REVERSIBLE INACTIVATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS BLOCKS REINSTATEMENT BUT NOT RENEWAL OF EXTINGUISHED FEAR

A Thesis

by

JANICE JUNGMIN KIM

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

MASTER OF SCIENCE

Chair of Committee,	Stephen Maren
Committee Members,	Mark Packard
	Paul Wellman
	Jane Welsh
Head of Department,	Doug Woods

December 2014

Major Subject: Psychology

ABSTRACT

The bed nucleus of the stria terminalis (BNST) is thought to be involved in the expression of fear to shock-associated contexts, but not to discrete conditional stimuli (CSs) paired with shock. Because context plays an important role in the extinction and relapse of fear, we sought to examine the contribution of the BNST to two different relapse phenomena: renewal and reinstatement. In the renewal experiment, male Long-Evans rats received 5 tone-shock trials for conditioning in “context A”; 24 hours later they received 45 tone–alone (extinction) trials in either “context B” or “context C”. Ten minutes prior to a retrieval test (5 tone-alone trials), rats were infused with either selective agonist for $GABA_A$ receptors, muscimol or vehicle in the BNST. In the reinstatement experiment, rats underwent a similar procedure, but were presented with an unsignaled ‘reminder’ shock in the extinction context to reinstate fear. As before, we examined the influence of muscimol inactivation of the BNST during a retrieval testing 24-hours after the reinstatement shock. In the reinstatement test, rats with muscimol infusion showed significantly less freezing than did rats with vehicle infusion. In contrast, BNST inactivation did not attenuate the renewal of fear to an extinguished CS outside the extinction context. These data indicate that the BNST is involved in forms of fear relapse that depend on direct associations of the test context with an aversive US.

DEDICATION

This thesis is dedicated to my parents: Young Ki Kim and Kyung Hye Ahn. This thesis would not have been possible without their constant support, encouragement, and guidance.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Stephen Maren for his supervision and patience. I also wish to thank my committee members, Dr. Mark Packard, Dr. Paul Wellman, and Dr. Jane Welsh for their guidance throughout the course of my degree program.

I am grateful to have fellow members of the Maren lab. It has been my pleasure sharing lab space with you all.

Travis, I have enjoyed sitting next to you and spending time with you in lab. I have had the good fortune to work with such a brilliant scientist. Your constant support and help made this thesis possible. I appreciate your patience and guidance throughout the work that we have done together.

Jingji, I have loved spending time with you in and out of main office. Thanks for your cheers of encouragement. I appreciate all of your assistance on many experiments.

Finally, thanks to my family for their faith in me, and also, I thank Damian for his constant love and support.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

The work of the thesis presented here is composed of two different experiments, and thus requires a broad background. In this introductory chapter, use of Pavlovian conditioning in fear learning and memory and neural circuitry involved in acquisition, extinction, and relapse of fear will be discussed. Then, the neural framework underlying fear learning, will be addressed. Finally, the role of bed nucleus of stria terminalis (BNST), the brain site of interest in this thesis work, in fear memory will be explained. The aim of this thesis work is to investigate the role of the BNST in renewal and reinstatement of extinguished fear.

I.1 Statement of Problem

Among psychiatric illnesses, anxiety disorders have a high incidence and an elevated societal cost (Pincus & Pettit, 2001). The lifetime prevalence of anxiety disorders is estimated at about 29% of the population in the United States and 4.5% of global population (Kessler, et al., 2005; Demyttenaere, et al., 2004). Of these, there are anxiety disorders which are driven with fear emotion. The representative of fear-driven mental disorder is posttraumatic stress disorder (PTSD). PTSD is a severe, chronic mental illness that develops after exposure to a traumatic event such as warfare, serious injury, or sexual assault and causes intense fear and a feeling of helplessness. In recent years, one of the vast interests in the field of behavioral neuroscience is to find an

effective approach to extinguish learned fear to develop effective behavioral therapies and pharmacological treatments for patients with anxiety disorders.

It is known that learning of fear memories is rapid and robust, but extinguishing these memories is slow and susceptible to disruption. Therefore, currently available psychotherapeutic approaches that are built upon extinction learning have major drawbacks such as context dependency. For instance, exposure therapy is not long lasting because it is not generalized across various environments and circumstances. Because extinction learning is found to only temporarily suppress fear memories, rather than erase them, fear can easily return as a consequence. This return, or "relapse" of fear is a great challenge for maintaining robust and long-lasting fear suppression after behavioral therapies. Therefore, from a clinical perspective, developing novel therapeutic interventions is essential. Furthermore, from a neuroscientific point of view, it is critical to find the neural circuitry underlying the relapse of extinguished fear.

I.2 Pavlovian Fear Conditioning, Extinction, and Fear Relapse

Learning from experience and consolidating memory is critical for survival, particularly when it involves threats in the environment. Fear is one of the most basic emotions; it is programmed into the nervous system. Fear emotion produces a physiological and behavioral response to the present or anticipated occurrence of a dangerous stimulus. Learning to fear is critical for survival. However, intense experience of fear may be deleterious or incapacitating, producing dysregulated fear response. Consequently, learning *not* to fear is critical as well in order to regulate and control emotion to modify behavioral responses appropriately.

Decades of work in animal fear models have relied on Pavlovian conditioning, which is a behavioral procedure to study the brain mechanisms underlying fear learning and memory. Pavlovian fear conditioning is a form of associative learning in which a neutral stimulus such as a tone, or a conditioned stimulus (CS), is paired with an aversive stimulus such as a footshock, or an unconditioned stimulus (US). One or more presentations of this CS-US paring enables the animal to rapidly acquire a robust association between the CS and the US (Bouton, 1988; Fanselow, 1998). In addition to the CS, the animal associates the US the environmental stimuli, or context, in which the footshock was presented (Maren, et al., 2013). The CS-US association results in the transformation of the animal's response to the CS which was previously neutral and did not pose a threat. After being presented with the pairing, the animal learns that the CS is a dangerous stimulus, predicting an unsafe event (i.e. footshock). Thus, the CS evokes conditioned responses (CR) of fear, including freezing, especially in the context the CS was encountered. After fear conditioning (FC), the learned CR can be suppressed by repeated presentation of CS in the absence of the US (Pavlov, 1927; Rescorla, 2000). This procedure is called extinction.

Similar to FC, extinction involves learning and memory. Historically, extinction was perceived as 'unlearning' of the previously learned CS-US association (Rescorla, 1969; Rescorla & Wagner, 1972). Researchers argued that the CS-US association that elicits the CR was weakened, and through extinction training, the CS loses its significance and no longer induces the CR. However, other researchers hypothesized that extinction is a form of active inhibitory learning that suppresses the previously learned

CS-US association (Bouton, 2004; Myers & Davis, 2002). The majority of investigations into extinction learning have supported the latter hypothesis that an organism learns an entirely new CS-*no* US association. In other words, extinction is not considered as an erasure of previously learned fear memories, but rather generates a new extinction memory. Over the course of the extinction, the original CS-US association is not degraded, and both excitatory and inhibitory representations of the CS remain (Bouton, 2004).

Convincing evidence behind the notion that extinction does not eliminate previously learned fear memories is based on relapse phenomena. There are four types of fear relapse phenomena: spontaneous recovery, reacquisition, renewal, and reinstatement. Spontaneous recovery occurs when significant amount in passage of time after extinction, and an extinguished CR to a CS is returned (Bouton, 1993; Rescorla, 1997). Reacquisition phenomenon is exhibited when the subject is reintroduced with the original CS-US pairing after extinction training is conducted, and the behavioral procedure induces reacquisition of CR (Bouton, 2002; Napier, et al., 1992). Renewal is observed when an extinguished CS is presented outside of the extinction context, and the fear to the tone 'renews' (Bouton & Nelson, 1994). Lastly, reinstatement of fear response is observed when the US is presented alone after extinction training, and it causes a return of extinguished CR to the CS (Bouton & Bolles, 1979; Westbrook, et al., 2002). These phenomena would not be possible if the original association between CS and US was degraded, and also, if fear extinction is characterized as forgetting of FC memories.

I.3 Renewal and Reinstatement of Extinguished Fear

The standard procedure for extinction is to expose a subject with repeated CS-alone trials to produce a reduction in CR. However, as discussed above, extinction is not permanent and does not indicate a total loss of CS-US association due to evidence of fear relapse. In addition, extinction memories are characterized as short-lived and highly context-dependent. Due to its context-dependence, extinction imposes a "mask" that is relatively specific to the context in which extinction training was given.

