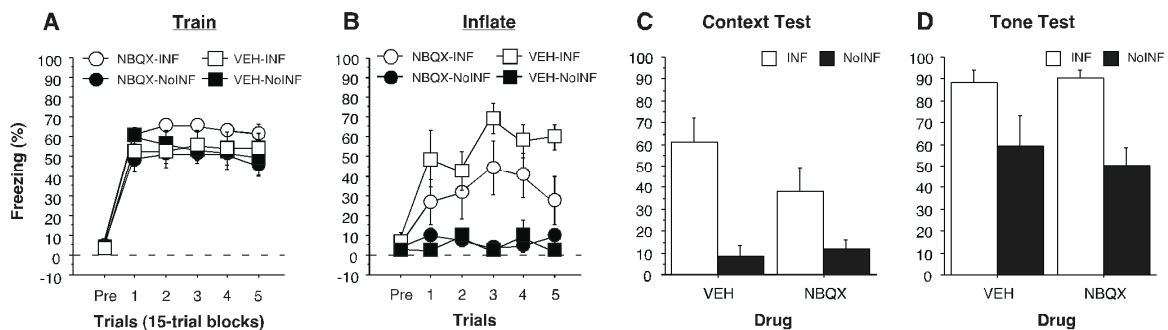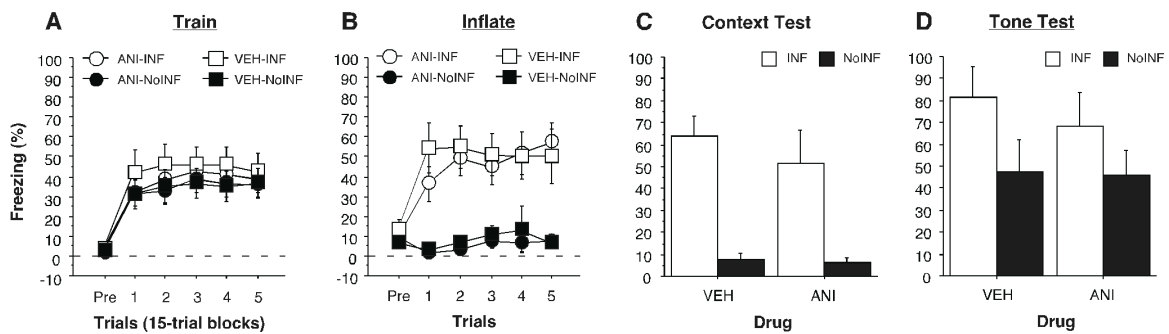
**Figure 3.8.** Conditioned freezing during the overtraining session, inflation session, context, and tone tests (left to right, respectively) (Experiment 3). *A,* Mean percentage of freezing (± SEM) during the 75-minute overtraining session (15-trial blocks). *B,* Mean percentage of freezing (± SEM) during the 5-trial inflation session. For both line graphs groups are denoted as follows: ANI-INF group (open circles), the VEH-INF group (open squares), the ANI-NoINF groups (closed circles), and the VEH-NoINF (closed squares). *C,* Mean percentage of freezing (± SEM) across the 10-minute context test. *D,* Mean percentage of freezing (± SEM) during the 2-trial tone test. Data are an average of freezing during the ITI periods. In both bar graphs data are shown for INF groups (white bar) and the NoINF groups (black bar) within each drug type.
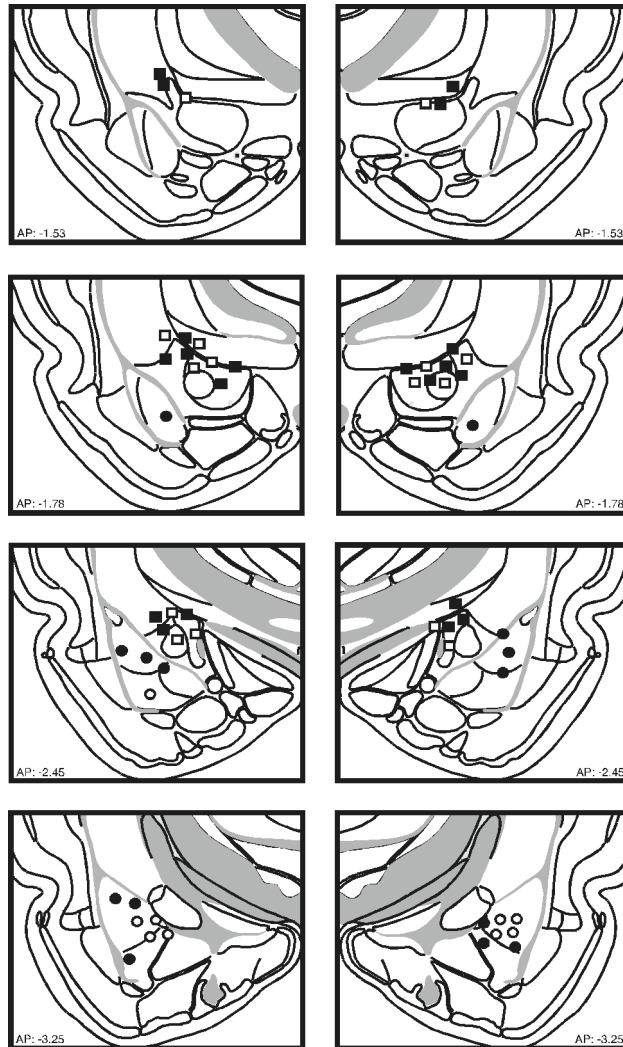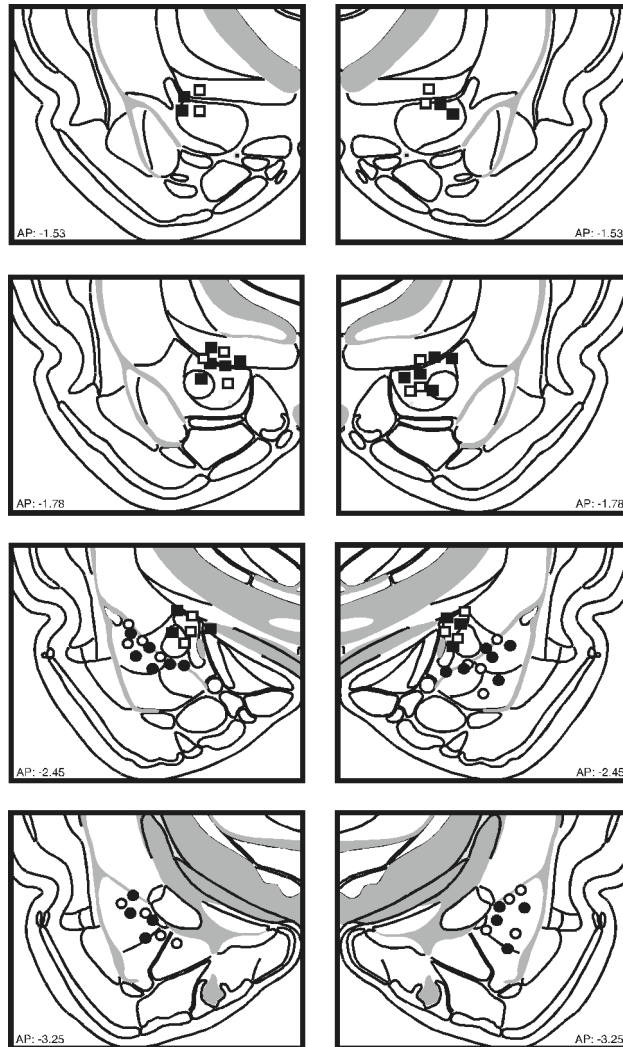
**Figure 3.9.** Schematic representation of the locations of included cannula placements for the infusion of ANI (closed) or VEH (open) in the BLA (circles) or CEA (squares) for Experiment 4. Coronal brain images were adapted from (Swanson, 2004).
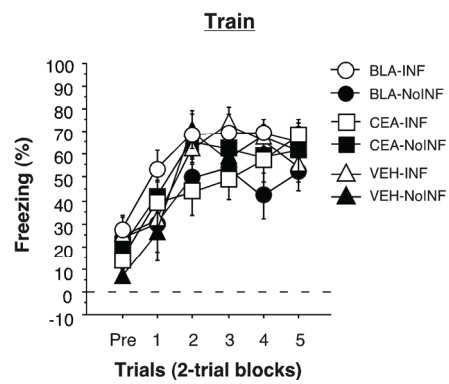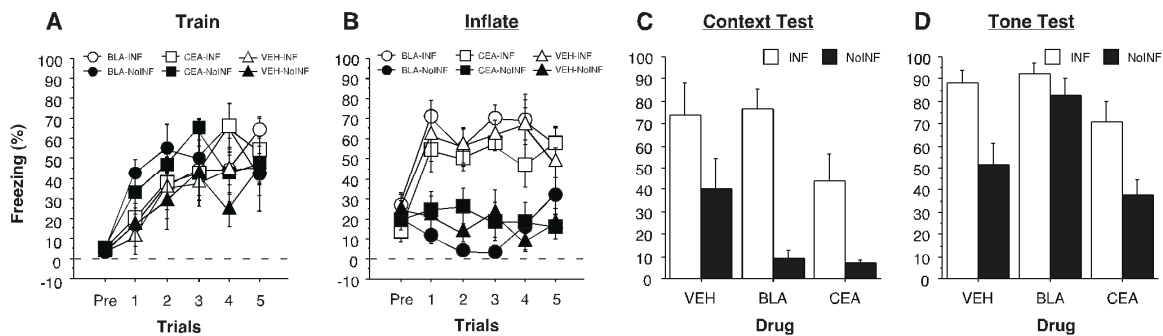
**Figure 3.10.** Conditioned freezing during the training session, inflation session, context, and tone tests (left to right, respectively) (Experiment 4). *A,* Mean percentage of freezing (± SEM) during the 5-minute overtraining session. *B,* Mean percentage of freezing (± SEM) during the 5-trial inflation session. For both line graphs groups are denoted as follows: BLA-INF group (open circles), CEA-INF (open squares), VEH-INF group (open triangles), BLA-NoINF groups (closed circles), CEA-NoINF (closed squares) and VEH-NoINF (closed triangles). *C,* Mean percentage of freezing (± SEM) across the 10-minute context test. *D,* Mean percentage of freezing (± SEM) during the 2-trial tone test. Data are an average of freezing during the ITI periods. In both bar graphs data are shown for INF groups (white bar) and the NoINF groups (black bar) within each drug type.

# References

Balleine, B. W., Killcross, A. S., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *J. Neurosci., 23*(2), 666-675.

Baxter, M. G., Bucci, D. J., Holland, P. C., & Gallagher, M. (1999). Impairments in conditioned stimulus processing and conditioned responding after combined selective removal of hippocampal and neocortical cholinergic input. *Behav Neurosci, 113*(3), 486-495.

Blundell, P., Hall, G., & Killcross, S. (2001). Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. *J. Neurosci., 21*(22), 9018-9026.

Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A modern learning theory perspective on the etiology of panic disorder. *Psychol Rev, 108*(1), 4-32.

Campeau, S., & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J. Neurosci., 15*(3), 2301-2311.

Cousens, G., & Otto, T. (1998). Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience, 112*(5), 1092-1103.

Davey, G. C. L. (1989). Ucs revaluation and conditioning models of acquired fears. *Behaviour Research and Therapy, 27*(5), 521-528.

Davis, M., & Whalen, P. J. (2001). The amygdala: Vigilance and emotion. *Mol Psychiatry, 6*(1), 13-34.

Everitt, B. J., Cardinal, R. N., Parkinson, J. A., & Robbins, T. W. (2003). Appetitive behavior: Impact of amygdala-dependent mechanisms of emotional learning. *Ann N Y Acad Sci, 985*, 233-250.

Fanselow, M. S., & Gale, G. D. (2003). The amygdala, fear, and memory. *Ann N Y Acad Sci, 985*, 125-134.

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews, 23*, 743-760.

Gale, G. D., Anagnostaras, S. G., Godsil, B. P., Mitchell, S., Nozawa, T., Sage, J. R., et al. (2004). Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. *J Neurosci, 24*(15), 3810-3815.

Gill, R., Nordholm, L., & Lodge, D. (1992). The neuroprotective actions of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline (nbqx) in a rat focal ischaemia model. *Brain Res, 580*(1-2), 35-43.

Goosens, K. A., & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn Mem, 8*(3), 148-155.

Goosens, K. A., & Maren, S. (2003). Pretraining nmda receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear. *Behav Neurosci, 117*(4), 738-750.

Gottfried, J. A., & Dolan, R. J. (2004). Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nat Neurosci, 7*(10), 1144-1152.

Gottfried, J. A., O'Doherty, J., & Dolan, R. J. (2003). Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science, 301*(5636), 1104-1107.

Grillon, C., Southwick, S. M., & Charney, D. S. (1996). The psychobiological basis of posttraumatic stress disorder. *Molecular Psychiatry, 1*(4), 278-297.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with pavlovian second-order conditioning and reinforcer devaluation effects. *Journal of Neuroscience, 16*(16), 5256-5265.

Helmstetter, F. J. (1992). The amygdala is essential for the expression of conditional hypoalgesia. *Behavioral Neuroscience, 106*(3), 518-528.

Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience, 108*(5), 1005-1009.

Holland, P. C. (2004). Relations between pavlovian-instrumental transfer and reinforcer devaluation. *Journal of Experimental Psychology:  Animal Behavior Processes, 30*, 104-117.

Holland, P. C., & Gallagher, M. (1993). Amygdala central nucleus lesions disrupt increments, but not decrements, in conditioned stimulus processing. *Behav Neurosci, 107*(2), 246-253.

Holland, P. C., & Gallagher, M. (1999). Amygdala circuitry in attentional and representational processes. *Trends in Cognitive Sciences, 3*(2), 65-73.

Holland, P. C., & Gallagher, M. (2004). Amygdala-frontal interactions and reward expectancy. *Current Opinion in Neurobiology, 14*(2), 148-155.

Hosoba, T., Iwanaga, M., & Seiwa, H. (2001). The effect of ucs inflation and deflation procedures on `fear' conditioning. *Behaviour Research and Therapy, 39*(4), 465-475.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature (London), 388*(6640), 377-380.

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in pavlovian fear conditioning: A critical review. *Neuroscience & Biobehavioral Reviews, 30*(2), 188-202.

Koo, J. W., Han, J. S., & Kim, J. J. (2004). Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. *J Neurosci, 24*(35), 7654-7662.

LeDoux, J. (1998). Fear and the brain: Where have we been, and where are we going? *Biol Psychiatry, 44*(12), 1229-1238.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184.

Lees, G. J. (2000). Pharmacology of ampa/kainate receptor ligands and their therapeutic potential in neurological and psychiatric disorders. *Drugs, 59*(1), 33-78.