Renewal is a key phenomenon that demonstrates that extinction involves acquisition of a context-specific inhibitory-like association. For instance, when the extinguished CS is given in a context that is different from the context where a subject received extinction, then the previously learned fear memory will be returned (Bouton, 2004; Neumann & Longbottom, 2008; Vervliet, et al., 2013). Thus, the subject re-establishes the CR to the CS.

In animal models, the "ABC design", which is a typical behavioral procedures, is designed to generate context-dependent retrieval of extinguished fear memory (Bouton & Bolles, 1979; Bouton & Ricker, 1994). All subjects receive conditioning training in Context A with tone-shock trials. Then, extinction training is performed either in Context B or Context C with tone-alone trials. Finally, all subjects are tested in Context B with tone-alone trials to examine renewal of fear to the CS. The group that received both extinction and renewal testing in the same context, Context B, does not express return of fear to the CS, tone. However, the group which received extinction in Context C, and renewal testing in Context B expresses fear renewal to the tone since the CS is

presented in the context that is different from the extinction context. The same tone in the testing context, which was presented equally for all subjects, retrieved two different memories. Based on the assignment to extinction context, one group which underwent an order of ABC contexts retrieved fear memory to the CS whereas a group which underwent an order ABB contexts retrieved safe memory to the CS. Again, this emphasizes that extinction is not erasing previously learned fear memories. The renewal of fear is also observed in human patients. Through exposure therapy, suppression of pathological fear that is acquired in a treatment context such as a therapist's office may not be exhibited in non-treatment contexts that patients would encounter daily life (Rodriguez, et al., 1999). Renewal shows that the breaking CS-US contingency by multiple series of CS-alone presentation does not block the retrieval of what had been learned about the original contingency (Rescorla & Wagner, 1972).

As renewal occurs due to context-dependence in extinction, *reinstatement* is attributed to context-US associations. Reinstatement occurs when a one or more presentations of the US results in recovery of extinguished CR. In other words, the CR can be 'reinstated' by administering an unsignaled US in absence of the CS, after extinction procedure is conducted (Rescorla & Heth, 1975). Previously, it has been shown that the unsignaled US must be delivered in the original conditioning context in order to produce reinstatement phenomenon because the US exposure in a novel context does not result in reinstatement (Bouton, 1984; Bouton & King, 1984).

However, recent findings from Westbrook and colleagues in 2002 revealed that the reinstatement phenomenon is not always context specific. Specifically, in

Experiment 4 of their study, rats were conditioned in Context A to two types of CSs: a clicker and white noise. For extinction, rats was extinguished to the clicker in Context B and white noise in Context C. Following extinction procedures, all rats were exposed to a reminder US, footshock in Context B. Lastly, they were tested to both CSs in a novel, 'irrelevant' context, Context D. Their data showed that rats had higher fear to the clicker than to white noise when they were tested in D. When extinction and US re-exposure occur in the same context, but testing is conducted in another context, the CR is reinstated in a CS-specific manner because the CS and US re-exposure are linked by their common context associate. In contrast to Bouton et al. (1984), results from Westbrook et al. (2002) supports the idea that reinstatement is not context specific.

In recent years, considerable effort has been focused on understanding the mechanisms underlying renewal and reinstatement. These two fear relapse phenomena share the importance of contextual information in fear responding. However, there are differences between them. Renewal is considered as similar to 'occasion setting' (Holland, 1992) in which a subject learns a CS-US association during FC and a CS-no US association during extinction, and that the different contexts determine which of these association is retrieved. The mechanism is different from the reinstatement which is considered to be a more specific phenomenon that is attributable to context-US associations (Frohardt, et al., 2000). Moreover, there is evidence showing that brain regions underlying renewal and reinstatement are different. Wilson, Brooks, and Bouton (1995) found that lesions of the fornix abolished reinstatement but not renewal effect. Because one route of hippocampal-amygdala connectivity is via the fimbria-fornix

fibers, Frohardt, et al. (2000) showed that reinstatement is abolished with hippocampal lesions because the amygdala and hippocampus are functionally dissociated, effectively preventing the CS-US association from reforming (LeDoux, 1993). Renewal may be mediated by entorhinal and cingulate cortices, which do not primarily communicate with the hippocampus via fimbria-fornix routes in rats (Swanson & Cowan, 1977). However, recent studies have found that lesions of the dorsal hippocampus impairs fear renewal in both ABA and AAB designs (Ji & Maren, 2005). Corcoran, et al. (2005) also has found that dorsal hippocampal lesions produced a significant impairment in context-dependent fear renewal.

I.4 Neural Circuitry of Fear Learning

Several decades of research have deciphered brain mechanisms related to fear learning. Studies on neural circuitry of fear learning have revealed that central amygdala (CeA) and basolateral amygdala (BLA) are essential for acquisition and expression of conditioned fear (Fanselow & Poulos, 2005; Maren & Quirk, 2004). Expression of conditioned fear involves CS transmission to BLA, connections from BLA to the CeA either directly or by way of intra-amygdala connections, and then output connections from CeA to various regions that control specific CRs.

On the other hand, extinction seems to be involved in plasticity in the amygdala (AMYG) (Falls, et al., 1992; Quirk & Mueller, 2008). Also, the neural basis for fear extinction is believed to involve connections between the medial prefrontal cortex (mPFC) and the AMYG (Savander, et al., 1996; Garcia, et al., 1999, Quirk & Gehlert, 2003). It has found that firing in BLA neurons increase in response to the CS after fear

conditioning, and the fear response decrease in probability and magnitude after extinction of CS-alone presentations (Quirk, et al., 1995). The CS-elicited firing in the AMYG is context-dependent in which there is return of CR to the CS when the CS is presented in a context that is different from extinction context (Hobin, et al., 2003). This renewal phenomenon induces a return of CS-elicited activity in BLA neurons in response to the extinguished CS. A recent study by Herry, et al. (2008), suggested that some AMYG neurons activate in response to the CS during renewal in context that is different from extinction context, and others fire in response to the CS during the reduction of fear in the extinction context.

Extinction produces a new CS-*no* US association that is encoded in the AMYG to induce a reduction in CR. There is consensus that the hippocampus (HIPP) is involved in regulating the context-dependence of extinction memory. Previous work on HIPP manipulation in the renewal paradigm indicated there was a reduction in renewal of fear to an extinguished CS under reversible inactivation of the HIPP (Corcoran & Maren, 2001). Thus, inactivation of HIPP impairs the retrieval of the CS-context associations. Considerable work has supported that CS-elicited firing in AMYG depends on the HIPP (Krasne, et al., 2011; Maren & Hobin, 2007).

The majority of the data in literature suggests that there are interactions among HIPP, mPFC, and AMYG involved in contextual retrieval of fear memory. For instance, pharmacological inactivation of the HIPP disrupts context-dependent firing in the AMYG (Maren, 2007) and blocks fear renewal (Corcoran & Maren, 2001). Also, the inactivation of the ventral HIPP modulates the activity in the prelimbic regions of the

mPFC, in turn impairs fear renewal (Hobin, et al., 2006). Furthermore, when the ventral HIPP afferent pathways to either the BLA or prelimbic of mPFC is disconnected, fear renewal would not be observed. Renewal and reinstatement shares the neural network of structures including HIPP, PFC, and AMYG. As renewal, inactivation of the HIPP disrupts the reinstatement (Frohardt, et al., 2000), suggesting that the HIPP plays a crucial role in encoding context representations.

I.5 Role of the BNST in Fear Learning

As reviewed above, extinction memories are highly context-dependent and only expressed in the context where the subject receives extinction. Learned fear to context appears to be a major cause of fear relapse. Given the critical role of context associations in fear relapse phenomena, the BNST may be critical for fear relapse.

In recent years, several studies have reported that the BNST may be important relay station linking critical forebrain structures involved in conditioned fear response to context such as the AMYG, HIPP and mPFC to the hypothalamus and autonomic regulatory brainstem areas (Fanselow, 2000; Huff & Rudy, 2004). In addition, the BNST also modulates behavioral responses to aversive stimuli. Studies using lesions or reversible inactivation demonstrated that the BNST is important for the expression of aversive responses such as freezing, mean arterial pressure (MAP) and heart rate increases. A recent study from Haufler, Nagy, and Pare (2013) found that activity and volume of the BNST are positively correlated with the level of anxiety. Also, the BNST is known to directly modulate stress responding via the hypothalamus (Casada & Dafny 1992; Erb et al. 2001).

The BNST is anatomically positioned to have a role in anxiety and the renewal and reinstatement of extinguished fear. The BNST is characterized by reciprocal connections with the medial and CeA (Alheid & Heimer, 1988; Shammah-Lagnado, et al., 2000) and receives projections from HIPP. Then, the BNST projects to brain sites that are responsible for the regulation of heart rate as well as brain areas that are critical for the vigilance exhibited in fearful and anxious states, such as the locus coeruleus, among other brain stem areas involved in the responses to fear and anxiety (Davis, 1998; Dong, et al., 2000).

Davis and colleagues study (1997) suggested that there are behavioral and neuroscientific data distinguishing between anxiety and fear. The amygdala has a critical role in the acquisition and expression of CR, but the BNST appears to have a role in aversive emotional states, similar to anxiety. The researchers suggested that the CeA and BLA are essential in the acquisition and expression of fear to CSs associated with a footshock, US, whereas, an intact BNST is not critical for freezing, the fear expression. The BNST is necessary, however, for unconditional enhancement of the startle reflex in rats through central administration of corticotrophin-releasing hormone and extended exposure to bright lights, whereas the central nucleus of the amygdala is not necessary. Davis and colleagues suggested that the latter manipulations initiate anxiety rather than fear. Sullivan and colleagues (2004) have found that a disruption of freezing to contextual cues but not a CS when rats were tested following lesions of the BNST. These findings argue that the BNST does not play a role in aversive states that are

elicited by brief, discrete CSs, but rather by cues that are diffuse and of a long duration – the context.

Collectively, it appears that the BNST activity is selectively involved in the expression of fear to conditioned contexts, but not to an auditory CS (Sullivan et al., 2004; Zimmerman & Maren, 2011). Zimmerman and Maren (2011) demonstrated that fear response to CS is intact after lesions or inactivation of the BNST, but the manipulation impaired expression of CR to the context. However, lesions of the BNST have been shown to attenuate shock-induced reinstatement of fear (Waddell, et al. 2006). This finding reveals that the BNST may be able to indirectly modulate response of fear relapse to the CS.

Here, I explored the role of the bed nucleus of the stria terminalis (BNST) in modulating two different forms of fear relapse in rats: *reinstatement* and *renewal* of fear. The research determined whether reversible inactivation of the BNST is capable of preventing reinstatement while leaving renewal of fear intact. In doing so, this thesis work illuminated a functional dichotomy in the role of the BNST in these different forms of relapse, which may serve to enhance selective brain treatments for anxiety disorders.

CHAPTER II

RENEWAL

This chapter consisted of Experiment 1 using the renewal paradigm.

II.1 Introduction

The aim of Experiment 1 was to examine role of the BNST in renewal of extinguished fear, using either muscimol or NBQX, as a pharmacological inactivation tool. A 2 × 2 factorial design was used with four groups: DRUG-Diff, DRUG-Same, VEH-Diff, and VEH-Same (see below). Corcoran and Maren (2001) used the same experimental design with a HIPP manipulation. They found that muscimol-induced inactivation of a dorsal hippocampus (DH) disrupts the contextual retrieval of extinction memory in the ABC design (see below). Because the BNST is innervated by the HIPP, it may participate in hippocampal-dependent renewal. Findings in the work of reversible inactivation as well as neurotoxic lesions of the BNST disrupt the expression of contextual but not cued fear (LeDoux, et al., 1988; Sullivan, et al., 2004; Walker & Davis, 1997). However, recent studies report that the BNST is functionally heterogeneous (Kim, et al., 2013), suggesting that neural activity within the BNST may not be necessary for generating learned fear, but it exerts a tonic inhibitory influence on fear output networks. Thus, the hypothesis for Experiment 1 is that reversible inactivation of the BNST in a familiar but different context from extinction context will not block fear renewal given that context fear is not required for renewal.

II.2 Methods

II.2.1 Subjects

All 35 subjects were adult (60-90 days of age) male Long-Evans (Blue Spruce) rats from Harlan Laboratories (Houston, TX, USA). Rats were individually housed in clear plastic cages on a rotating rack (Animal Care Systems, Inc.) Group assignments for behavioral procedures were randomized for cage position on the rack. Each rat was handled for 1 min per day for at least 5 days prior to the start of surgery. In addition, rats were habituated to the infusion procedures in the infusion room prior to behavioral procedures. Rats were maintained on a 14 h light/dark cycle (lights come on at 7:00 AM). At the time of surgery, rats weighted between 200 and 250 g. All handling, surgeries, and behavioral procedures were conducted during the light phase of the light/dark cycle. The procedures were approved by the Institutional Animal Care and Use Committee at the Texas A&M University.

II.2.2 Surgery

After a period of handling, rats underwent intracranial stereotaxic surgery to implant 23 gauge guide cannulae bilaterally into the BNST. Prior to surgery, rats were anesthetized with intraperitoneal (i.p.) injections of ketamine (100 mg/kg) and muscle relaxant xylazine (10 mg/kg), and they were also injected i.p. with atropine methyl nitrate (0.02 mg/kg). Eye lubricant was applied for each rat. The area of incision above the skull was shaved with a hair trimmer. Rats were placed in a stereotaxic frame (Kopf Instruments), and blunt ear-bars were maintained to fix a head position. Povidone-iodine pad was applied prior to injection of 0.5 mL of lidocaine in the tissue above the skull as

a local anesthetic. A small incision was made with a scalpel. The skull was leveled with bregma and λ on an even horizontal plane to achieve a flat position. A hand drill was used to make five small holes. Two holes were drilled for steel guide cannulae, and three holes were drilled for stainless steel screws. Rats were implanted bilaterally with 23-gauge steel guide cannulae (Small Parts, Inc.) The cannulae were slowly lowered into the BNST over a minute. The stereotaxic coordinates for the BNST, in mm from bregma and dura, were: AP: 0 mm; ML: ±2.7 mm; DV: -6.9 mm. Also, the cannulae were angled at 10°. Three 3.175 mm stainless steel screws were embedded in the skull in order to help secure the guide cannulae in place once cemented with dental cement. Dental cement (methyl methacrylate liquid and powder compound) was applied on top of the skull to secure the cannulae and screws. A dummy cannula, or stainless steel obturators (30 gauge, 9 mm; Small Parts, Inc.), was kept in each guide cannula at all times, except during infusions. The dummy cannulae were changed every two days prior to the start of behavioral procedures. Immediately after surgery, rats were given a single bacon-flavored Rimadyl tablet (2 mg/tablet; Bio-Serv). Rats were allowed at least seven days to recover from surgery prior to the start of behavioral procedures.

II.2.3 Behavioral Apparatus

For all experiments, behavioral training and testing were conducted in either one of two rooms in the laboratory: Room 1 (smaller size) or Room 2 (larger size). Eight identical experimental chambers (30 cm [width] × 24 cm [length] × 21 cm [height]; Med Associates, Inc.) were placed in each room. Inside the behavioral chambers, including ceiling, rear wall, and hinged front door, were constructed with Plexiglas. Side walls

were constructed with aluminum. The bottom of each chamber, or grid floor, was built of nineteen stainless steel rods that are 4 mm in diameter, spaced 15 mm apart. The grid floor was connected to a constant-current generator, or a shock source, in order to deliver the US (Med Associates, Inc.). Beneath the rods, an aluminum pan was placed in each chamber to collect animal waste and hold context odor (see below). Each chamber is equipped with a speaker to provide the CS, 15-W house bulb for contextual lighting, and a small fan to provide background noise (~70 dB).

Each chamber rests upon a load-cell platform, which responds to the animal's movement on the grid floor. Load-cell activity values (ranging from -10 V to +10 V) are sent to and digitized by Threshold Activity Software (Med Associates, Inc.) on a remote computer. Threshold Activity Software converts load-cell activity into absolute values and multiplies the numbers by ten to generate a range of activity from 0 to 100 in every 200 msec. The higher values indicate greater in the animal's movement. Rats are considered to be freezing when the absolute values of load-cell activity are ≤10 for 2 sec or more. Each chamber was enclosed in wooden cabinets (59 cm [width] × 83 cm [height] × 59 cm [height]). The chambers were wiped down and cleaned with assigned context odor between squads.

Experimental contexts were designed to be distinct from one another by use of different odors and visual cues. Testing chamber assignments were randomized for group assignments. When the same context was used, rats were returned to the same testing chamber. For Experiment 1, Context A was used for conditioning procedure. Context A was assigned in Room 1 and consisted of acetic acid odor (~50 mL of 1.5%

acetic acid solution) which is poured in the pans beneath the grid floor, with the lights of the chambers turned off, and white lights of Room 1 was on. The cupboard doors of the chambers were left open throughout the behavior procedure conducted in Context A. When Context A was in use for behavioral procedure, rats were transported in white transport containers from the vivarium and to the laboratory, using a cart.