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *Journal of Neuroscience, 18*(8), 3088-3097.

Maren, S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J. Neurosci., 19*(19), 8696-8703.

Maren, S. (2001a). Is there savings for pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? *Neurobiology of Learning and Memory, 76*(3), 268-283.

Maren, S. (2001b). Neurobiology of pavlovian fear conditioning. *Annual Review of Neuroscience, 24*(1), 897-931.

Maren, S. (2005). Building and burying fear memories in the brain. *Neuroscientist, 11*(1), 89-99.

Maren, S., Aharonov, G., & Fanselow, M. S. (1996a). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: Absence of a temporal gradient. *Behavioral Neuroscience, 110*(4), 718-726.

Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996b). N-methyl-d-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience, 110*(6), 1365-1374.

Muller, J., Corodimas, K. P., Fridel, Z., & LeDoux, J. E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behavioral Neuroscience, 111*(4), 683-691.

Nader, K., Majidishad, P., Amorapanth, P., & LeDoux, J. E. (2001). Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. *Learn Mem, 8*(3), 156-163.

Pickens, C. L., Saddoris, M. P., Gallagher, M., & Holland, P. C. (2005). Orbitofrontal lesions impair use of cue-outcome associations in a devaluation task. *Behav Neurosci, 119*(1), 317-322.

Pickens, C. L., Saddoris, M. P., Setlow, B., Gallagher, M., Holland, P. C., & Schoenbaum, G. (2003). Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. *J. Neurosci., 23*(35), 11078-11084.

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of pavlovian fear conditioning: A cellular and molecular perspective. *Trends in Neurosciences, 24*(9), 540-546.

Swanson, L. W. (2004). *Brain maps: Structure of the rat brain* (Third ed.). San Diego: Academic Press.

Unger, W., Evans, I. M., Rourke, P., & Levis, D. J. (2003). The s-s construct of expectancy versus the s-r construct of fear: Which motivates the acquisition of avoidance behavior? *J Gen Psychol, 130*(2), 131-147.

Walker, D. L., & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J. Neurosci., 17*(23), 9375-9383.

Wang, S., Marin, M., & Nader, K. (2005). *Memory strength as a transient boundary condtion of reconsolidation of auditory fear memories and its molecular correlates.* Paper presented at the Society for Neuroscience, Washington, D.C.

White, K., & Davey, G. C. L. (1989). Sensory preconditioning and ucs inflation in human `fear' conditioning. *Behaviour Research and Therapy, 27*(2), 161-166.

Wilensky, A. E., Schafe, G. E., Kristensen, M. P., & LeDoux, J. E. (2006). Rethinking the fear circuit: The central nucleus of the amygdala is required for the acquisition, consolidation, and expression of pavlovian fear conditioning. *J. Neurosci., 26*(48), 12387-12396.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (1999). Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. *J. Neurosci., 19*(24), 48RC-.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (2000). Functional inactivation of amygdala nuclei during acquisition of pavlovian fear conditioning. *Society for Neuroscience Abstracts, 30*, 465.469.

Zimmerman, J. M., Rabinak, C. A., McLachlan, I. G., & Maren, S. (2007). The central nucleus of the amygdala is essential for acquiring and expressing conditional fear after overtraining. *Learn Mem, 14*(9), 634-644.

**CHAPTER IV**


**PROTEIN SYNTHESIS WITHIN THE CENTRAL NUCLEUS OF THE AMYGDALA IS NECESSARY FOR CONSOLIDATION AND RECONSOLIDATION OF PAVLOVIAN FEAR MEMORIES IN RATS**


Many anxiety disorders are triggered by aversive or traumatic experiences. Pavlovian fear conditioning is a behavioral model used to investigate the neurobiology underlying the development and maintenance of fear learning and memory (Bouton et al., 2001; Grillon et al., 1996; Kim & Jung, 2006; LeDoux, 1998; LeDoux, 2000; Maren, 2001b; Maren, 2005). In this paradigm an innocuous conditioned stimulus (CS), such as a tone, is paired with an aversive unconditioned stimulus (US), such as a footshock. After one or more pairings the rat learns that the CS predicts the US, and CS presentations alone elicit a conditioned fear response (CR), which includes increases in heart rate, arterial blood pressure, hypoalgesia, potentiated acoustic startle, stress hormone release, and freezing (somatomotor immobility).

The amygdala is involved in the formation, consolidation, and retrieval of fear memories. Within the amygdala there are two sub-regions that contribute to fear learning and expression of learned fear responses. The basolateral complex of the amygdala (BLA) and the central nucleus of the amygdala (CEA) of which both are necessary for the acquisition, expression, and consolidation of fear memories

(Campeau & Davis, 1995; Cousens & Otto, 1998; Davis & Whalen, 2001; Fanselow & Gale, 2003; Fendt & Fanselow, 1999; Gale et al., 2004; Goosens & Maren, 2001, 2003; Helmstetter, 1992; Helmstetter & Bellgowan, 1994; Killcross et al., 1997; Koo et al., 2004; LeDoux, 1998; LeDoux, 2000; Maren, 1998; Maren, 1999, 2001a, 2001b; Maren et al., 1996a; Maren et al., 1996b; Muller et al., 1997; Nader et al., 2001; Schafe et al., 2001; Walker & Davis, 1997; Wilensky et al., 2006; Wilensky et al., 1999; Wilensky et al., 2000; Zimmerman et al., 2007).

Considerable evidence indicates that within the BLA inhibiting protein synthesis in the BLA immediately after training eliminates the consolidation of fear memory (Bailey et al., 1999; Goosens et al., 2000; Lin et al., 2001; Maren et al., 2003; Nader et al., 2000a; Schafe et al., 2000; Schafe & LeDoux, 2000; Wei et al., 2002). Once a memory is consolidated it is stable and long lasting. However, retrieval of memory appears to return it to an active state. Inhibition of protein synthesis after memory retrieval produces profound impairments in the retention of the fear memory (Alberini, 2005; Duvarci & Nader, 2004; Fanselow & Gale, 2003; Nader et al., 2000b).

Although studies of the cellular basis of fear conditioning have focused on the BLA, several recent studies suggest the CEA may have a role in the acquisition of long-term fear memories (Pare et al., 2004; Samson & Pare, 2005; Wilensky et al., 2006; Zimmerman et al., 2007). For example, rats with BLA lesions can still acquire fear if given overtraining and we have found that CEA lesions block acquisition and expression of fear memory even after overtraining. Our data also indicate that temporary inactivation of the CEA prevents both acquisition and expression of overtrained fear memories (Gale et al., 2004; Maren, 1998; Maren, 1999; Zimmerman et al., 2007). In

addition, Wilensky and colleagues have recently shown that temporary inactivation of the CEA produced impairments in acquisition of fear responses (Wilensky et al., 2006). Like the BLA, protein synthesis within the CEA is also involved in consolidation of fear memories (Wilensky et al., 2006). Furthermore, in Chapter II we found that the CEA appears to encode S-S memories like the BLA, however we don't know if the mechanisms of memory consolidation observed in another S-S system (i.e. the BLA) also operates in the CEA to store fear memories. That is, are the cellular mechanisms of memory consolidation and reconsolidation the same in the CEA as they are in the BLA? The present experiments were designed to answer this question about the role of the CEA in learning.

## General Methods

*Subjects*

The subjects were 123 adult male Long-Evans rats (60-90 days old; 200-224 grams; Blue Spruce) obtained from a commercial supplier (Harlan Sprague-Dawley, Indianapolis, IN). Upon arrival all rats were individually housed in conventional Plexiglas hanging cages and kept on a 14 hr light/10 hr dark cycle (lights on at 7:00am) with free access to food and tap water. To acclimate the rats to the experimenter they were handled daily (10-15 sec per rat) for 5 days following their arrival. All experimental procedures were conducted in accordance with the approved guidelines as stated by the University of Michigan Committee on Use and Care of Animals (UCUCA).

*Behavioral Apparatus*

All sessions were conducted in eight identical rodent conditioning chambers (30 x 24 x 21 cm; MED Associates, St. Albans, VT). The chambers were positioned inside sound-attenuating cabinets located in an isolated room. Each chamber was constructed of aluminum (2 side walls) and Plexiglas (rear wall, ceiling, and hinged front door); the floor consisted of 19 stainless-steel rods, (4 mm diameter) spaced apart 1.5 cm (center to center). The grid floor was connected to a shock source and solid-state grid scrambler (MED Associates), which delivered the footshock US. Mounted on one wall of the chamber was a speaker to provide a distinct auditory CS and on the opposite wall was a 15-W house light; a fan provided background noise (65dB).

Three distinct contexts were created by manipulating multiple visual, olfactory and tactile cues: 1) Context A: 1% acetic acid odor in the chamber, houselights and room lights on, fans on in the cabinets, cabinet doors open, and grid floors; 2) Context B: 1% ammonium hydroxide odor in the chamber, red lights on in the room, houselights off, fans off in the cabinets, cabinet doors closed, and Plexiglas floors; 3) Context C: 70% ethanol odor in the chamber, house lights on, room lights off, fans off in the cabinets, cabinet doors open, and grid floors.

Each chamber rested on a load-cell platform, which was used to record chamber displacement in response to each rat's motor activity. The output from each load-cell was amplified to a level previously established to detect freezing responses. For each chamber, the load-cell amplifier output was digitized at 5 Hz (300 observations per minutes per rat) and acquired online using Threshold Activity software (MED Associates). Locomotor activity was quantified by the raw load cell values (range = 0 - 100) and freezing behavior was quantified by calculating the number of load cell values

below the freezing threshold (threshold = 10).  However, to prevent the inclusion of momentary bouts of inactivity as freezing, (i.e., < 1 sec) freezing was only scored after five or more contiguous observations below the freezing threshold (for details see (Maren, 1998; Maren, 1999, 2001a).  Freezing observations during each session were transformed into a percentage of total observations.

*Data Analysis*

Freezing data were converted to a percentage of total observations, which is a probability estimate that is amenable to analysis with parametric statistics.  These values were analyzed using analysis of variance (ANOVA) and post hoc comparisons using Fishers LSD tests were performed after a significant overall $F$ ratio was obtained.  All data are represented as means ± SEMs.

**Experiment 1**

Wilensky and colleagues have recently shown that inhibition of protein synthesis within the CEA blocks consolidation of Pavlovian fear memories (Wilensky et al., 2006). The purpose of Experiment 1 was to confirm the consolidation impairments seen by Wilensky and colleagues (2006) when anisomycin is infused into the CEA.  However, we used a lower dose of anisomycin infused into the CEA to minimize the spread of the drug to structures outside of the target site (Wanisch & Wotjak, 2008).

*Method*

*Subjects and design.*  Prior to conditioning, 24 rats were divided into three equal groups. One group received bilateral infusions of a vehicle control (VEH; ACSF; Sigma). The other two groups second group received bilateral infusions of anisomycin (12.0 μg;

48 μg/μL); Sigma, St. Louis, MO) infused into the either the CEA or the caudate

putamen (CPu; as a control site).

    *Surgery.* One week prior to training and after having been handled for 1 week

each rat was anesthetized with an intraperitoneal (i.p.) injection of a Nembutal (sodium

pentobarbital; 65 mg/kg body weight) and atropine methyl nitrate (0.4 mg/kg body

weight) cocktail. Ocular lubricant was used to moisten the eyes. The scalp was shaved,

cleaned with antiseptic (Betadine) and the rat was mounted in a stereotaxic apparatus

(David Kopf Instruments, Tujunga, CA). After the scalp was incised and retracted, the

skull was positioned so that bregma and lambda were in the same horizontal plane. Small

burr holes were drilled bilaterally in the skull to allow for the placement of 26-gauge

guide cannulae (Plastics One, Roanoke, VA) in the CEA (2.0 mm posterior to bregma,

4.0 mm lateral to the midline, and 5.7 mm ventral to the brain surface) or the CPu (2.3

mm posterior to bregma, 5.0 mm lateral to the midline, and 3.7 mm ventral to the brain

surface), along with holes for 3 small jeweler's screws. Dental acrylic was applied to the

cannulae, screws, and skull surface to hold the guide cannulae in place. After surgery,

33-gauge dummy cannulae (16 mm; Plastics One) were inserted into the guide cannulae

and the rats were kept on a heating pad until they recovered from anesthesia before

returning to their home cages. Dummy cannulae were replaced daily during the week of

recovery.

    *Conditioning and test procedure.* Fear conditioning was conducted using limited

training procedure. Rats were transported from their home cages in squads of eight and

placed in the conditioning chambers (Context A). Chamber position and experimental

group were counterbalanced for each squad. Rats received 1 paired presentation of a tone

(30 seconds, 2kHz, 85dB) that co-terminated with a footshock (1.5mA, 2 seconds) beginning 3 minutes after being placed in the chambers. The animals remained in the boxes 60 seconds after the last footshock presentation. Immediately following conditioning, the rats were transported to the infusion room in white 5-gallon buckets. Hamilton syringes (10 μL; Harvard Apparatus, South Natick, MA) were mounted in two infusion pumps (10 syringes/pump; Harvard Apparatus) and connected to 33-gauge internal cannula (1.0 mm longer than the implanted guide cannulae) with polyethylene tubing (A-M Systems). Dummy cannulae were removed from each rat and internal cannulae were inserted into each guide cannula. Either anisomycin, (48 μg/μl; dissolved in ACSF, pH 7.4) or ACSF (same volume and rate) was infused bilaterally into the CEA (0.25 μl/side; 0.1 μl/min). In addition, anisomycin (48 μg/μl; same volume and rate) was infused bilaterally into the CPu as a control. One minute was allowed for diffusion of the drug into the target structure before the injectors were removed. Dummy cannulae were inserted into the guide cannulae once the injectors were removed and the rats were taken back to their home cages. Twenty-four hours after conditioning, fear to the tone was tested by placing the rats into a novel context (Context B) and presenting 3 tone alone presentations (30 seconds, 2kHz, 85dB, 60 sec ITI) beginning 3 minutes after being placed into the chambers. Freezing behavior was measured throughout all experimental sessions.

*Histology.* After behavioral testing, rats were euthanized with an overdose of sodium pentobarbital (i.p. 100 mg/kg) and were transcardially perfused with physiological saline followed by 10 % formalin. Brains were removed and post-fixed in 10% formalin followed by 10% formalin/30% sucrose solution until sectioning. Coronal

brain sections (45 µm) were cut on a cryostat and wet-mounted with 70% ethanol on glass microscope slides.  Once dry, the sections were stained with 0.25% thionin to visualize neuronal cell bodies and identify lesion sites.


### Results and Discussion

*Histology.*  Two rats were excluded from the analyses because their cannulae placements missed the CEA or CPu (Figure 4.1).  This yielded the following groups designated by drug type following conditioning: vehicle (VEH; $n = 7$); anisomycin infused into the CPu (CPu; $n = 8$); and anisomycin infused into the CEA (CEA; $n = 7$).