Context B and Context C (as per group assignment) were used for extinction and renewal testing procedure. Context B was assigned in Room 2 and consisted of ammonium hydroxide odor (~50 mL of 1% ammonium hydroxide solution) which is poured in pans beneath the grid floor, with the lights of the chambers turned on, and red lights of Room 2 was on. The cupboard doors of the chambers were closed throughout the behavior procedure conducted in Context B. When Context B was in use for behavioral procedure, rats were transported in black plastic containers from the vivarium and to the laboratory. Context C was assigned in Room 1 and consisted of ethanol odor (~50 mL of 70% ethanol solution) which is poured in pans beneath the grid floor, with the lights of the chambers turned on, and red lights of Room 1 was on. Additionally, a thin, black plastic sheet was placed on the grid floor to add uniqueness to Context C. The cupboard doors of the chambers were closed throughout the behavior procedure conducted in Context C. When Context C was in use for behavioral procedure, rats were transported in black plastic containers from the vivarium and to the laboratory, using a cart.

The experimenters were not present in the rooms at the time of behavioral procedures, and rats' behavior was recorded remotely.

II.2.4 Behavioral Procedures

Table 1 is an overview of the protocol that is designed for behavioral paradigm used for Experiment 1.

Group	Phase				Context Design
	Day1	Day2	Day3		
	Conditioning	exposure, then Extinction	Infusion	Renewal Testing	
○ DRUG-Diff	AT+	B−, then CT− or C−, then BT−	DRUG	○ T− in exposure context	ABC/ ACB
□ DRUG-Same			DRUG	□ T− in Extinction context	ABB/ ACC
○ VEH-Diff			VEH	○ T− in exposure context	ABC/ ACB
□ VEH-Same			VEH	□ T− in Extinction context	ABB/ ACC

Table 1. Experimental design for Experiment 1. The behavioral procedures begin from left to right of Table 1. Each phase is separated by 24-hours. However, to note, infusion phase occurred 10-minute prior to renewal testing. (A, B & C = experiment context; T = tone, CS; + = US presentation; − = US absence.)

II.2.4.1 Group Assignment

Experiment 1 consisted of 2 × 2 design with variables of drug infusion and renewal testing context. Rats were randomly assigned to an infusion group (DRUG or VEH) and a renewal testing context group (Diff or Same). The rats in Same group received renewal testing in the context where they received extinction procedure. On the other hand, the rats in Diff group received renewal testing in the context where they received exposure procedure. A total of 76 rats underwent the surgeries and behavioral procedures. Of those, 41 rats were excluded from the results based on their cannulae placements. As seen in Table 2, following four groups of subjects were formed in order to carry out Experiment 1: DRUG-Diff, n = 11; DRUG-Same, n = 6; VEH-Diff, n = 8;

VEH-Same, *n* = 10. For Experiment 1, rats assigned to DRUG group received 0.3 µL of 1.0 µg/µL muscimol or 0.3 µL of 10.0 µg/µL 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) per hemisphere 10 min prior to renewal testing procedure.

Muscimol is a potent, selective agonist for $GABA_A$ receptors. NBQX is a potent AMPA and kainite receptor antagonist. Of the seventeen rats assigned for DRUG, eleven were infused with NBQX, and six were infused with muscimol. Rats assigned to VEH group received 0.3 µL of physiological saline which is also referred to vehicle. For first three cohorts of Experiment 1 (each cohort is consisted of 16 rats), muscimol was used to infuse DRUG group. For following two cohorts of Experiment 1, NBQX was used to infuse DRUG group. The reason for using two different types of pharmacological inactivation tool was that muscimol may have affected locomotor activity of rats in DRUG group. In order to rule out that freezing behavior is not due to lack of locomotor activity that is induced by muscimol, NBQX was chosen to be infused. Thus, rats in DRUG group were either infused with muscimol or NBQX.

Testing / **Drug**	○ **Diff** (ABC/ ACB)	□ **Same** (ABB/ ACC)
DRUG	DRUG-Diff	DRUG-Same
VEH	VEH-Diff	VEH-Same

Table 2. Group assignment for Experiment 1

Table 3 represents number of subjects in each group based on drug type and context design for behavioral procedures.

Group	Drug Type	Testing	Context Design	# of subjects (*n*)	*n* per group
○ **DRUG-Diff**	MUS	Diff	ACB	4	11
	NBQX		ACB	3	
	NBQX		ABC	4	
□ **DRUG-Same**	MUS	Same	ABB	2	6
	NBQX		ABB	2	
	NBQX		ACC	2	
○ **VEH-Diff**	VEH	Diff	ACB	4	8
	VEH		ABC	4	
□ **VEH-Same**	VEH	Same	ABB	7	10
	VEH		ACC	3	
				Total (*N*)	**35**

Table 3. Number of subjects in each group for Experiment 1.

II.2.4.2 Conditioning

On Day 1 of behavioral procedure, animals were fear conditioned to an auditory tone, with group assignments counterbalanced by behavioral squad. Each squad was consisted of maximum of eight rats and can be trained at the same time. After three minutes of habituation to the chamber, all rats received five CS-US pairings (CS = 80 dB, 10 sec, 2 kHz, auditory tone; US = 1.0 mA, 2.0 sec, footshock) pairings in Context A. Following the final, fifth CS-US pairing, rats were left in the chamber for one minute before being returned to their home cages.

II.2.4.3 Extinction

On Day 2, when is twenty-four hours after Day 1, all rats were extinguished either in Context B or Context C. The assignment for extinction context was counterbalance by group. In Experiment 1, a single day of extinction procedure was conducted. Prior to extinction, rats that receive extinction in Context B were exposed to Context C for equal amount of duration, and rats that receive extinction in Context C were exposed to Context B as well. The reason behind giving Exposure phase was to minimize the effect of potential problems for generating fear to the novel context when renewal testing procedure is conducted. Extinction consisted of forty-five CS alone trials, with each trial separated by 30 sec intervals. Rats were given with three minutes for habituation to the extinction context prior to the start of CS alone trials. After final, forty-fifth presentation of the CS, rats were left in the chamber for three minutes before being returned to home cages.

II.2.4.4 Renewal Testing

On Day 3, renewal testing was conducted. Based on group assignment, rats were infused with either drug (muscimol or NBQX) or vehicle 10 min prior to renewal testing procedure. Infusion phase took a place in a separate room from behavioral testing. Rats were infused with drug or vehicle at a rate of 0.3 μL/min for a total volume of 0.3 μL per hemisphere. For the infusion procedure, experimenters utilized an infusion syringe pump (KD Scientific, Inc.) to draw up either drug or vehicle into stainless steel injection needles (30 gauge, 9.0 mm; Small Parts, Inc.) immediately prior to infusion. The injection needles were attached to polyethylene tubing (PE-20; Braintree Scientific,

Inc.); the tubing was inserted over gastight 10 μL syringes (Hamilton, Co.) that were resting on the infusion pump. Experimenters gently removed the dummy cannulae from the guide cannulae on the rats, and the stainless steel injection needles were inserted into the guide cannulae. Following the infusion of drug or vehicle, the injection needles remained in the guide cannulae for one minute for diffusion before being removed and clean dummy cannulae inserted. Tubing and injection needles were flushed with water between each infusion cohort and cleaned with ethanol after the entire set of infusions.

Following infusion phase, rats underwent renewal testing procedure. All subjects were tested to the five CS alone trials either in the context which was used for extinction to the CS for the SAME group or in the context which was used for exposure phase for the DIFF group to induce renewal to the extinguished CS. In detail, rats that received extinction in Context B were tested for renewal in the same Context B if they were assigned to SAME. On the other hand, rats that received extinction Context C were tested for renewal in familiar but different Context B. Thus, there were two types of behavioral procedure design for each group; DIFF group underwent either ABC or ACB design, and SAME group underwent either ABB or ACC design. Rats were given a three-minute baseline prior to the start of CS alone trials during the renewal testing. The intertrial intervals were 30 sec. After final, fifth presentation of the CS, rats were left in the chamber for three minutes before being returned to home cages.

II.2.5 Data Analysis

Freezing behavior served as the index of fear and the dependent variable in the statistical analyses. Rats were considered to be freezing if immobile (i.e., values of ≤10

in Threshold Activity Software; refer to Behavioral Apparatus) for ≥2 sec. The number and type of testing trials used for the analyses for each day of training is described under Behavioral Procedures. Analysis of variance (ANOVA) and post-hoc analyses (Fisher's Protected LSD) identified significant *p*-values (set at <0.05) when critical *F* ratios were revealed in the data set (statistical software StatView 5.0, SAS Institute, NC, USA). All data are represented as means ±SEMs. Effect size was calculated by partial eta squared (η_p^2) for ANOVA results (SPSS 20.0, IBM Corporation). Only rats with injection sites localized within the BNST (in both hemispheres) are included as part of the data analyses of this thesis document.

II.2.5.1 Conditioning

Freezing (%) for conditioning to the CS was analyzed across six trials: one trial accounted for baseline activity within the three-minute period of habituation, five more trials were generated for freezing (%) during each minute following the CS-US pairings.

II.2.5.2 Extinction

Freezing (%) for extinction was analyzed across eleven trials: one trial for baseline activity within the three-minute period of habituation, nine more trials accounted for mean levels of freezing (%) across blocks of five post-CS intervals; a final trial accounted for mean levels of freezing (%) during the final two minutes of rats in the extinction chamber.

II.2.5.3 Renewal Testing

Freezing (%) for renewal testing data was analyzed along seven trials: a single trial accounted for freezing (%) during the three-minute baseline, five more trials were

generated for freezing (%) during the 30 sec following each of the post-CS intervals, and a final trial accounted for freezing (%) in the 150 sec following the final post-CS interval. In addition to the post-CS intervals, freezing (%) during each 10-sec tone was calculated and analyzed separately (five tone trials in total).

II.3 Results

II.3.1 Histology

Following the conclusion of behavioral procedures, all rats were overdosed on pentobarbital and transcardial perfusions were performed with physiological saline and 10% formalin solution. Brains were dissected from the skull and placed in 10% formalin for 24 hrs at 4° C. After, brains were placed in a solution of 10% formalin and 30% sucrose for at least 3 days before sectioning. Coronal sections (40 µm) were collected on a cryostat (Leica Microsystems) at –20°C and mounted onto subbed slides. Sliced tissue was stained with 0.25% thionin to identify cannula tracts in the tissue and to localize injection sites. Refer to Figure 1 for an example of cannulae tracts localized within the BNST.

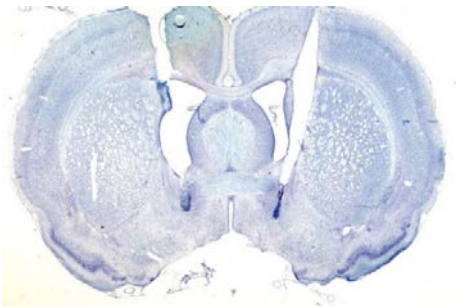

Figure 1. Representative photomicrograph of a thionin-stained coronal section. The coronal section (40 µm) of the tissue is from the brain of a rat with cannulae placements in the BNST.

Figure 2A represents histological placements from Experiment 1. Of all subjects, 35 rats were included in the data analysis based on their correct cannulae placements.

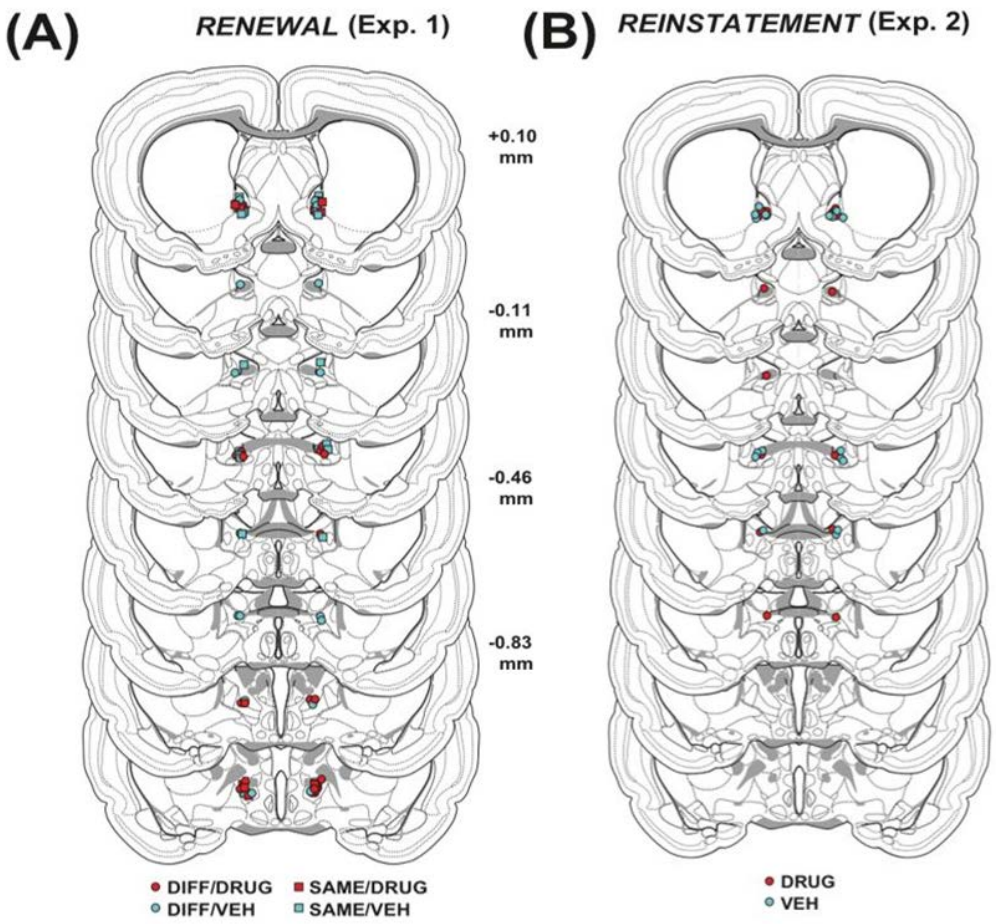

Figure 2. Illustration of guide cannulae placements in the BNST (split by Experiment 1 and 2). For Experiment 1 **(A)**, placements are representative of all rats included in the final analyses in *renewal* paradigm. For Experiment 2 **(B)**, placements are representative of all rats included in the final analyses in *reinstatement* paradigm. Adapted from Swanson (1992). Distances shown are relative to bregma.