*Behavior.*  An ANOVA of the average post-shock freezing during the conditioning session revealed a significant main effect of time [$F_{(1,19)} = 8317.1$; $p < 0.0001$] (Figure 4.2a), but no main effect of drug group.  These results indicated that all groups displayed similar levels of freezing during overtraining.

Conditioned freezing during the tone test is displayed in Figure 4.2b.  Rats in both the VEH and CPu groups exhibited more freezing than the CEA group.  These observations were confirmed by a two-way ANOVA that revealed a significant main effect of drug group [$F_{(2,19)} = 9692.7$; $p < 0.02$].  Indeed planned comparisons revealed no significant different between the VEH and CPu groups and significantly lower levels of freezing displayed by the CEA group when compared to either the VEH [$t_{(54)} = -3.4$; $p < 0.001$] or CPu [$t_{(58)} = -5.0$; $p < 0.0001$] groups.  These data indicate that protein synthesis inhibition within the CEA following fear conditioning blocked consolidation of fear memory.

**Experiment 2**

Experiment 1 indicated a necessary role of protein synthesis within the CEA for consolidation of fear memories in intact rats.  Rats with BLA lesions can still acquire fear if given overtraining and we have found that CEA lesions block acquisition and expression of fear memory even after overtraining.  These data also indicate that temporary inactivation of the CEA prevents both acquisition and expression of overtrained fear memories (Gale et al., 2004; Maren, 1998; Maren, 1999; Zimmerman et al., 2007).  Therefore, the purpose of Experiment 2 was to determine if protein synthesis within the CEA is also required for consolidation of fear memories acquired in rats with BLA lesions.

*Method*

*Subjects and design.* Prior to training 24 rats were divided into two groups: one group that received bilateral neurotoxic lesions of the BLA and second group that underwent sham surgery (SHAM).  Then rats with BLA lesions were further divided into two equal groups: One of the groups received bilateral infusions of anisomycin into the CEA (12.0 μg; 48 μg/μL) and the other group received bilateral infusions of a vehicle control (VEH; ACSF) into the CEA.  All rats in the SHAM group received bilateral infusions of a vehicle control (VEH) into the CEA.

*Surgery.*  One week prior to training and after having been handled for 1 week the rats were anesthetized and prepared for surgery as described in Experiment 1.  After the scalp was incised and retraced, the skull was positioned so that bregma and lambda were in the same horizontal plane.  Small burr holes were drilled bilaterally in the skull to allow placement of 28-gauge injectors in the BLA (3.3 mm posterior to bregma and 5.0

mm lateral to the midline).  Injectors were attached to polyethylene tubing and connected

to 10 µL syringes mounted to an infusion pump (Harvard Apparatus).  NMDA (20

mg/mL dissolved in 100 mM PBS, pH 7.4; Sigma) was infused (0.1 µL/min) at two sites

for each BLA lesion: 8.0 mm ventral to the brain surface (0.2 µL) and 7.5 mm ventral to

the brain surface (0.1 µL).  Five minutes were allowed for diffusion of the drug into the

target structure before the injectors were removed.  SHAM rats received a similar surgery

except that the injectors were not lowered.  Bilateral cannulae targeting the CEA were

implanted in all rats as described in Experiment 1.

*Conditioning and test procedure.*  Fear conditioning was conducted using an

overtraining procedure.  Prior to overtraining intracranial microinfusions were conducted

as described in Experiment 1. Either anisomycin (48 µg/µL; dissolved in ACSF, pH 7.4)

or ACSF (same volume and rate) was infused bilaterally into the CEA (0.25 µL/side; 0.1

µL/min) of rats with BLA lesions.  SHAM rats all received bilaterally infusions of VEH

into the CEA (same volume and rate).  One minute was allowed for diffusion of the drug

into the target structure before the injectors were removed.  Dummy cannulae were

inserted into the guide cannulae once the injectors were removed and the rats were

immediately transported to the conditioning chambers (Context A).  Chamber position

and experimental group were counterbalanced for each squad.  Rats received 75 paired

presentations of a tone (10 seconds, 2kHz, 85dB) that co-terminated with a footshock

(1.0mA, 2 seconds) beginning 3 minutes after being placed in the chambers.  There was a

60 second ITI and the animals remained in the boxes 60 seconds after the last footshock

presentation.  Twenty-four hours after conditioning, all rats were placed back into

Context A for 10 minutes to assess contextual fear.  Forty-eight hours after conditioning,

fear to the tone was tested by placing the rats into a novel context (Context B) and presenting 30 tone alone presentations (10 seconds, 2kHz, 85dB, 60 second ITI) 3 minutes after placement into the chambers. Freezing behavior was measured throughout all experimental sessions.

   *Histology.* Histology was conducted as described in Experiment 1.

### *Results and Discussion*

   *Histology.* One rat was excluded from the analyses because his cannulae placements missed the CEA (Figure 4.3). This yielded the following groups designated by lesion type and drug type during overtraining: rats with sham lesions and received vehicle infusions (SHAM; $n = 8$); rats with BLA lesions and received vehicle infusions (VEH; $n = 7$) and received anisomycin infusions (12 μg; $n = 8$). Successful lesions were generally confined to the targeted structure, although some rats in the BLA group had damage to the rostral endopiriform nucleus and caudate putamen (Figure 4.3). NMDA infusions into the BLA spared the CEA.

   *Behavior.* A two-way ANOVA of the average post-shock freezing during the overtraining session revealed a significant main effect of time (15-trial blocks) [$F_{(5,105)} = 99.6$; $p < 0.0001$], but no significant main effect of lesion type (Figure 4.4a). These results indicated that both groups displayed similar levels of freezing during overtraining.

   Conditioned freezing during the context test is displayed in Figure 4.4b. An ANOVA revealed a significant main effect of group [$F_{(2,20)} = 4.5$; $p < 0.03$]. The SHAM group displayed significantly higher levels of freezing when compared to the 12 μg anisomycin group ($p < 0.008$), while the SHAM group was not significantly different

from the VEH group (p = 0.26).  These data indicate that protein synthesis inhibition

within the CEA of rats with BLA lesions disrupts consolidation of fear memories.

Similar results were found during the tone test (Figure 4.4c).  An ANOVA

revealed a significant main effect of group [$F_{(2,20)}$ = 14.3; p < 0.0001].  Both vehicle

groups (SHAM and VEH) displayed significantly higher levels of freezing when

compared to the 12 µg anisomycin group (p < 0.01 for all comparisons).  Like the context

test, these data suggest that anisomycin within the CEA of rats with BLA lesions during

overtraining blocks consolidation of fear memories.

**Experiment 3**

Experiment 1 confirmed the findings of Wilensky and colleagues (2006) that, like

the BLA, protein synthesis within the CEA is involved in the consolidation of fear

memories.  Previous studies have shown that inhibition of protein synthesis within the

BLA after memory retrieval produces profound impairments in the retention of the fear

memory (Alberini, 2005; Duvarci & Nader, 2004; Fanselow & Gale, 2003; Nader et al.,

2000b), however it is unknown if the CEA plays a role in reconsolidation.  Therefore, the

purpose of Experiment 3 was to determine whether protein synthesis within the CEA is

necessary for reconsolidation of fear memories.

*Method*

*Subjects and design.*  Prior to CS reactivation, 75 rats were divided into five

groups.  Three of the groups each received bilateral infusions of anisomycin (31.25 µg;

125 µg/µl) into either the CEA, BLA, or CPu.  The other two groups received bilateral

135

infusions of a vehicle control into either the CEA or BLA (brain areas collapsed into one group; VEH; ACSF).

Surgery.  One week prior to training and after having been handled for 1 week the rats were anesthetized and prepared for surgery as described in Experiment 1.  Bilateral cannulae targeting the CEA, CPu, or BLA (3.3 mm posterior to bregma, 5.0 mm lateral to the midline, and 6.5 mm ventral to the brain surface) were implanted also as described in Experiment 1.

Conditioning, CS reactivation, and test procedure.  Fear conditioning was conducted using a limited training procedure.  Rats were transported from their home cages in squads of eight and placed in the conditioning chambers (Context A).  Chamber position and experimental group were counterbalanced for each squad.  Rats received 3 paired presentations of a tone (30 seconds, 2kHz, 85dB) that co-terminated with a footshock (1.0 mA, 1 second) beginning 3 minutes after being placed in the chambers.  There was a 60 second intertrial interval (ITI) and the animals remained in the boxes 60 seconds after the last footshock presentation.  Some of the rats received 1 paired presentation of a tone (30 seconds, 2kHz, 85dB) that co-terminated with a footshock (1.5mA, 2 seconds) beginning 3 minutes after being placed in the chambers and remained in the boxes 60 seconds after the last footshock presentation.  Twenty-four hours after conditioning, all rats were placed were placed in another, novel environment (Context C) for CS reactivation.  The reactivation session consisted of 1 tone alone presentation (30 seconds, 2kHz, 85dB) beginning 3 minutes after being placed in the chambers.  Immediately following CS reactivation intracranial microinfusions were conducted as described in Experiment 1.  Either anisomycin, (125 µg/µL dissolved in ACSF, pH 7.4)

or ACSF (same volume and rate) was infused bilaterally into the CEA, BLA, or CPu

(0.25 μL/side; 0.1 μL/min). One minute was allowed for diffusion of the drug into the

target structure before the injectors were removed.  Dummy cannulae were inserted into

the guide cannulae once the injectors were removed and the rats were transported back to

their home cages.  Forty-eight hours after conditioning, fear to the tone was tested by

placing the rats into a third novel context (Context B) and presenting 3 tone alone

presentations (30 seconds, 2kHz, 85dB, 60 second ITI) beginning 3 minutes after being

placed into the chambers.  Anisomycin infused into the CEA produced deficits in

conditioned freezing during the first tone test (see results) therefore, we conducted a

second tone test, as described above in this group as well as the CEA vehicle group

(VEH) one week following the first tone test.  In addition, to determine whether freezing

deficits were due to inhibition of reconsolidation or anisomycin-induced damage to the

CEA or BLA, rats in the CEA, BLA, and VEH groups were re-trained to 3 paired

presentations of a tone co-terminated with a footshock, as described above.  Twenty-four

hours after re-training, another tone test was conducted, as described above.  Freezing

behavior was measured throughout all experimental sessions.

   *Histology.* Histology was conducted as described in Experiment 1.


### Results and Discussion

   *Histology.*  Twenty-three rats were excluded from the analyses because their

cannulae placements missed the CEA, BLA, or CPu (Figure 4.5).  This yielded the

following groups designated by drug type following CS reactivation: vehicle infusions

into the BLA or CEA (collapsed across brain areas; VEH; $n = 19$); anisomycin infused

into the CPu (CPu; $n = 7$); anisomycin infused into the CEA (CEA; $n = 19$); and anisomycin infused into the BLA (BLA; $n = 7$).

*Behavior.* An ANOVA of the average post-shock freezing during the conditioning session revealed a significant main effect of time [$F_{(3,108)} = 78.4$; $p < 0.0001$], (Figure 4.6a), but no main effect of drug group. These results indicated that all groups displayed similar levels of freezing during overtraining.

Conditioned freezing during the CS reactivation session is shown in Figure 4.6b. An ANOVA of the average post-shock freezing during the CS reactivation session revealed a significant main effect of time [$F_{(1,48)} = 141.2$; $p < 0.0001$], but no main effect of drug group. These results indicated that all groups displayed similar levels of freezing during CS reactivation.

Conditioned freezing during the first tone test is displayed in Figure 4.6c. Rats in both the VEH and CPu groups exhibited more freezing than the CEA and BLA groups. These results were confirmed by an ANOVA that revealed a significant main effect of drug group [$F_{(3,48)} = 3.4$; $p < 0.03$]. The VEH group displayed significantly higher levels of freezing when compared to either the BLA ($p < 0.04$) or CEA groups ($p < 0.007$) and there was no significant difference of freezing between the BLA and CEA groups ($p = 0.98$). These data indicated that protein synthesis within the CEA is necessary for reconsolidation of fear memories.

Similar results were found for the spontaneous recovery test conducted one week after the initial tone test (Figure 4.7a). Rats in the VEH group displayed significant higher levels of freezing in response to the tone than the CEA group ($p < 0.03$). These results were confirmed by an ANOVA that revealed a significant main effect of drug

group [$F_{(1,13)}$ = 5.8; p < 0.03].  Even up to one week after CS reactivation animals that had received anisomycin infused into the CEA still showed impairments in conditioned freezing, suggesting that blocking reconsolidation is long lasting.

Conditioned freezing during the tone test after re-training is displayed in Figure 4.7b.  An ANOVA of the post-shock freezing during the tone test revealed no significant main effect of drug type.  These results indicated that all groups displayed similar levels of freezing during the tone test following re-training, suggesting that freezing deficits were due to inhibition of reconsolidation rather than anisomycin-induced damage.

### General Discussion

The present experiments used anisomycin to block protein synthesis within the CEA to assess the role of the CEA in consolidation and reconsolidation.  We found that protein synthesis within the CEA is necessary for consolidation of Pavlovian fear memories in intact rats (Experiment 1) and in rats with BLA lesions (Experiment 2).  In addition, we also found that protein synthesis within the CEA after memory retrieval is also necessary for reconsolidation of Pavlovian fear memories (Experiment 3).  In conjunction with these results other evidence has emerged that reveals that the CEA is not just important for expression of fear but that the role the CEA plays in Pavlovian fear conditioning is similar to that of the BLA.

Considerable evidence indicates that within the BLA, long-term potentiation (LTP) of synaptic transmission is the underlying mechanism associated with acquisition and consolidation of fear.  Activity at glutamate receptors is important for the induction of LTP in the amygdala (Goosens & Maren, 2002; Maren, 2001b).  For example, during

fear conditioning, the influx of calcium that results from NMDA activation initiates a cascade of intracellular events, such as protein synthesis and gene expression (Abel & Lattal, 2001; LeDoux, 1998). Blocking NMDA receptors or downstream cascades in the BLA impairs long-term memory formation (Bourtchuladze et al., 1994; Campeau et al., 1992; Fanselow & Kim, 1994; Goosens & Maren, 2003, 2004; LeDoux et al., 1990; Lee & Kim, 1998; Lee et al., 2001; Maren et al., 1996b; Rodrigues et al., 2001; Schafe et al., 1999). Importantly, inhibiting protein synthesis in the BLA immediately after training eliminates the consolidation of fear memory (Bailey et al., 1999; Goosens et al., 2000; Lin et al., 2001; Maren et al., 2003; Nader et al., 2000a; Schafe et al., 2000; Schafe & LeDoux, 2000; Wei et al., 2002).