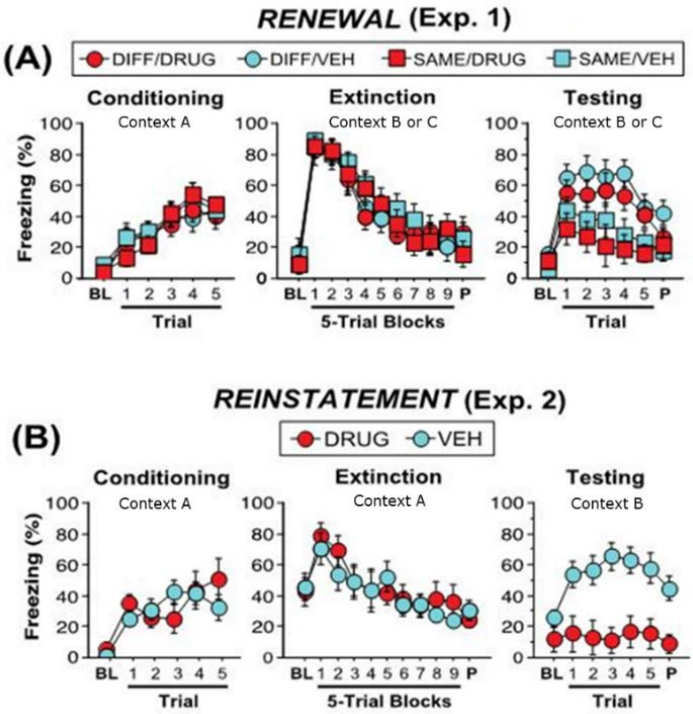

Figure 3. Conditioned freezing behavior in rats throughout the behavioral procedures. **(A)** Data from each phase in Experiment 1 with *renewal* paradigm are represented. Conditioning graph depicts the mean ±SEM percentage of freezing during fear conditioning, which consisted of a 3-min baseline (BL) period followed by five tone–shock pairings. Freezing was averaged across the pre-CS BL as well as during each of the five 1-min post-CS−US intervals. Extinction graph is consisted of mean ±SEM percentage of freezing during the 45 tone-alone extinction session. Freezing was averaged across the BL period as well as during each of 45 30-sec post-CS intervals. Data in extinction phase were binned into 9 blocks of 5-trial averages. Lastly, freezing was measured during a 150-sec post-trial phase (P). Testing graph is consisted of mean ±SEM percentage of freezing during the renewal testing session which involves five CS-alone trials with 30-sec intervals. Freezing was measured during the BL period, during the five 30-sec post-CS intervals, and during the P. Data are shown for rats that were tested outside the extinction context after Drug infusion (DIFF/DRUG; red circles), tested outside the extinction context after Vehicle infusion (DIFF/VEH; blue circles), tested within the extinction context after Drug infusion (SAME/DRUG; red squares), or tested within the extinction context after Vehicle infusion (SAME/VEH; blue squares). **(B)** Data from each phase in Experiment 2 with reinstatement paradigm are represented. Conditioning graph is consisted of mean ±SEM percentage of freezing during fear conditioning. Freezing was measured the 3- min BL period and during each of five 1-min post-CS−US intervals. Extinction graph in Experiment 2 represents data from the Day 3, which is the second day of extinction session. Extinction graph is consisted of mean ±SEM percentage of freezing during the 45 tone-alone extinction session. Freezing was averaged across the BL period as well as during each of 45 30-sec post-CS intervals. Data in extinction phase were binned into 9 blocks of 5-trial averages. Lastly, freezing was measured during the P. Testing graph is consisted of mean ±SEM percentage of freezing during the renewal testing session which involves 10-min BL followed by five CS-alone trials with 30-sec intervals. Freezing was measured during the BL, five 30-sec post-CS intervals, and the P of a 150-sec post-trial interval. Data are shown for rats that were infused with Drug (DRUG; red circles) or were infused with Vehicle (VEH; blue circles).

II.3.2 Data Report

On Day 1 of Experiment 1, all rats acquired the CS-US associations robustly (Figure 3A). There was a significant main effect of trial as rats increased in freezing level over the course of CS-US pairings, $F_{(5,155)} = 31.193$, $p < 0.0001$, $\eta_p^2 = 0.502$. No significant difference was observed between the groups for renewal testing context; In other words, freezing levels during conditioning were not significantly different between Diff and Same groups, $F_{(1,155)} = 0.293$, n.s.. Also, freezing levels did not differ based on group assignments for infusion, $F_{(1,155)} = 0.193$, n.s.. Fear to the CS is shown in Figure 3A for extinction phase. All rats showed a significant reduction in their freezing level across trials. There was a main effect of trial, $F_{(10,310)} = 50.607$, $p < 0.0001$, $\eta_p^2 = 0.62$. As expected, there was no main effect of context assignment, $F_{(1,310)} = 0.449$, n.s.. Also, there was no difference between Drug and Veh groups, $F_{(1,310)} = 0.083$, n.s..

Immediately prior to renewal testing, rats were infused either with drug or vehicle. Responses during renewal testing procedure is depicted in Figure 3A. The results revealed that, as predicted, pharmacological inactivation of the BNST was not sufficient to block renewal of extinguished fear. Specifically, there was a significant difference between group assignments based on renewal testing context. Rats that were in Diff group had significantly higher level of fear than rats that were in Same group overall across trials, $F_{(1,186)} = 12.843$, $p = 0.0011$, $\eta_p^2 = 0.327$. However, there was no significant interaction between infusion and renewal testing context, $F_{(1,186)} = 0.080$, n.s., indicating that infusion of drug to the BNST did not significantly affect the expression of fear renewal. There was a significant interaction between trial and renewal testing

context, $F_{(6,186)} = 2.647$, $p = 0.0173$, $\eta_p^2 = 0.079$, indicating that the higher fear response of rats in the renewal condition was specific to the post-CS intervals. For all groups, baseline of context fear was low. Although Drug groups exhibited a trend of less fear overall compared to Veh groups, there was no main effect of infusion assignment, $F_{(1,186)} = 1.669$, n.s.. Moreover, there was no significant difference between rats that were infused with muscimol and rats that were infused with NBQX across all renewal testing trials, $F_{(1,90)} = 0.035$, n.s.. For renewal testing context assignments, there was no main effect, $F_{(1, 90)} = 0.058$, n.s.. Taken together, the results from Experiment 1 show that reversible pharmacological inactivation of the BNST does not block renewal of extinguished fear.

II.4 Discussion

The results of Experiment 1 revealed that when the testing context is different from the extinction context but is not directly associated with the US, footshock, the BNST inactivation did not prevent fear renewal. The hypothesis was supported by the current results. Previous research has indicated that the lesions of the BNST did not attenuate tone fear (Sullivan, et al., 2004) and has found that there is no role for the BNST in fear responses to an over trained CS (Zimmerman & Maren, 2011). These results are in agreement that the BSNT does not appear to interact with cued fear, directly. However, it is interesting to note that current results from this thesis work and work by others (Waddell, et al., 2006) suggest that BNST manipulations are capable of mediating relapse to an extinguished CS, at least indirectly. Data from Experiment 1

suggest that the BNST has a role in modulating the expression of fear to the CS when the context holds no history of US presentation.

CHAPTER III

REINSTATEMENT

This chapter consisted of Experiment 2 using the reinstatement paradigm.

III.1 Introduction

The aim of Experiment 2 was to examine role of the BNST in reinstatement of extinguished fear. A between-subjects design was used with two groups: DRUG and VEH (see below).

As it was discussed above, when CS-US pairings are followed by repeated presentations of CS-alone, then the animal will exhibit a reduction in CR. However, this can be reversed when the US is presented alone between extinction and testing, which is called reinstatement. As renewal, reinstatement of the CR depends heavily on contextual learning (Bouton & Bolles, 1979). In the human literature, amnesic patients fail to show reinstatement extinguished fear after presentations of the aversive US alone outside the conditioning context despite being able to acquire the original fear association. This suggests that hippocampal damage produces deficits in context encoding or conditioning, which is consistent with findings from many animal studies (Maren, et al., 2013).

Given the important role of context associations in the reinstatement paradigm and that the BNST is both innervated by the HIPP and the AMYG and plays a role in contextual fear, the hypothesis for Experiment 2 is that inactivation of the BNST with

muscimol will reduce contextual fear preceding presentations of the extinguished CS in a conditioning context; thus, BNST inactivation would block reinstatement of extinguished fear.

III.2 Methods

III.2.1 Subjects

The subjects were 17 experimentally naïve, adult, male Long-Evans (Blue Spruce) rats from the same source and maintained under the same conditions as Experiment 1.

III.2.2 Surgery

Rats underwent the same surgery procedure as Experiment 1. After surgery, rats were allowed at least seven days to recover from surgery prior to the start of behavioral procedures. A dummy cannula, or stainless steel obturators (30 gauge, 9 mm; Small Parts, Inc.), was kept in each guide cannula at all times, except during infusions. The dummy cannulae were changed every two days prior to the start of behavioral procedures.

III.2.3 Behavioral Apparatus

The apparatus and stimuli used were the same as that used in Experiment I. However, for Experiment 2, Context A and Context B were used as experimental contexts. In reinstatement paradigm, Context A was used for conditioning and extinction procedures. Context B was used for reinstating shock phase and testing phase for reinstatement. Both Context A and Context B consisted of same elements and uniqueness as described in Experiment 1.

III.2.4 Behavioral Procedures

Table 4 is an overview of the protocol that is designed for behavioral paradigm used for Experiment 2.

Group	Phase				
	Day1	Day2	Day3	Day4	
	Conditioning	Extinction	Reinstating Shock	Infusion	Reinstatement Testing
DRUG	AT+	AT−	B+	Muscimol	BT−
VEH				Vehicle	

Table 4. Experimental design for Experiment 2. The behavioral procedures begin from left to right of Table 2. Each phase is separated by 24-hours. However, to note, infusion phase occurred 10-minute prior to reinstatement testing. (A & B = experiment context; T = tone, CS; + = US presentation; − = US absence.)

III.2.4.1 Group Assignment

For Experiment 2, rats were randomly assigned to two different infusion groups prior to the start of behavioral procedures. After undergoing cannulae surgery, rats were either assigned to DRUG group or VEH group: DRUG, $n = 7$; VEH, $n = 10$. A total of 32 rats underwent the surgeries and behavioral procedures. Of those, 15 rats were excluded from the results based on their cannulae placements.