Furthermore, inhibition of protein synthesis within the BLA after memory retrieval produces profound impairments in the retention of the fear memory (Alberini, 2005; Duvarci & Nader, 2004; Nader et al., 2000b; Schafe et al., 2001). For example, Nader and colleagues have shown that infusions of anisomycin into the BLA immediately after recall (i.e. exposure to the CS) causes retrograde amnesia regardless if the memory is activated 1 or 14 days after initial consolidation (Nader et al., 2000a). Additional studies also support that protein synthesis is necessary for re-stabilizing fear memories (Alberini, 2005; Alberini et al., 2006; Debiec et al., 2006; Duvarci et al., 2006; Duvarci & Nader, 2004; Nader et al., 2000a; Pedreira & Maldonado, 2003; Pedreira et al., 2002; Przybyslawski & Sara, 1997; Riccio et al., 2006; Suzuki et al., 2004).

Theses earlier studies investigating the role of the BLA in reconsolidation have not ruled out the possibility that drug effects were in fact mediated in the CEA. We have shown that the dose of anisomycin (62.5 μg) used in most BLA reconsolidation

experiments impairs protein synthesis in structures well outside of the BLA, including the CEA (Maren et al., 2003).  Therefore, deficits in conditional responding after infusions of a protein synthesis inhibitor into the amygdala may be due to protein synthesis inhibition in the CEA.  Recently, Wanisch and Wotjak (2008) have shown that lower doses of anisomycin (20 μg) infused into the hippocampus are more precisely confined to the target structures instead of the commonly used dose of 62.5 μg.  Therefore, in Experiment 3 we investigated the effects of a low-dose of anisomycin (31.25 μg) infused into the CEA, BLA, or CPu following memory reactivation.  We found similar deficits in freezing during test in rats that received anisomycin into the BLA or CEA, confirming the results of earlier studies that used the higher dose of anisomycin in the BLA, while implicating a role for the CEA in reconsolidation.  We found no effect on conditioned freezing when anisomycin was infused into the CPu, a structure located in close proximity to the amygdala, suggesting that anisomycin-induced deficits were not due to impairment of protein synthesis inhibition in structure well outside of the amygdala.

Interestingly, in a higher dose of anisomycin (31.25 μg) was needed to impair reconsolidation (Experiment 3), as compared to consolidation, which was easily blocked with a lower dose of anisomycin (12 μg; Experiments 1 & 2). Stafford and Lattal (2008) revealed that impairments in consolidation are generally larger and more persistent than reconsolidation deficits.  Mice infused with anisomycin either after consolidation or reconsolidation displayed deficits in freezing compared to vehicle controls, however the impairment in freezing was larger in the consolidation group compared to the reconsolidation group.  When tested again 17 days later the freezing deficit produced by anisomycin was still present in the consolidation group and not the reconsolidation group

(Stafford & Lattal, 2008). Consolidation may be easier to disrupt because the entire memory is sensitive to disruption, whereas when as memory is reactivated it is not clear how much of the entire memory is returned to a labile state. If only part of the memory is reactivated only that part is affected by manipulations during reconsolidation, but the part that is not reactivated allows at least a trace of the original memory to persist making it more difficult to get rid of the entire memory completely.

In addition a recent study indicates that NMDA receptors are also involved in the reconsolidation of fear memories. Nader and colleagues found that APV infused into the BLA prior to recall prevented consolidated memories from returning to a labile state during reactivation, but did not prevent re-stabilization of a memory after reactivation. In addition, NBQX did not prevent the induction of labiality indicating that AMPA receptors do not play a role in reconsolidation. They concluded that NMDA receptor activation is the critical process initiating reconsolidation, but protein synthesis within the BLA is the process that re-stabilizes memory (Ben Mamou et al., 2006). Others have shown that NMDA receptor activation is involved in re-stabilization of memory (Lee et al., 2006; Pedreira et al., 2002; Przybyslawski & Sara, 1997; Summers et al., 1997; Suzuki et al., 2004; Torras-Garcia et al., 2005). Experiments need to be conducted to determine whether and/or how NMDA receptors within the CEA are involved in reconsolidation of fear memories.

A common treatment for anxiety disorders such as post-traumatic stress disorder (PTSD) or phobia is exposure therapy. The patient is exposed repeatedly to stimuli associated with the traumatic memory in a safe setting. After repeated presentations the patient learns that the stimulus no longer predicts a negative outcome and his or her

anxiety is reduced.  However, a major limitation of this type of therapy is that it is context-specific.  Although the patient may extinguish his or her fear in the therapist's office as soon as he or she is in a different environment, such as at home, and he or she is presented with the "extinguished" stimulus there is a renewal of anxiety.  While extinction is context-specific, reconsolidation deficits do not show renewal after a contextual shift.  In addition, reminder shocks will reinstate extinction memory, but do not reinstate fear memory after reconsolidation inhibition (Duvarci & Nader, 2004).  Interrupting reconsolidation after a memory is reactivated has the potential to be a more effective treatment for anxiety disorder than just extinction.  The present experiments were designed to identify the neural substrates and cellular mechanisms necessary for consolidating and reconsolidating traumatic memories.  Understanding these mechanisms may provide novel tools to eradicate traumatic memories long after they are acquired.
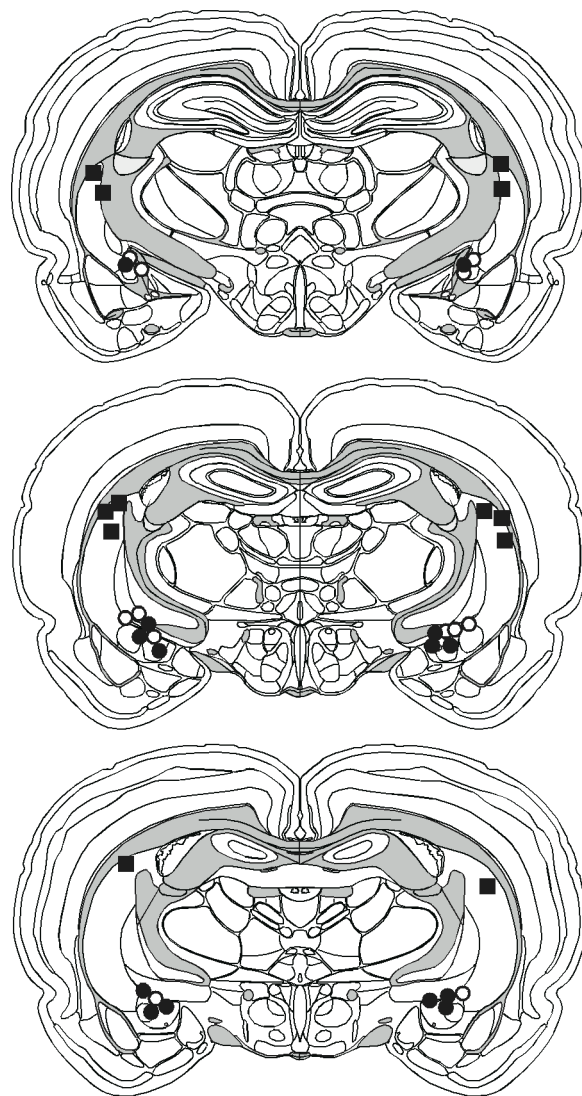
**Figure 4.1.** Schematic representation of the locations of included cannula placements for the infusion of anisomycin (closed circles) or VEH (open circles) in the CEA and for the infusion of anisomycin (closed squares) into the CPu for Experiment 1. Coronal brain images were adapted from (Swanson, 2004).
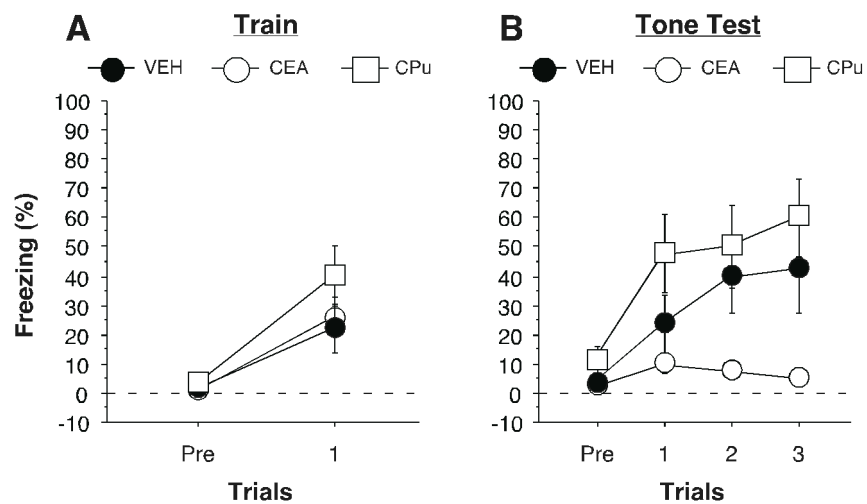
**Figure 4.2.** Conditioned freezing during the training session and tone test (Experiment 1). *A,* Mean percentage of freezing (± SEM) during the 1-trial training session. *B,* Mean percentage of freezing (± SEM) during the 3-trial tone test. For both line graphs groups are denoted as follows: VEH (closed circles), CEA (open circles), and CPu (open squares).
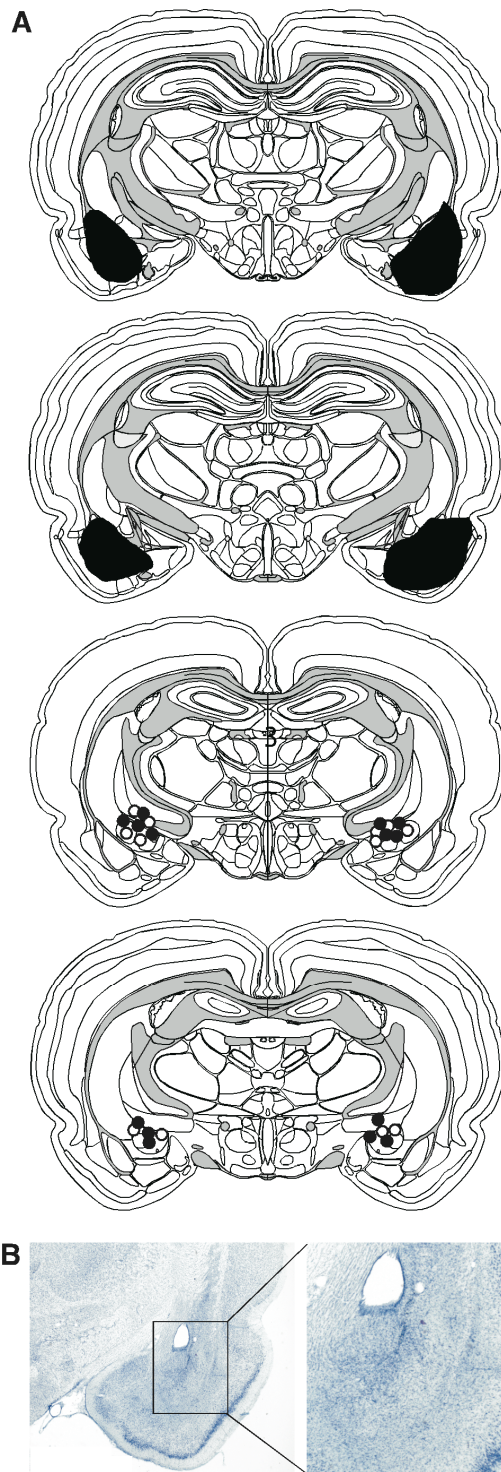
**Figure 4.3.** *A,* Schematic representation of the extent of pre-training NMDA lesions in the BLA and included cannula placements for the infusion of anisomycin (closed circles) or VEH (open circles) in the CEA for Experiment 1.  Coronal brain images were adapted from (Swanson, 2004).  *B,* Representative thionin-stained section from rats that received lesions of the BLA.

**Figure 4.4.** Conditioned freezing during the overtraining session, context, and tone tests (left to right, respectively) (Experiment 2). *A,* Mean percentage of freezing (± SEM) during the 75-trial training session (15-trial blocks). Data are shown for SHAM rats (closed circles) and rats with BLA lesions (open circles). *B,* Mean percentage of freezing (± SEM) during the 10-minute context test. *C,* Mean percentage of freezing (± SEM) during the 5-trial tone test. Data are an average of freezing during the ITI periods for the vehicle groups (SHAM and VEH; black bar) and the anisomycin group (12 µg; white bar).
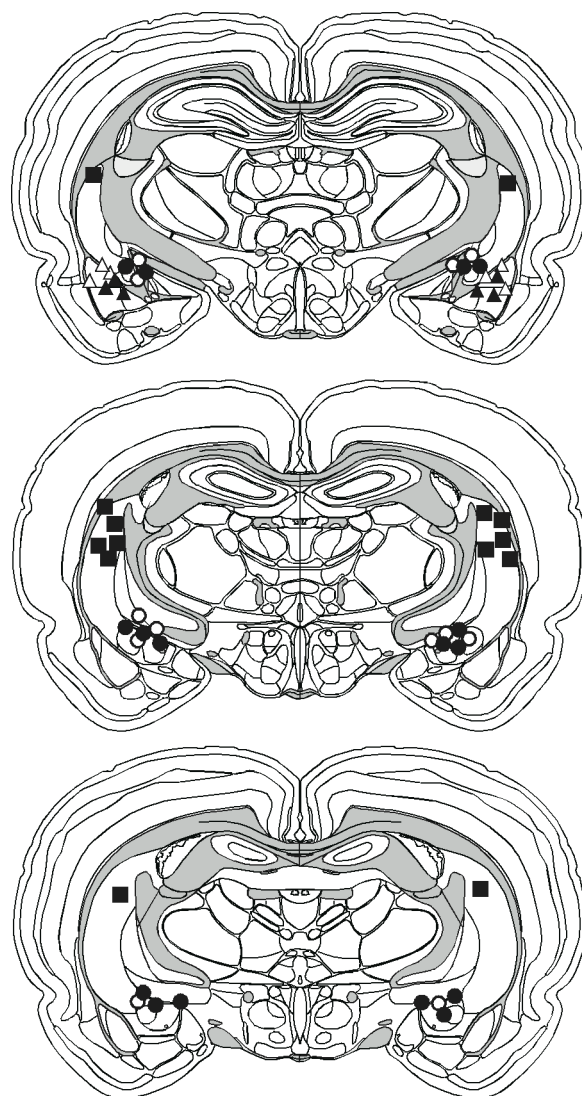
**Figure 4.5.** Schematic representation of the locations of included cannula placements for the infusion of anisomycin into the CEA (closed circles), BLA (closed triangles), or CPu (closed squares) and for the infusion of VEH into the CEA (open circles) or BLA (open triangles) for Experiment 3. Coronal brain images were adapted from (Swanson, 2004).
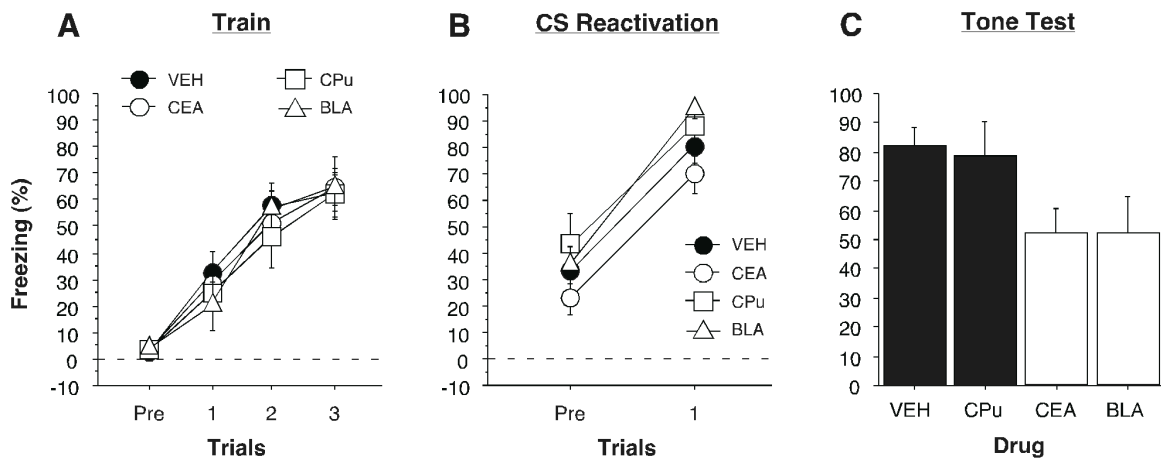
**Figure 4.6.** Conditioned freezing during the training session, CS reactivation session, and tone test (left to right, respectively) (Experiment 3). *A,* Mean percentage of freezing (± SEM) during the 3-trial training session. *B,* Mean percentage of freezing (± SEM) during the 1-trial CS reactivation session. For both line graphs groups are denoted as follows: VEH (closed ci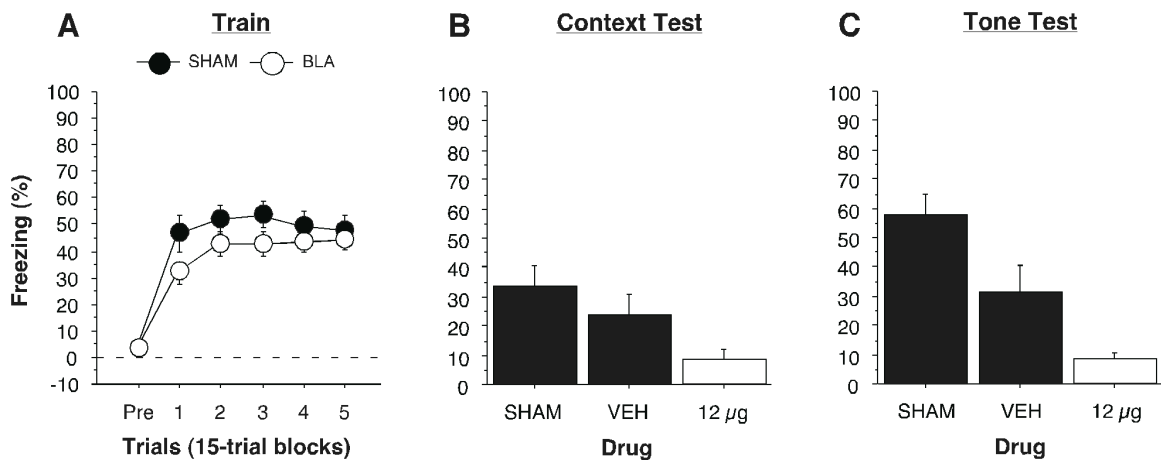rcles), CPu (open squares), CEA (open circles), and BLA (open triangles). *C,* Mean percentage of freezing (± SEM) during the 3-trial tone test. Data are an average of freezing during the ITI periods for the control groups (VEH and CPu; black bar) and the experimental groups (CEA and BLA; white bar).

**A** <u>Spontaneous Recovery</u>
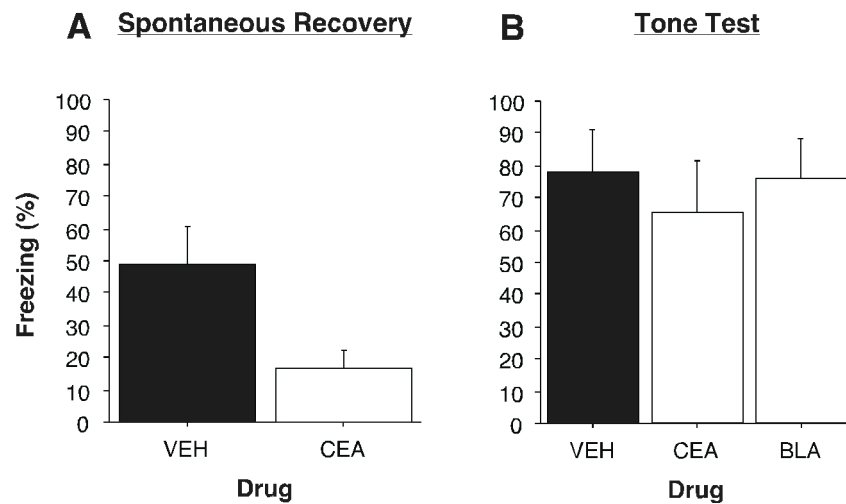
**B** <u>Tone Test</u>

**Figure 4.7.** Conditioned freezing during the spontaneous recovery test and the tone test following re-training (Experiment 3). *A,* Mean percentage of freezing (± SEM) during the 3-trial tone test. *B,* Mean percentage of freezing (± SEM) during the 3-trial tone test following re-training. Data are an average of freezing during the ITI periods. In both bar graphs data are shown for the control groups (VEH and CPu; black bar) and the experimental groups (CEA and BLA; white bar).

# References

Abel, T., & Lattal, K. M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol, 11*(2), 180-187.

Alberini, C. M. (2005). Mechanisms of memory stabilization: Are consolidation and reconsolidation similar or distinct processes? *Trends Neurosci, 28*(1), 51-56.

Alberini, C. M., Milekic, M. H., & Tronel, S. (2006). Mechanisms of memory stabilization and de-stabilization. *Cell Mol Life Sci, 63*(9), 999-1008.

Bailey, D. J., Kim, J. J., Sun, W., Thompson, R. F., & Helmstetter, F. J. (1999). Acquisition of fear conditioning in rats requires the synthesis of mrna in the amygdala. *Behavioral Neuroscience, 113*(2), 276-282.

Ben Mamou, C., Gamache, K., & Nader, K. (2006). Nmda receptors are critical for unleashing consolidated auditory fear memories. *Nat Neurosci, 9*(10), 1237-1239.

Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., & Silva, A. J. (1994). Deficient long-term memory in mice with a targeted mutation of the camp-responsive element-binding protein. *Cell, 79*(1), 59-68.

Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A modern learning theory perspective on the etiology of panic disorder. *Psychol Rev, 108*(1), 4-32.

Campeau, S., & Davis, M. (1995). Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J. Neurosci., 15*(3), 2301-2311.

Campeau, S., Miserendino, M. J., & Davis, M. (1992). Intra-amygdala infusion of the n-methyl-d-aspartate receptor antagonist ap5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behav Neurosci, 106*(3), 569-574.

Cousens, G., & Otto, T. (1998). Both pre- and posttraining excitotoxic lesions of the basolateral amygdala abolish the expression of olfactory and contextual fear conditioning. *Behavioral Neuroscience, 112*(5), 1092-1103.

Davis, M., & Whalen, P. J. (2001). The amygdala: Vigilance and emotion. *Mol Psychiatry, 6*(1), 13-34.

Debiec, J., Doyere, V., Nader, K., & Ledoux, J. E. (2006). Directly reactivated, but not indirectly reactivated, memories undergo reconsolidation in the amygdala. *Proc Natl Acad Sci U S A, 103*(9), 3428-3433.

Duvarci, S., Mamou, C. B., & Nader, K. (2006). Extinction is not a sufficient condition to prevent fear memories from undergoing reconsolidation in the basolateral amygdala. *Eur J Neurosci, 24*(1), 249-260.

Duvarci, S., & Nader, K. (2004). Characterization of fear memory reconsolidation. *J Neurosci, 24*(42), 9269-9275.

Fanselow, M. S., & Gale, G. D. (2003). The amygdala, fear, and memory. *Ann N Y Acad Sci, 985*, 125-134.

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neuroscience and Biobehavioral Reviews, 23*, 743-760.

Gale, G. D., Anagnostaras, S. G., Godsil, B. P., Mitchell, S., Nozawa, T., Sage, J. R., et al. (2004). Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. *J Neurosci, 24*(15), 3810-3815.

Goosens, K. A., Holt, W., & Maren, S. (2000). A role for amygdaloid pka and pkc in the acquisition of long-term conditional fear memories in rats. *Behavioural Brain Research, 114*(1-2), 145-152.

Goosens, K. A., & Maren, S. (2001). Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. *Learn Mem, 8*(3), 148-155.

Goosens, K. A., & Maren, S. (2002). Long-term potentiation as a substrate for memory: Evidence from studies of amygdaloid plasticity and pavlovian fear conditioning. *Hippocampus, 12*(5), 592-599.

Goosens, K. A., & Maren, S. (2003). Pretraining nmda receptor blockade in the basolateral complex, but not the central nucleus, of the amygdala prevents savings of conditional fear. *Behav Neurosci, 117*(4), 738-750.

Goosens, K. A., & Maren, S. (2004). Nmda receptors are essential for the acquisition, but not expression, of conditional fear and associative spike firing in the lateral amygdala. *Eur J Neurosci, 20*(2), 537-548.

Grillon, C., Southwick, S. M., & Charney, D. S. (1996). The psychobiological basis of posttraumatic stress disorder. *Molecular Psychiatry, 1*(4), 278-297.

Helmstetter, F. J. (1992). The amygdala is essential for the expression of conditional hypoalgesia. *Behavioral Neuroscience, 106*(3), 518-528.

Helmstetter, F. J., & Bellgowan, P. S. (1994). Effects of muscimol applied to the basolateral amygdala on acquisition and expression of contextual fear conditioning in rats. *Behavioral Neuroscience, 108*(5), 1005-1009.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature (London), 388*(6640), 377-380.

Kim, J. J., & Jung, M. W. (2006). Neural circuits and mechanisms involved in pavlovian fear conditioning: A critical review. *Neuroscience & Biobehavioral Reviews, 30*(2), 188-202.

Koo, J. W., Han, J. S., & Kim, J. J. (2004). Selective neurotoxic lesions of basolateral and central nuclei of the amygdala produce differential effects on fear conditioning. *J Neurosci, 24*(35), 7654-7662.

LeDoux, J. (1998). Fear and the brain: Where have we been, and where are we going? *Biol Psychiatry, 44*(12), 1229-1238.

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184.

LeDoux, J. E., Cicchetti, P., Xagoraris, A., & Romanski, L. M. (1990). The lateral amygdaloid nucleus: Sensory interface of the amygdala in fear conditioning. *Journal of Neuroscience, 10*(4), 1062-1069.

Lee, H., & Kim, J. J. (1998). Amygdalar nmda receptors are critical for new fear learning in previously fear-conditioned rats. *Journal of Neuroscience, 18*(20), 8444-8454.

Lee, H. J., Choi, J. S., Brown, T. H., & Kim, J. J. (2001). Amygdalar nmda receptors are critical for the expression of multiple conditioned fear responses. *Journal of Neuroscience, 21*(11), 4116-4124.

Lee, J. L., Milton, A. L., & Everitt, B. J. (2006). Reconsolidation and extinction of conditioned fear: Inhibition and potentiation. *J Neurosci, 26*(39), 10051-10056.

Lin, C. H., Yeh, S. H., Lin, C. H., Lu, K. T., Leu, T. H., Chang, W. C., et al. (2001). A role for the pi-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. *Neuron, 31*(5), 841-851.

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *Journal of Neuroscience, 18*(8), 3088-3097.

Maren, S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J. Neurosci., 19*(19), 8696-8703.

Maren, S. (2001a). Is there savings for pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? *Neurobiology of Learning and Memory, 76*(3), 268-283.

Maren, S. (2001b). Neurobiology of pavlovian fear conditioning. *Annual Review of Neuroscience, 24*(1), 897-931.

Maren, S. (2005). Building and burying fear memories in the brain. *Neuroscientist, 11*(1), 89-99.

Maren, S., Aharonov, G., & Fanselow, M. S. (1996a). Retrograde abolition of conditional fear after excitotoxic lesions in the basolateral amygdala of rats: Absence of a temporal gradient. *Behavioral Neuroscience, 110*(4), 718-726.

Maren, S., Aharonov, G., Stote, D. L., & Fanselow, M. S. (1996b). N-methyl-d-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behavioral Neuroscience, 110*(6), 1365-1374.

Maren, S., Ferrario, C. R., Corcoran, K. A., Desmond, T. J., & Frey, K. A. (2003). Protein synthesis in the amygdala, but not the auditory thalamus, is required for consolidation of pavlovian fear conditioning in rats. *European Journal of Neuroscience, 18*(11), 3080-3088.

Muller, J., Corodimas, K. P., Fridel, Z., & LeDoux, J. E. (1997). Functional inactivation of the lateral and basal nuclei of the amygdala by muscimol infusion prevents fear conditioning to an explicit conditioned stimulus and to contextual stimuli. *Behavioral Neuroscience, 111*(4), 683-691.

Nader, K., Majidishad, P., Amorapanth, P., & LeDoux, J. E. (2001). Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. *Learn Mem, 8*(3), 156-163.

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000a). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*(6797), 722-726.

Nader, K., Schafe, G. E., & LeDoux, J. E. (2000b). The labile nature of consolidation theory. *Nat Rev Neurosci, 1*(3), 216-219.

Pare, D., Quirk, G. J., & Ledoux, J. E. (2004). New vistas on amygdala networks in conditioned fear. *J Neurophysiol, 92*(1), 1-9.

Pedreira, M. E., & Maldonado, H. (2003). Protein synthesis subserves reconsolidation or extinction depending on reminder duration. *Neuron, 38*(6), 863-869.

Pedreira, M. E., Perez-Cuesta, L. M., & Maldonado, H. (2002). Reactivation and reconsolidation of long-term memory in the crab chasmagnathus: Protein synthesis requirement and mediation by nmda-type glutamatergic receptors. *J Neurosci, 22*(18), 8305-8311.

Przybyslawski, J., & Sara, S. J. (1997). Reconsolidation of memory after its reactivation. *Behav Brain Res, 84*(1-2), 241-246.