As seen in Table 5, following two groups of subjects were formed. Rats assigned to DRUG group were infused with 0.3 µL of 1.0 µg/µL muscimol, a selective $GABA_A$ receptor agonist, into the BNST per hemisphere immediately prior to testing, whereas 'VEH' rats were infused with 0.3 µL of physiological saline per hemisphere. Because there was no effect of locomotor activity by muscimol, NBQX was not used in Exp. 2.

Table 5. Number of subjects in each group for Experiment 2.

Group	# of subjects (*n*)
DRUG	7
VEH	10
Total (*N*)	**17**

III.2.4.2 Conditioning

On Day 1 of behavioral procedure, animals were conditioned via five CS-US pairings as described in Experiment 1.

III.2.4.3 Extinction

Twenty-four hours later, and over the course of two days, all rats underwent extinction to the CS. Rats were given two consecutive days of extinction to avoid ceiling effects at the time of reinstatement testing. For each day of extinction, rats were placed back in the conditioning context (Context A), with three minutes of acclimation to the testing chamber before the first CS presentation. Forty-five CS presentations were administered per day of extinction, separated by 30 sec intertrial intervals. Following the final CS presentation, rats remained in the testing chamber for three minutes.

III.2.4.4 Reinstating Shock

On Day 4, when is twenty-four hours after the final day of extinction, rats were placed in Context B for three minutes. After three minutes in the chamber, all rats experienced a weak unsignaled footshock (1 sec, 0.4 mA).

III.2.4.5 Reinstatement Testing

On Day 5, reinstatement testing was conducted. Based on group assignment, rats were infused with either drug or vehicle 10 min prior to reinstatement testing procedure. Infusion phase took a place in the same room as described in Experiment 1. The experimenters followed the same infusion protocol as explained in Experiment 1. Rats were infused with drug or vehicle at a rate of 0.3 μL/min for a total volume of 0.3 μL per hemisphere.

Following infusion phase, rats underwent reinstatement testing procedure. Rats were placed in Context B for ten minutes prior to the start of the first of five CS alone trials; The ten-minute baseline is given to ensure that reinstatement would not be masked by high levels of context fear preceding CS onset. CS presentations were separated by 30 sec intervals, and rats remained in the testing chambers for three minutes following the final CS presentation.

III.2.5 Data Analysis

Data analysis was performed as same as described in Experiment 1.

III.2.5.1 Conditioning

Freezing (%) was analyzed along the same number and type of trials as described for Experiment 1.

III.2.5.2 Extinction

Freezing (%) for extinction training in Experiment 2 was analyzed per day along the same number and type of trials as for a single day of extinction as described for Experiment 1.

III.2.5.3 Reinstating Shock

The level of fear was analyzed across two trials for Day 4. First trial was generated for baseline freezing (%) in Context B prior to footshock, and a second trial was generated for freezing (%) during a minute following footshock.

III.2.5.4 Reinstatement Testing

Freezing (%) for reinstatement testing was analyzed along the same number and type of trials as for Experiment 1. But the baseline trial in Experiment 2 refers to freezing (%) during ten minutes of exposure prior to CS onset.

III.3 Results

III.3.1 Histology

Figure 1 shows the representative histology with cannulae tracts localized in the BNST.

III.3.2 Data Report

Figure 2B represents histological placements from Experiment 2. On Day 1 of behavioral procedures, rats exhibited normal and robust conditioning to the auditory CS (Figure 3B), without significant differences between rats assigned to drug or vehicle. There was a significant main effect of trial as rats increased in freezing level over the course of CS-US pairings, $F_{(5,75)} = 9.060$, $p < 0.0001$, $\eta_p^2 = 0.377$. As expected, no significant difference was observed between the groups for infusion; In other words, freezing levels during conditioning were not significantly different between DRUG and VEH groups, $F_{(1,75)} = 0.096$, n.s.. Additionally, there was no trial × infusion group interaction effect for conditioning phase, $F_{(5,75)} = 1.623$, n.s..

Over the course of Day 2 and Day 3, all rats received extinction in Context A. Figure 3B shows freezing level of rats on Day 3. Extinction was robust for each day. Specifically, on Day2, there was a main effect of extinction trial as there was a reduction in freezing level of rats to multiple presentations of the CS, $F_{(10,150)} = 10.613$, $p < 0.0001$, $\eta_p^2 = 0.414$. No difference was detected between the two infusion groups, $F_{(1,150)} = 0.079$, n.s.. There was no trial × infusion group interaction effect for extinction phase, $F_{(10,150)} = 0.265$, n.s.. Both infusion groups extinguished equally. On Day 3, there also was a main effect of extinction trial, $F_{(10,150)} = 7.938$, $p < 0.0001$, $\eta_p^2 = 0.346$. There was neither a main effect of infusion group [$F_{(1,150)} = 0.083$, n.s.] nor a trial × infusion group interaction [$F_{(10,150)} = 0.630$, n.s.]. Taken together, extinction in Experiment 2 was normal.

On Day 4, when all rats received reinstating shock, rats experienced a weak reinstating footshock in a novel context, or Context B. A main effect of trial was detected [$F_{(1,15)} = 49.531$, $p < 0.0001$, $\eta_p^2 = 0.768$] as rats increased in freezing level following the reinstating footshock. This fear response did not differ based on infusion assignment, indicating that there was no main effect of infusion group assignment, $F_{(1,15)} = 0.644$, n.s.. Also, there was no trial × infusion interaction [$F_{(1,15)} = 2.061$, n.s.] for Day 4.

On Day 5, rats were tested to the CS in Context B. Results indicate that BNST inactivation effectively blocked reinstatement of fear to the extinguished CS (see Figure 3B). Specifically, there was a main effect of infusion across all testing trials [$F_{(1,90)} = 12.668$, $p = 0.0029$, $\eta_p^2 = 0.729$], and also a significant trial × infusion group interaction

[$F_{(6,90)}$ = 3.633, p = 0.0028, η_p^2 = 0.195]. In other words, rats infused with drug into the BNST immediately prior to reinstatement testing to the CS showed significantly less fear to the CS than rats infused with vehicle. A main effect of trial was detected [$F_{(6,90)}$ = 4.998, p = 0.0002, η_p^2 = 0.250] as most of the rats increased in freezing level following CS onset. A similar pattern was observed for freezing (%) during the tones, such that DRUG rats showed significantly less fear response than VEH rats, $F_{(1,60)}$ = 8.508, p = 0.0106, η_p^2 = 0.585. A data analysis of responses at the baseline trial indicated that there is a trend but not significant enough to see a main effect of infusion group, $F_{(1,15)}$ = 2.298, n.s. DRUG rats exhibited a trend of having less fear prior to CS onset. Taken together, the results from Experiment 2 indicate that pharmacological inactivation of the BNST can block reinstatement that results from testing the extinguished CS in a shock-associated context.

III.4 Discussion

The results of Experiment 2 support the hypothesis that reversible inactivation of the BNST would prevent reinstatement of fear to the CS, even if the testing context is different from the context where extinction procedure was conducted. As shown in the previous literature, the contextual fear preceding CS onset in the testing context is a primary mechanism for reinstatement of fear an extinguished CS. Although there was no significant main effect of infusion group at the baseline, DRUG rats showed a trend of less fear prior to CS onset compared to VEH group. In Experiment 2, a weaker shock (compared to the footshock that was given in conditioning procedure) was presented to rats in order to prevent VEH rats to have high responding to the extinguished CS. If rats

were given a stronger shock, then there might be a significant different between the two infusion groups. In current study, BNST inactivation may be sufficient to block reinstatement of fear to the CS in the context where it has a history of unsignaled shock.