Riccio, D. C., Millin, P. M., & Bogart, A. R. (2006). Reconsolidation: A brief history, a retrieval view, and some recent issues. *Learn Mem, 13*(5), 536-544.

Rodrigues, S. M., Schafe, G. E., & LeDoux, J. E. (2001). Intra-amygdala blockade of the nr2b subunit of the nmda receptor disrupts the acquisition but not the expression of fear conditioning. *Journal of Neuroscience, 21*(17), 6889-6896.

Samson, R. D., & Pare, D. (2005). Activity-dependent synaptic plasticity in the central nucleus of the amygdala. *J. Neurosci., 25*(7), 1847-1855.

Schafe, G. E., Atkins, C. M., Swank, M. W., Bauer, E. P., Sweatt, J. D., & LeDoux, J. E. (2000). Activation of erk/map kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning. *Journal of Neuroscience, 20*(21), 8177-8187.

Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase a in the amygdala. *Journal of Neuroscience, 20*(18), RC96.

Schafe, G. E., Nadel, N. V., Sullivan, G. M., Harris, A., & LeDoux, J. E. (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, pka, and map kinase. *Learn Mem, 6*(2), 97-110.

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of pavlovian fear conditioning: A cellular and molecular perspective. *Trends in Neurosciences, 24*(9), 540-546.

Stafford, J. M., & Lattal, K. M. (2008). *Direct comparisons of the size and persistence of anisomycin-induced consolidation and reconsolidation deficits.* Paper presented at the Society for Neuroscience, Washington, D.C.

Summers, M. J., Crowe, S. F., & Ng, K. T. (1997). Administration of dl-2-amino-5-phosphonovaleric acid (ap5) induces transient inhibition of reminder-activated memory retrieval in day-old chicks. *Brain Res Cogn Brain Res, 5*(4), 311-321.

Suzuki, A., Josselyn, S. A., Frankland, P. W., Masushige, S., Silva, A. J., & Kida, S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci, 24*(20), 4787-4795.

Swanson, L. W. (2004). *Brain maps: Structure of the rat brain* (Third ed.). San Diego: Academic Press.

Torras-Garcia, M., Lelong, J., Tronel, S., & Sara, S. J. (2005). Reconsolidation after remembering an odor-reward association requires nmda receptors. *Learn Mem, 12*(1), 18-22.

Walker, D. L., & Davis, M. (1997). Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J. Neurosci., 17*(23), 9375-9383.

Wanisch, K., & Wotjak, C. T. (2008). Time course and efficiency of protein synthesis inhibition following intracerebral and systemic anisomycin treatment. *Neurobiol Learn Mem, 90*(3), 485-494.

Wei, F., Qiu, C. S., Liauw, J., Robinson, D. A., Ho, N., Chatila, T., et al. (2002). Calcium-calmodulin-dependent protein kinase iv is required for fear memory. *Nature Neuroscience, 5*(6), 573-579.

Wilensky, A. E., Schafe, G. E., Kristensen, M. P., & LeDoux, J. E. (2006). Rethinking the fear circuit: The central nucleus of the amygdala is required for the acquisition, consolidation, and expression of pavlovian fear conditioning. *J. Neurosci., 26*(48), 12387-12396.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (1999). Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. *J. Neurosci., 19*(24), 48RC-.

Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (2000). Functional inactivation of amygdala nuclei during acquisition of pavlovian fear conditioning. *Society for Neuroscience Abstracts, 30*, 465.469.

Zimmerman, J. M., Rabinak, C. A., McLachlan, I. G., & Maren, S. (2007). The central nucleus of the amygdala is essential for acquiring and expressing conditional fear after overtraining. *Learn Mem, 14*(9), 634-644.

# CHAPTER V

## CONCLUSION

### The Nature of Learned Associations

In Chapter I, an argument was presented that the nature of learned associations could be explained in two different ways. Tolman argues that learning occurs through S-S associations, whereby the CS directly activates the representation of the specific US in order to produce a CR (Tolman & Postman, 1954). Hull, however, argues that learning occurs through S-R associations, whereby the CS directly evokes a CR without accessing the US (Hull, 1943). We also discussed that, at least in terms of instrumental learning, the type of associative representation mediating learning is a function of the amount of training. Specifically, S-S associations mediate responding early in training, while S-R associations mediate performance in extensively trained animals (Adams, 1982; Dickinson et al., 1995; Holland, 2004; Holland & Gallagher, 2004).

Therefore, when these ideas were applied to Pavlovian fear conditioning the following logic was used to create the central focus of this dissertation: Animals with BLA lesions can acquire conditioned fear responses if given overtraining and this type of learning is CEA-dependent (Maren, 1998; Maren, 1999; Zimmerman et al., 2007), which suggests that the associative basis of fear conditioning may also change as a function of

training. On the other hand, it is possible that the associative basis of conditioned fear in rats with BLA lesions is different from that in intact rats. Together these ideas led to the formation of our initial hypothesis that S-S associations (mediated by the BLA) may underlie fear memory in intact rats, whereas S-R associations (mediated by the CEA) may underlie memory in rats with BLA lesions.

However, we found that despite being overtrained, that the fear memory of intact rats is sensitive to the inflation procedure, which suggests it is mediated by an S-S association (Chapter II, Experiment 1). Moreover, lesions of the BLA during overtraining did not prevent inflation of fear memory (Chapter II, Experiments 2 & 3). These results reveal that S-S associations mediate conditional fear not only in intact rats, but also in rats with BLA lesions. In addition, this suggests that brain structures, such as the CEA, that mediate fear in the absence of the BLA encode CS-US association during fear conditioning. Furthermore, Pavlovian fear responses are notoriously insensitive to instrumental contingencies (Bolles et al., 1974), and appear to require US representations for expression in behavior even after overtraining.

Our results would follow Tolman's S-S theory that during conditioning animals learn about the relationship between the CS and the US in order to produce CRs that flexibly reflect this association (Tolman & Postman, 1954). However, the way in which the CS accesses properties of the US can occur by different psychological processes. For example, Bindra (1974) suggests that there is a simple associative transfer of incentive properties between the CS and the US, so that the CS becomes the incentive. He provides a nice example to illustrate this theory, whereby a rat learns that a light CS predicts the occurrence of a food US. Early in training, each stimulus has its own

emotional or motivational representation and the occurrence of the CR (i.e. approach, eating, salivating) depends on the presentation of the food US. At this point the light CS alone is not capable of producing any aspect of the CR that is produced by the presentation of the food US. However, once the association is learned, the onset of the light CS excites the central representation of the light and through its association with the food US, also excites the central motivational state of the hedonic food US, causing the animal to perceive the CS as the US (i.e. approach) (Bindra, 1974, 1978). These incentive properties that CS takes on are attractive to the animal and elicit goal-directed behavior.

Toates (1986) adopted Bindra's theory, but also argued that drive states mediate the value of the incentive stimuli through a cognitive expectancy (Toates, 1986). For example, when we are hungry, a turkey on Thanksgiving may be delicious, but after stuffing our faces full of Thanksgiving dinner, that same turkey may not seem so pleasurable, even though sensory properties of the turkey are basically the same as they were before we were sated. Overall, the physiological drive states only change the pleasurably properties of the sensation (Berridge, 2001). If we apply this drive state to the example above in a rat that receives light CS and food US pairings, then the light CS that predicts food to a hungry rat becomes attractive, maybe even edible and possibly tastes like the food. Normally directed towards the food US, approach and possibly even consummatory behaviors are now elicited towards the light CS. However, if the animal is no longer hungry then the CS no longer carries any motivational value and is merely a predictive signal. The rat is not only learning a Pavlovian association but is also engaged in a motivational change.

Toates also suggested that the ability of the CS to acquire the incentive value of the US is very strong, so much so that it can "overcome any existing hedonic value that signal stimulus may already have" (Berridge, 2001, pg. 240). Holland and Straub (1979) demonstrated an excellent example of how the CS takes on altered incentive value of the US through an US devaluation procedure. Rats first learned that a tone CS predicted a food US. Then, in the absence of the tone CS, the food US was paired with an injection of a toxin, LiCl. As a result, the rats developed an aversion towards the food US and decreased consumption. During a subsequent test of conditioned responding to the tone CS (no food present), rats also showed a similar decrease in CRs as well (Holland & Straub, 1979). When the tone CS was presented during test it evoked a sensory representation of the food US, specifically it's taste, which was now devalued; the tone CS adopted this change in incentive value.

The Bindra-Toates model of incentive motivation suggests that Pavlovian CSs that are initially neutral can acquire incentive salience through associations with biologically significant USs, in which the CSs then can become motivational triggers. However, USs are represented by multiple features and these different features can enter into independent associations with the CSs. In 1967, Konorski argued that independent associations are formed between the CS and the sensory features and motivational properties of the US and explained this relationship in terms of consummatory and preparatory conditioning. Konorski believed that specific consummatory responses, such as chewing, biting, and licking, are mediated by the sensory properties of the US, whereas general preparatory responses, such as changes in heart rate, blood pressure,

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approach, and withdrawal, are mediated by the general affective properties (Balleine & Killcross, 2006; Konorski, 1967).

Recent studies, in both appetitive and aversive learning, suggest that the amygdala is involved in these independent associations with the CS, in that the BLA is involved in the associative processes that mediate consummatory conditioning and the CEA is involved in the associative processes that mediate preparatory conditioning (Balleine & Killcross, 2006). For example, BLA lesions do not affect the acquisition of food aversion or conditioned orienting responses to a light CS or food-cup CRs, but do impair the ability to adjust CRs appropriately to post-training changes in US value (i.e. US devaluation). These results support that preparatory responses remain intact in rats with BLA lesions, but the BLA is necessary for the CS to gain access to the specific sensory features of the US (Hatfield et al., 1996). In the same experiment, CEA lesions impaired preparatory responses, such as conditioned orienting, but did not alter the devaluation effect, suggesting that the CEA is not involved in the transfer of value from the US to the CS (Hatfield et al., 1996).

This same theory can be applied to aversive learning, whereby the BLA mediates US-specific (i.e. nociceptive) association with the CS and the CEA mediates associations with the more general affective aspects of the US (i.e. aversive) (Balleine & Killcross, 2006). For example, rats with BLA lesions are unable to bias their choice of action away from a lever that produced an aversive, punishing stimulus, but display normal conditioned suppression to this stimulus. In contrast, rats with CEA lesions are able to direct their actions to avoid further presentations of an aversive stimulus, but are impaired on conditioned suppression to CS that was paired with an aversive US (Killcross et al.,

1997). These results demonstrate that both the BLA and CEA are not only capable of independently supporting CS-US associations, but also independently mediate different forms of defense CRs. Furthermore, rats with BLA lesions can acquire conditioned fear responses if given overtraining and this type of learning is CEA-dependent (Maren, 1999; Zimmerman et al., 2007). If the CEA is using general affective aspects of the US in association with the CS to guide learning then overtraining in the absence of the BLA may take longer to acquire because it may take the animal longer to identify the specific CS. However, in Pavlovian fear conditioning BLA lesions block conditioned freezing, which could be categorized as more of a preparatory response, which is arguably not a response generated by the sensory aspects of the US. Perhaps, the BLA encodes both sensory and affective aspects of the US. We found that both the BLA and CEA appear to encode S-S associations during Pavlovian fear conditioning (Chapter II), each may do so by accessing different properties of the US (e.g. sensory and general affective aspects).

In addition the Bindra-Toates model for explaining incentive salience mechanisms, in reward learning, a cognitive expectation of a reward for goal-directed behavior can also be added. Cognitive incentive learning means that you have a declarative goal already known to you. Specifically, you know what the reward is that you are working for and this type of cognitive expectation gives the representation of a stimulus incentive value (i.e. cognitive value) (Berridge, 2001). Dickinson (1989) referred to the cognitive understanding of the causal relationship between working as a cause for gaining an incentive reward, as an act-outcome (A-O) representation (Dickinson, 1989). Therefore, instrumental behavior is guided by A-O representations and the incentive value (i.e. cognitive expectation) of the outcome (Berridge, 2001;

Dickinson & Balleine, 1995).  In the procedure of outcome devaluation Dickinson and

colleagues argue that animals must learn about changes in the incentive value of the

outcome through consummatory contact with that outcome before this change can affect

goal-directed performance (Adams & Dickinson, 1981; Balleine & Killcross, 2006;

Dickinson & Balleine, 1994).

Multiple psychological processes have emerged from the above mentioned studies

in reward learning, such as S-S associations described by Bindra, Toates and Konorski

that posit that the CS acquires flexible and state-dependent properties of the US to drive

CRs based on this associative transfer (Bindra, 1974, 1978; Konorski, 1967; Toates,

1986).  Dickinson and colleagues further transformed this idea with the incorporation of

cognitive expectation mechanisms to guide motivated behavior via representations of a

causal relationship between an act and its expected outcome (Adams & Dickinson, 1981;

Balleine et al., 2003; Balleine & Killcross, 2006; Blundell et al., 2003; Dickinson, 1989;

Dickinson & Balleine, 1994, 1995).  Therefore, researchers should not dilute associative

learning to one explanation.  Instead further research should explore "…the nature and

features of each of these psychological processes, their relation to brain mechanisms, and

the rules that govern interactions between them" (Berridge, 2001, pg. 272).

In 1989, Graham C. L. Davey discussed how the strength of a CR could be

determined by many factors other than the strength of the CS-US association, such as the

processes that lead the individual to revalue the US itself (Davey, 1989).  He proposed

that when the CS is presented it elicits a cognitive representation of the US, much like

Dickinson (1989) suggested, which then leads to the evaluation of the US (i.e. aversive,

appetitive, palatable, painful, etc) and ultimately produces a CR that reflects the current

value of the US. Processes that lead to the evaluation of the US include the subject's

experience with the US alone, any socially or verbally transmitted information about the

US and response attribution processes. Furthermore, Davey suggested, "any factor which

influences the individual's evaluation of the US will affect response strength to

subsequent presentation of the CS" (Davey, 1989, pg. 527). For example, there are

situations in which there is a failure to develop a phobia following a traumatic experience

with a stimulus situation or spontaneous fluctuations in the fear response (i.e. latent

inhibition, whereby exposure to a CS prior to conditioning prevents conditioned

associations with that CS from being formed; or US devaluation). The acquisition of the

fear associations and modulation of US value "is no longer bound by the need to discover

contiguous stimulus-trauma experiences in the histories of clinical phobias" (Davey,

1989, pg. 527). Elucidating the mechanisms by which the amygdala encodes fear

memory is critical for developing effective treatments for anxiety disorders, including

PTSD (Davey, 1989; Hosoba et al., 2001; Unger et al., 2003; White & Davey, 1989).


### Amygdala Involvement in US Revaluation

As mentioned above, recent work in appetitive conditioning paradigms suggest

that the BLA has a role in representing US value. That is, BLA inactivation or lesions

reduces the decrement in conditioned responding after devaluation of a food US (Balleine

et al., 2003; Blundell et al., 2001; Everitt et al., 2003; Hatfield et al., 1996; Holland,

2004; Killcross et al., 1997; Pickens et al., 2003). In addition, Fanselow and Gale (2003)

found that BLA inactivation prior to an inflation session blocked the enhancement in

conditioned fear responding (Fanselow & Gale, 2003). However, in contrast to these

devaluation studies, we found that rats with BLA lesions exhibit US inflation after an overtraining procedure, suggesting that the BLA may not be necessary for coding the value of the aversive USs (Chapter II). Furthermore, BLA inactivation did not impair the inflation of conditioned freezing, nor did inhibition of protein synthesis in the BLA after the inflation session prevent inflation fear memories (Chapter III). Together these results suggest that BLA neurotransmission is not necessary for coding changes in US value and synaptic plasticity within the BLA is not necessary for consolidating or maintaining representations of revalued USs. These data contradict Fanselow and Gale (2003), however the exact reasons why these results are conflicting are not clear; it may be due to potential differences within the experimental protocol (Fanselow and Gale (2003) protocol unpublished).

There are many differences between appetitive and aversive conditioning that could account for these differences in whether the BLA is involved in US revaluation. For example, devaluation procedures in appetitive conditioning typically rely upon an instrumental component. In food devaluation the animal voluntarily approaches a food reward and consumes it. The food US is then devalued by either an injection of LiCl following consumption or allowing the animal to consume more food pellets until it is sated. Thus, on subsequent presentations of the food, approach towards and consummation of the food pellet is decreased. Perhaps BLA dysfunction only impairs "instrumental" devaluation. Another difference includes the direction in which the US is revalued. In appetitive studies, the US experiences a decrease in value, whereas in our experiments the US experiences an increase in value. Studies of attention have revealed differential involvement of neural structures that depends on whether there are increases

or decreases in attention (Baxter et al., 1999; Holland & Gallagher, 1993). For example, the CEA mediates increasing attentional performance when there is a shift from a predictive relationship between stimuli to a surprising relationship, but not when the predictive relationship is consistent. Rats with CEA lesions do not show effects of increase attentional performance and in fact display deficits in learning (Holland & Gallagher, 1999). The hippocampus also mediates decrements and not increases in attention (Baxter et al., 1999; Holland & Gallagher, 1993). Like the CEA and hippocampus in attention, the BLA may be differentially involved in US revaluation depending on the direction the US is revalued. Human neuroimaging studies have shown that responding in the amygdala is altered after US devaluation in an appetitive olfactory conditioning paradigm, whereas amygdala responses are unaltered after US inflation in an aversive olfactory conditioning paradigm (Gottfried & Dolan, 2004; Gottfried et al., 2003). These authors provide similar explanations for the differential amygdala involvement in US revaluation as we suggest above (Gottfried & Dolan, 2004).

**What Neural Substrate Mediates US Inflation of Aversive Memories?**

If the BLA is not necessary for US inflation in Pavlovian fear conditioning, then what neuroanatomical structure(s) is/are involved in this process? Cortical structures, such as the orbitofrontal cortex (OFC), may be involved in US revaluation. Evidence from appetitive studies suggest that the OFC plays a role in maintaining associative representations and/or coding and updating US value (Delamater, 2007; Holland & Gallagher, 2004; Pickens et al., 2005; Pickens et al., 2003; Rolls, 2004; Schoenbaum et al., 2003b). For example, rats with OFC lesions made prior to devaluation of a food US

are not sensitive to subsequent food devaluation (Pickens et al., 2005; Pickens et al., 2003). These studies suggest the OFC might be involved in the maintenance of information about the current incentive value of reinforcers, and/or the use of that information in guiding behavior. Monkeys with combined, unilateral lesions to the amygdala-orbitofrontal circuit in either hemisphere displayed incorrect responses in a discrimination task when the identity of the predicted outcome was reversed or when the predicted outcome was devalued by satiation (Baxter et al., 2000; Izquierdo & Murray, 2004). These impairments are similar to those caused by bilateral lesions of either the amygdala or the OFC alone in rats (Gallagher et al., 1999; Schoenbaum et al., 2003a). Specifically in these studies, lesions of the OFC impair the ability of a cue to access representational information about the incentive value of the reward.

Furthermore, imaging studies in humans have revealed a greater response of medial OFC activity to high-incentive food items, whereas activity within the lateral OFC was greater when subjects had to suppress responses to other desirable food items in order to select their preferred food (Arana et al., 2003). This study suggests there is a differential contribution within the OFC that is essential for selection of goals based on the prospective incentive value of the reward when compared to the value of other, less desirable rewards. Furthermore, activity within the amygdala is increased when a face CS predicts an aversive noise US (CS+) compared to another non-predictive face CS (CS-). However, when the contingency is reversed there is enhanced activation in the OFC, while the original predictive CS+ continues to evoke increased responding in the amygdala (Morris & Dolan, 2004). The OFC displays rapid reversal of conditioned fear

responses, whereas the amygdala shows a persistent memory for previous aversive stimulus associations.

In addition to the extensive literature on OFC involvement in reward representations, lesions of the gustatory cortex (Balleine & Dickinson, 2000), or nucleus accumbens core (Corbit et al., 2001) have also been shown to produce deficits in reward devaluation.  The findings in this dissertation support amygdala involvement in fear learning, but not US inflation; therefore another structure outside of the amygdala must be working in parallel to the amygdala during Pavlovian fear conditioning to encode changes in US value.  However, experiments will need to be conducted to determine whether the OFC or another cortical/subcortical structure plays a role in coding US value and/or maintenance of updated aversive USs in Pavlovian fear conditioning.

**Encoding Fear Associations**

In Chapter II we found that the CEA appears to encode S-S memories like the BLA, however we did not know if the mechanisms of memory consolidation observed in another S-S system (i.e. the BLA) also operates in the CEA to store fear memories.  That is, are the cellular mechanisms of memory consolidation and reconsolidation the same in the CEA as they are in the BLA?  We found that, like the BLA, protein synthesis within the CEA is necessary for consolidation and reconsolidation of Pavlovian fear memories (Chapter IV).

Interestingly, a higher dose of anisomycin (31.25 $\mu$g) was needed to impair reconsolidation (Chapter IV, Experiment 3), as compared to consolidation, which was easily blocked with a lower dose of anisomycin (12 $\mu$g; Chapter IV, Experiments 1 & 2).

Stafford and Lattal (2008) revealed that impairments in consolidation are generally larger and more persistent than reconsolidation deficits. Mice infused with anisomycin either after consolidation or reconsolidation displayed deficits in freezing compared to vehicle controls, however the impairment in freezing was larger in the consolidation group compared to the reconsolidation group. When tested again 17 days later, the freezing deficit produced by anisomycin was still present in the consolidation group and not the reconsolidation group (Stafford & Lattal, 2008). Consolidation may be easier to disrupt because the entire memory is sensitive to disruption, whereas when memory is reactivated it is not clear how much of the entire memory is returned to a labile state. If only part of the memory is reactivated only that part is affected by manipulations during reconsolidation, but the part that is not reactivated allows at least a trace of the original memory to persist making it more difficult to get rid of the entire memory completely. This begs the question: is reconsolidation a recapitulation of consolidation or is it a distinct process?

Inhibiting protein synthesis in the BLA immediately after training or after memory retrieval produces profound impairments in the consolidation or reconsolidation of fear memory, respectively (Alberini, 2005; Anokhin et al., 2002; Bailey et al., 1999; Cestari et al., 2006; Duvarci & Nader, 2004; Goosens et al., 2000; Lin et al., 2001; Maren et al., 2003; Nader et al., 2000a; Nader et al., 2000b; Schafe & LeDoux, 2000; Schafe et al., 2001; Wei et al., 2002). For example, cyclic-AMP-response-element-binding protein (CREB) is necessary for consolidation and reconsolidation of contextual fear conditioning in mice (Kida et al., 2002). Consolidation and reconsolidation of conditioned taste aversion requires amygdala protein kinase A (PKA) (Koh & Bernstein,

2003).  Like the acquisition of fear conditioning, retrieval of a fear memory also induces synaptic potentiation in the LA that is selective to the reactivated memory.  Furthermore disruption of reconsolidation, via a mitogen-activated protein kinase (MAPK) inhibitor, is correlated with a decrease in synaptic potentiation in the LA at the reactivated synapses (Doyere et al., 2007).  However, whether the potentiation that is induced by retrieval and by initial learning share similar characteristics warrants further investigation.  While both new and reactivated memories may incorporate similar underlying mechanisms and neurocircuitry, there is evidence that supports differences in the underlying processes (Alberini, 2005; Alberini et al., 2006).

In conditioned taste aversion, protein synthesis in the CEA is required for consolidation but not for reconsolidation (Bahar et al., 2004), which differs from our findings in Pavlovian fear conditioning; the difference could be attributed to the different behavioral paradigms and the neural substrates they incorporate (Chapter IV).  von Herzen and Giese (2005) investigated the levels of two specific context-shock transcripts, the serum and glucocorticoid-induced kinase 3 (SGK3) and nerve growth factor-inducible gene B (NGFI-B), which are upregulated in the mouse hippocampus during contextual fear conditioning.  While both transcripts were regulated during consolidation, only the SGK3 genes were regulated during reconsolidation (von Hertzen & Giese, 2005).  This supports that the processes of reconsolidation is only partly similar to consolidation. Furthermore, zif268, an activity-dependent inducible immediate early gene (IEG) in the hippocampus, is required for reconsolidation but not consolidation of contextual fear memory, whereas brain-derived neurotrophin factor (BDNF) is required for consolidation but not reconsolidation (Lee et al., 2004).

Colón-Cesario and colleagues found that unlike general protein synthesis, DNA recombination, which may contribute to long-term memory storage, is a process specific to consolidation and is not involved in post-reactivation editing of fear memories (Colon-Cesario et al., 2006). They suggested that by restricting DNA recombination to the consolidation of new learning this would prevent increased drastic changes in the original memory that could be induced by other events at the time of recall. Furthermore, Tronel and colleagues found that memory reactivation mediates two independent processes: "the formation of a new association composed of new and old and reactivated information and the reconsolidation of the old memory" (Tronel et al., 2005, pg. 1636), suggesting that "…post-retrieval stabilization is a process distinct from consolidation, although overlap in both its function (storage) and underlying mechanisms (protein synthesis)…" (Tronson & Taylor, 2007, pg. 262). The process of reconsolidation can also be conceptualized as acting to maintain and strengthen retrieved memories and/or it acts to update them, much like in the case of US revaluation (Tronson & Taylor, 2007). This insight can help in the development of specific pharmacological treatments for anxiety disorders.

The β-adrenergic receptor antagonist, propranolol, has been commonly used after a traumatic experience in humans suffering from PTSD (Pitman & Delahanty, 2005; Pitman et al., 2002), and may be useful in blocking reconsolidation of fear memories associated with PTSD (Debiec & Ledoux, 2004; Miller et al., 2004; Pitman & Delahanty, 2005; Przybyslawski et al., 1999). In addition, benzodiazepines disrupt reconsolidation of contextual fear memory in rats (Bustos et al., 2006), thus providing useful role for anxiolytic agents in eliminating fear memories after retrieval. Bucherelli and colleagues have shown that cholinergic and histaminergic neurons within the amygdala appear to

modulate only consolidation, whereas cannabinoids (CB), specifically CB1 receptors, within the amygdala are involved in both consolidation and reconsolidation of aversive memories in rats (Bucherelli et al., 2006). Attenuating aversive memories in humans could benefit from the use of CB1 receptor antagonists, such as Rimonabant, which is currently used for weight disorders, such as obesity, in humans (Pertwee, 2001; Phan et al., 2008; Rumsfeld & Nallamothu, 2008; Wadman, 2006). Together these results provide insight towards the development of drugs targeted for therapeutic disruption of a specific memory after retrieval.

A common behavioral treatment for anxiety disorders such as PTSD or phobia, is exposure therapy. The patient is exposed repetitively to stimuli associated with the traumatic memory in a safe setting. After repeated presentations the patient learns that the stimulus no longer predicts a negative outcome and his or her anxiety is reduced. However, a major limitation of this type of therapy is that it is context-specific. Although the patient may extinguish their fear in the therapist's office as soon as they are in a different environment, such as at home, and they are presented with the "extinguished" stimulus there is a renewal of anxiety. While extinction is context-specific, reconsolidation deficits do not show renewal after a contextual shift. In addition, reminder shocks will reinstate extinction memory, but do not reinstate memory after reconsolidation inhibition (Duvarci & Nader, 2004). Interrupting reconsolidation after a memory is reactivated has the potential to be a more effective treatment for anxiety disorders than just extinction alone.

However, reconsolidation can be used in conjunction with extinction to enhance extinction of fear memories. LeDoux and colleagues have shown that a single retrieval

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trial given prior to an extinction session (i.e. multiple presentations of the CS-alone) attenuates freezing, blocks reconsolidation, and prevents the return of fear in rats (Monfils et al., 2008). These findings were adapted to a fear conditioning study in humans where subjects were presented with two CSs that were paired with a shock. The next day, subjects were given a single presentation of one of the CSs and afterwards both CS were extinguished. Upon testing only fear to the non-reactivated CS returned, suggesting that reconsolidation enhances the effects of extinction (Schiller et al., 2008). These results are promising towards the treatment of anxiety disorders without the use of drugs. Understanding these mechanisms may provide novel tools towards eradicate traumatic memories long after they are acquired (Tronson & Taylor, 2007).

# References

Adams, C. D. (1982). Variations in the sensitivity of instrumental responding to reinforcer devaluation. *Quarterly Journal of Experimental Psychology Section B-Comparative and Physiological Psychology, 34*, 77-98.

Adams, C. D., & Dickinson, A. (1981). Instrumental responding following reinforcer devaluation. *Quarterly Journal of Experimental Psychology Section B-Comparative and Physiological Psychology, 33*, 109-122.

Alberini, C. M. (2005). Mechanisms of memory stabilization: Are consolidation and reconsolidation similar or distinct processes? *Trends Neurosci, 28*(1), 51-56.

Alberini, C. M., Milekic, M. H., & Tronel, S. (2006). Mechanisms of memory stabilization and de-stabilization. *Cell Mol Life Sci, 63*(9), 999-1008.

Anokhin, K. V., Tiunova, A. A., & Rose, S. P. (2002). Reminder effects - reconsolidation or retrieval deficit? Pharmacological dissection with protein synthesis inhibitors following reminder for a passive-avoidance task in young chicks. *Eur J Neurosci, 15*(11), 1759-1765.

Arana, F. S., Parkinson, J. A., Hinton, E., Holland, A. J., Owen, A. M., & Roberts, A. C. (2003). Dissociable contributions of the human amygdala and orbitofrontal cortex to incentive motivation and goal selection. *J. Neurosci., 23*(29), 9632-9638.

Bahar, A., Dorfman, N., & Dudai, Y. (2004). Amygdalar circuits required for either consolidation or extinction of taste aversion memory are not required for reconsolidation. *Eur J Neurosci, 19*(4), 1115-1118.

Bailey, D. J., Kim, J. J., Sun, W., Thompson, R. F., & Helmstetter, F. J. (1999). Acquisition of fear conditioning in rats requires the synthesis of mrna in the amygdala. *Behavioral Neuroscience, 113*(2), 276-282.

Balleine, B. W., & Dickinson, A. (2000). The effect of lesions of the insular cortex on instrumental conditioning: Evidence for a role in incentive memory. *J Neurosci, 20*(23), 8954-8964.

Balleine, B. W., Killcross, A. S., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *J. Neurosci., 23*(2), 666-675.

Balleine, B. W., & Killcross, S. (2006). Parallel incentive processing: An integrated view of amygdala function. *Trends Neurosci, 29*(5), 272-279.

Baxter, M. G., Bucci, D. J., Holland, P. C., & Gallagher, M. (1999). Impairments in conditioned stimulus processing and conditioned responding after combined

selective removal of hippocampal and neocortical cholinergic input. *Behav Neurosci, 113*(3), 486-495.

Baxter, M. G., Parker, A., Lindner, C. C. C., Izquierdo, A. D., & Murray, E. A. (2000). Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. *J. Neurosci., 20*(11), 4311-4319.

Berridge, K. C. (2001). Reward learning: Reinforcement, incentives, and expectations. In *The psychology of learning and movtivation* (Vol. 40, pp. 223-278). San Diego: Academic Press.

Bindra, D. (1974). A motivational view of learning, performance, and behavior modification. *Psychol Rev, 81*(3), 199-213.

Bindra, D. (1978). How adaptive behavior is produced: A perceptual-motivation alternative to response reinforcement. *Behavioral and Brain Sciences, 1*, 41-91.

Blundell, P., Hall, G., & Killcross, S. (2001). Lesions of the basolateral amygdala disrupt selective aspects of reinforcer representation in rats. *J. Neurosci., 21*(22), 9018-9026.

Blundell, P., Hall, G., & Killcross, S. (2003). Preserved sensitivity to outcome value after lesions of the basolateral amygdala. *J. Neurosci., 23*(20), 7702-7709.

Bolles, R. C., Riley, A. L., Cantor, M. B., & Duncan, P. M. (1974). The rat's failure to anticipate regularly scheduled daily shock. *Behav Biol, 11*(3), 365-372.

Bucherelli, C., Baldi, E., Mariottini, C., Passani, M. B., & Blandina, P. (2006). Aversive memory reactivation engages in the amygdala only some neurotransmitters involved in consolidation. *Learn Mem, 13*(4), 426-430.

Bustos, S. G., Maldonado, H., & Molina, V. A. (2006). Midazolam disrupts fear memory reconsolidation. *Neuroscience, 139*(3), 831-842.

Cestari, V., Costanzi, M., Castellano, C., & Rossi-Arnaud, C. (2006). A role for erk2 in reconsolidation of fear memories in mice. *Neurobiol Learn Mem, 86*(2), 133-143.

Colon-Cesario, M., Wang, J., Ramos, X., Garcia, H. G., Davila, J. J., Laguna, J., et al. (2006). An inhibitor of DNA recombination blocks memory consolidation, but not reconsolidation, in context fear conditioning. *J Neurosci, 26*(20), 5524-5533.

Corbit, L. H., Muir, J. L., & Balleine, B. W. (2001). The role of the nucleus accumbens in instrumental conditioning: Evidence of a functional dissociation between accumbens core and shell. *J Neurosci, 21*(9), 3251-3260.

Davey, G. C. L. (1989). Ucs revaluation and conditioning models of acquired fears. *Behaviour Research and Therapy, 27*(5), 521-528.

Debiec, J., & Ledoux, J. E. (2004). Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience, 129*(2), 267-272.

Delamater, A. R. (2007). The role of the orbitofrontal cortex in sensory-specific encoding of associations in pavlovian and instrumental conditioning. *Ann N Y Acad Sci, 1121*, 152-173.

Dickinson, A. (1989). Expectancy theory in animal conditioning. In R. R. M. Stephen & B. Klein (Eds.), *Contemporary learning theories: Pavlovian conditioning and the status of traditional learning theory* (pp. 279-308). Hillsdale, NJ: Lawrence Erlbaum Associates, Inc.

Dickinson, A., & Balleine, B. (1994). Motivational control of goal-directed action. *Animal Learning & Behavior, 22*, 1-18.

Dickinson, A., & Balleine, B. (1995). Motivational control of instrumental action. *Current Directions in Psychological Science, 4*, 162-167.

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., & Boakes, R. A. (1995). Motivational control after extended instrumental training. *Animal Learning & Behavior, 23*, 197-206.

Doyere, V., Debiec, J., Monfils, M. H., Schafe, G. E., & LeDoux, J. E. (2007). Synapse-specific reconsolidation of distinct fear memories in the lateral amygdala. *Nat Neurosci, 10*(4), 414-416.

Duvarci, S., & Nader, K. (2004). Characterization of fear memory reconsolidation. *J Neurosci, 24*(42), 9269-9275.

Everitt, B. J., Cardinal, R. N., Parkinson, J. A., & Robbins, T. W. (2003). Appetitive behavior: Impact of amygdala-dependent mechanisms of emotional learning. *Ann N Y Acad Sci, 985*, 233-250.

Fanselow, M. S., & Gale, G. D. (2003). The amygdala, fear, and memory. *Ann N Y Acad Sci, 985*, 125-134.

Gallagher, M., McMahan, R. W., & Schoenbaum, G. (1999). Orbitofrontal cortex and representation of incentive value in associative learning. *J. Neurosci., 19*(15), 6610-6614.

Goosens, K. A., Holt, W., & Maren, S. (2000). A role for amygdaloid pka and pkc in the acquisition of long-term conditional fear memories in rats. *Behavioural Brain Research, 114*(1-2), 145-152.

Gottfried, J. A., & Dolan, R. J. (2004). Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nat Neurosci, 7*(10), 1144-1152.

Gottfried, J. A., O'Doherty, J., & Dolan, R. J. (2003). Encoding predictive reward value in human amygdala and orbitofrontal cortex. *Science, 301*(5636), 1104-1107.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., & Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with pavlovian second-order conditioning and reinforcer devaluation effects. *Journal of Neuroscience, 16*(16), 5256-5265.

Holland, P. C. (2004). Relations between pavlovian-instrumental transfer and reinforcer devaluation. *Journal of Experimental Psychology:  Animal Behavior Processes, 30*, 104-117.

Holland, P. C., & Gallagher, M. (1993). Amygdala central nucleus lesions disrupt increments, but not decrements, in conditioned stimulus processing. *Behav Neurosci, 107*(2), 246-253.

Holland, P. C., & Gallagher, M. (2004). Amygdala-frontal interactions and reward expectancy. *Current Opinion in Neurobiology, 14*(2), 148-155.

Holland, P. C., & Straub, J. J. (1979). Differential effects of two ways of devaluing the unconditioned stimulus after pavlovian appetitive conditioning. *J Exp Psychol Anim Behav Process, 5*(1), 65-78.

Hosoba, T., Iwanaga, M., & Seiwa, H. (2001). The effect of ucs inflation and deflation procedures on `fear' conditioning. *Behaviour Research and Therapy, 39*(4), 465-475.

Hull, C. L. (1943). *Principles of behavior: An introduction to behavior theory*. New York: D. Appleton-Century Co.

Izquierdo, A., & Murray, E. A. (2004). Combined unilateral lesions of the amygdala and orbital prefrontal cortex impair affective processing in rhesus monkeys. *J Neurophysiol, 91*(5), 2023-2039.

Kida, S., Josselyn, S. A., de Ortiz, S. P., Kogan, J. H., Chevere, I., Masushige, S., et al. (2002). Creb required for the stability of new and reactivated fear memories. *Nat Neurosci, 5*(4), 348-355.

Killcross, S., Robbins, T. W., & Everitt, B. J. (1997). Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature (London), 388*(6640), 377-380.

Koh, M. T., & Bernstein, I. L. (2003). Inhibition of protein kinase a activity during conditioned taste aversion retrieval: Interference with extinction or reconsolidation of a memory? *Neuroreport, 14*(3), 405-407.

Konorski, J. (1967). *Integrative activity of the brain*. Chicago: University of Chicago Press.

Lee, J. L., Everitt, B. J., & Thomas, K. L. (2004). Independent cellular processes for hippocampal memory consolidation and reconsolidation. *Science, 304*(5672), 839-843.

Lin, C. H., Yeh, S. H., Lin, C. H., Lu, K. T., Leu, T. H., Chang, W. C., et al. (2001). A role for the pi-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. *Neuron, 31*(5), 841-851.

Maren, S. (1998). Overtraining does not mitigate contextual fear conditioning deficits produced by neurotoxic lesions of the basolateral amygdala. *Journal of Neuroscience, 18*(8), 3088-3097.

Maren, S. (1999). Neurotoxic basolateral amygdala lesions impair learning and memory but not the performance of conditional fear in rats. *J. Neurosci., 19*(19), 8696-8703.

Maren, S., Ferrario, C. R., Corcoran, K. A., Desmond, T. J., & Frey, K. A. (2003). Protein synthesis in the amygdala, but not the auditory thalamus, is required for consolidation of pavlovian fear conditioning in rats. *European Journal of Neuroscience, 18*(11), 3080-3088.

Miller, M. M., Altermus, M., Debiec, J., LeDoux, J. E., & Phelps, E. A. (2004). *Propranolol impairs reconsolidation of conditioned fear memory in humans.* Paper presented at the Society for Neuroscience, San Diego.

Monfils, M. H., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2008). *Behavioral blockade of reconsolidation prevents return of fear.* Paper presented at the Society for Neuroscience, Washington D.C.

Morris, J. S., & Dolan, R. J. (2004). Dissociable amygdala and orbitofrontal responses during reversal fear conditioning. *NeuroImage, 22*(1), 372-380.

Nader, K., Schafe, G. E., & Le Doux, J. E. (2000a). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature, 406*(6797), 722-726.

Nader, K., Schafe, G. E., & LeDoux, J. E. (2000b). The labile nature of consolidation theory. *Nat Rev Neurosci, 1*(3), 216-219.

Pertwee, R. G. (2001). Cannabinoid receptors and pain. *Prog Neurobiol, 63*(5), 569-611.

Phan, K. L., Angstadt, M., Golden, J., Onyewuenyi, I., Popovska, A., & de Wit, H. (2008). Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. *J Neurosci, 28*(10), 2313-2319.

Pickens, C. L., Saddoris, M. P., Gallagher, M., & Holland, P. C. (2005). Orbitofrontal lesions impair use of cue-outcome associations in a devaluation task. *Behav Neurosci, 119*(1), 317-322.

Pickens, C. L., Saddoris, M. P., Setlow, B., Gallagher, M., Holland, P. C., & Schoenbaum, G. (2003). Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. *J. Neurosci., 23*(35), 11078-11084.

Pitman, R. K., & Delahanty, D. L. (2005). Conceptually driven pharmacologic approaches to acute trauma. *CNS Spectr, 10*(2), 99-106.

Pitman, R. K., Sanders, K. M., Zusman, R. M., Healy, A. R., Cheema, F., Lasko, N. B., et al. (2002). Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biol Psychiatry, 51*(2), 189-192.

Przybyslawski, J., Roullet, P., & Sara, S. J. (1999). Attenuation of emotional and nonemotional memories after their reactivation: Role of beta adrenergic receptors. *J Neurosci, 19*(15), 6623-6628.

Rolls, E. T. (2004). The functions of the orbitofrontal cortex. *Brain and Cognition, 55*(1), 11-29.

Rumsfeld, J. S., & Nallamothu, B. K. (2008). The hope and fear of rimonabant. *Jama, 299*(13), 1601-1602.

Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase a in the amygdala. *Journal of Neuroscience, 20*(18), RC96.

Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of pavlovian fear conditioning: A cellular and molecular perspective. *Trends in Neurosciences, 24*(9), 540-546.

Schiller, D., Monfils, M. H., JOhnson, D. C., Raio, C. M., LeDoux, J. E., & Phelps, E. A. (2008). *Behavioral blockade of the return of fear in humans.* Paper presented at the Society for Neuroscience, Washington D.C.

Schoenbaum, G., Setlow, B., Nugent, S. L., Saddoris, M. P., & Gallagher, M. (2003a). Lesions of orbitofrontal cortex and basolateral amygdala complex disrupt acquisition of odor-guided discriminations and reversals. *Learn. Mem. %R 10.1101/lm.55203, 10*(2), 129-140.

Schoenbaum, G., Setlow, B., & Ramus, S. J. (2003b). A systems approach to orbitofrontal cortex function: Recordings in rat orbitofrontal cortex reveal interactions with different learning systems. *Behavioural Brain Research, 146*(1-2), 19-29.

Toates, F. (1986). *Motivational systems*. Cambridge, UK: Cambridge University Press.

Tolman, E. C., & Postman, L. (1954). Learning. *Annu Rev Psychol, 5*, 27-56.

Tronel, S., Milekic, M. H., & Alberini, C. M. (2005). Linking new information to a reactivated memory requires consolidation and not reconsolidation mechanisms. *PLoS Biol, 3*(9), e293.

Tronson, N. C., & Taylor, J. R. (2007). Molecular mechanisms of memory reconsolidation. *Nat Rev Neurosci, 8*(4), 262-275.

Unger, W., Evans, I. M., Rourke, P., & Levis, D. J. (2003). The s-s construct of expectancy versus the s-r construct of fear: Which motivates the acquisition of avoidance behavior? *J Gen Psychol, 130*(2), 131-147.

von Hertzen, L. S., & Giese, K. P. (2005). Memory reconsolidation engages only a subset of immediate-early genes induced during consolidation. *J Neurosci, 25*(8), 1935-1942.

Wadman, M. (2006). Rimonabant adds appetizing choice to slim obesity market. *Nat Med, 12*(1), 27.

Wei, F., Qiu, C. S., Liauw, J., Robinson, D. A., Ho, N., Chatila, T., et al. (2002). Calcium-calmodulin-dependent protein kinase iv is required for fear memory. *Nature Neuroscience, 5*(6), 573-579.

White, K., & Davey, G. C. L. (1989). Sensory preconditioning and ucs inflation in human `fear' conditioning. *Behaviour Research and Therapy, 27*(2), 161-166.

Zimmerman, J. M., Rabinak, C. A., McLachlan, I. G., & Maren, S. (2007). The central nucleus of the amygdala is essential for acquiring and expressing conditional fear after overtraining. *Learn Mem, 14*(9), 634-644.