CHAPTER IV

CONCLUSIONS

The current experiments reveal that when the testing context is directly associated with the US (footshock) reversible inactivation of the BNST prevents reinstatement of fear to the CS, even if the testing context is different from the extinction context (Exp. 2). In contrast, BNST inactivation does not prevent this renewal of fear (Exp. 1), when the testing context is different from the context where extinction procedures were performed. These two sets of experiments implicate that the BNST in various innate anxiety responses, including vigilance in the presence of unconditioned threats (Fendt, et al., 2003; Fendt et al., 2005; Kenny, et al., 2004; Somerville, et al., 2010; Walker & Davis, 1997). The results suggest that the BNST may play a role in anxiety-eliciting activity with relation to sustained cues (Davis, et al., 2010; Walker, et al., 2009). In contrast, the BNST is not involved in acute conditioned fear responses *per se* (Sullivan, et al., 2004; Treit, et al., 1998; Zimmerman & Maren, 2011). Sullivan and colleagues (2004) found that lesions of the BNST did not disrupt normal acquisition of conditioned fear to a cue, nor did it impair acquisition or retention of extinction memory. Sullivan and colleagues (2004) did not attempt to induce reinstatement or renewal of extinguished fear in these animals. However, lesions of the BNST did attenuate contextual fear. In rats with BLA lesions, Zimmerman and Maren (2011) found no role for the BNST in responding to an over-trained CS. This indicates that even under

extreme input of the CS, the BNST does not appear to directly modulate acute fear responses to the CS. From this current set of data and previous work by others (Waddell, et al., 2006), manipulations to the BNST are able to mediate fear relapse to an extinguished CS, indirectly. The BNST may indirectly affect CS responding in paradigms of reinstatement by attenuating the context fear preceding CS onset. Indeed, others have argued that context fear preceding CS onset in the test context is a primary mechanism through which reinstatement manifests to the extinguished cue (Bouton & Bolles, 1979; Waddell, et al, 2006; Westbrook, et al., 2002). Although there was no significant effect of drug solely at the baseline trial, rats on drug trended towards less fear prior to CS onset. In this particular case, rats were presented with a weak reinstating shock so that context fear in vehicle rats would not mask an increase in responding to the extinguished CS. Therefore, if animals were presented with a stronger shock, or the same shock they received on Day 1, baseline of contextual fear might differ significantly between drug and vehicle groups.

In the case of renewal, inactivation of the HIPP, prefrontal cortex (PFC), or AMYG is known to disrupt renewal of fear (Corcoran & Maren, 2004; Ji & Maren, 2005; Ji & Maren, 2007; Maren, 2014; Orsini, et al., 2011; Orsini, et al., 2013). Interestingly, the BNST receives input from these brain regions. However, the current results indicate that the BNST does not play a role in the renewal of extinguished fear. The HIPP, PFC, and AMYG are all highly interconnected, suggesting that during the renewal testing, these regions act uniquely to facilitate the relapse of responding (Knapska & Maren, 2009; Orsini, et al., 2011; Orsini, et al., 2013).

In addition to the experience of reinstating shock, rats in Exp. 2 were tested to the CS outside of its extinction context. This experiment was designed and conducted in order to build on the work of Waddell and colleagues (2006), which showed that BNST lesions prevented reinstatement of fear in the extinction context once the extinction context had been conditioned with shock. Specifically, in Exp. 2 of this report, rats were conditioned in Context A, extinguished in Context A, and tested to the CS in Context B (i.e., "AAB" design) following the reinstating shock in Context B. In Exp. 1, rats were conditioned in Context A, extinguished in Context B or C, and tested to the CS in either the same or different context as used for extinction (i.e., "ABC" or "ACB" renewal). Is Exp. 2 confounded by renewal? Indeed, others have shown renewal effects with "AAB design" (Maren, 2014). However, renewal appears to be more robust in ABC (or ACB) designs when compared to "AAB design" (Maren, 2014).

In the current study, the BNST inactivation may have been sufficient to attenuate relapse in Exp. 2 because unsignaled shock was the most salient and latest threat to the animals. The inefficacy of the BNST inactivation in curbing renewal in Exp. 1 suggests that if renewal were a major factor in Exp. 2, there would not be such a robust effect of drug between two groups. Thus, while not tested in these experiment, this present work suggests that, in the absence of a reinstating shock, BNST inactivation does not attenuate "AAB" renewal. Additionally, although there was no control of "no-shock" group in Exp.2, the data from Exp. 1 indicates that the animals are capable of freezing with muscimol (or NBQX) in the BNST. In other words, under infusion of drug into the BNST, freezing behavior does not appear to be due to sedative effect from muscimol (or

NBQX). The drug effects are likely specific to the role of the BNST in reinstatement. Likewise, the levels of fear of vehicle rats in Experiment 2 are on par with the levels of relapse observed in Experiment 1.

Although the drug effect was not significant in Exp. 1, rats on muscimol or NBQX showed a tendency to freeze less overall as compared to vehicle rats regardless of the renewal condition. This may be related to a non-specific reduction in stress responses as a result of the BNST manipulation. In particular, the BNST is known to directly modulate stress and autonomic responses via its connections with hypothalamic and brain stem structures (Crestani, et al., 2013; Lezak, et al., 2014, Roman, et al., 2014). Lesions of the BNST are known to disrupt corticosterone signaling in rats associated with contextual fear (Sullivan, et al., 2004). In this case, it might be that pharmacological inactivation of the BNST may have blunted stress responses overall, which may have slightly, but not significant enough, blunted fear responses, independent of whether rats were tested in a renewal context or not.

Regions that neighbor the BNST include the hypothalamus, caudoputamen, substantia innominata, and the preoptic area (Canteras, et al., 1995). While it is possible that drug may have spread into these neighboring regions at the time of test, the drug effects is expected to be limited specifically to the BNST based on previous BNST manipulations (e.g., Zimmerman & Maren, 2011). The angle of the cannulae placements helped to precisely target the site and avoid non-BNST-specific effects by reducing the possibility of leakage of drug into the ventricles. On a similar note, others have reported unique behavioral effects based on subregion-specific manipulations of the BNST (Kim,

et al., 2013). Kim and colleagues (2013) reported that photostimulation of activity in the oval nucleus of the BNST resulted in the manifestation of anxiety-driven behaviors, while activity in the anterodorsal region of the BNST resulted in anxiolytic behaviors. In line with this evidence, electrophysiological recordings by Haufler and colleagues in 2013 found unique patterns of activity within the BNST that suggest that certain subregions may oppose one another in response to a CS. Additionally, there is evidence which indicates that anterior and posterior regions of the BNST may be differentially involved in certain behaviors, such as feeding (Kocho-Schellenberg, et al., 2014). These effects, in part, may be related to the relative expression of anxiety- and stress-related signaling systems within regions of the BNST. Based on the histology in the current study, the cannulae placements were located closely within the anterior portion of the BNST, though spread of drug likely affected both anterior and posterior regions of the BNST. It is interesting to note that afferent and internal projections of the BNST are largely GABAergic (Sajdyk, et al., 2008), though the BNST has glutamatergic projections as well (Jennings, et al., 2013; Sparta, et al., 2013). Taken together, these findings suggest that the net behavioral result of BNST manipulations likely depends heavily on the task at hand and which subregions are affected by the manipulation.

Even though it was not examined here, research indicates that the BNST is sexually dimorphic (Allen & Gorski, 1990; Hines, et al., 1992). It is possible that the relapse of fear phenomena that depends on the BNST may interact with the sex of the animal. Future research work should explore this possibility. In addition to future research, substantial research showed the BNST in various reinstatement paradigms of

drug-seeking behavior (Buffalari & See, 2011; Erb, et al., 2001; Stamatakis, et al., 2014). In light of the comorbidity of addiction with anxiety disorders (Conway, et al., 2006), the BNST may be a critical region for the treatment of complex psychiatric disorders that include features of addiction.

In conclusion, this thesis work suggests that selective manipulations of the BNST may be particularly effective in preventing reinstatement of extinguished fear when contextual fear is major factor. However, renewal of fear appears to rely on circuits that may be independent of the BNST. Future studies may need to examine whether subregions of the BNST plays a selective role in these fear relapse phenomena. At large, in order to be effective, the future of brain-specific manipulations aimed at reducing fear-related anxiety should be particularly mindful of the circumstances that may give rise to relapse.

REFERENCES

Alheid, G. F., & Heimer, L. (1988). New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders – The striatopallidal, amygdaloid, and corticopetal components of substantia innominata. *Neuroscience, 27*(1), 1-39.

Allen, L. S., & Gorski, R. A. (1990). Sex difference in the bed nucleus of the stria terminalis of the human brain. *Journal of Comparative Neurology, 302*(4), 697-706.

Bouton, M. E. (1984). Differential control by context in the inflation and reinstatement paradigms. *Journal of Experimental Psychology-Animal Behavior Processes, 10*(1), 56-74.

Bouton, M. E. (1988). Context and ambiguity in the extinction of emotional learning – Implications for exposure therapy. *Behaviour Research and Therapy, 26*(2), 137-149.

Bouton, M. E. (1993). Context, time, and memory retrieval in the interference paradigms of Pavlovian learning. *Psychological Bulletin, 114*(1), 80-99.

Bouton, M. E. (2002). Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. *Biological Psychiatry, 52*(10), 976-986.

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learning & Memory, 11*(5), 485-494.

Bouton, M. E., & Bolles, R. C. (1979). Role of conditioned contextual stimuli in reinstatement of extinguished fear. *Journal of Experimental Psychology-Animal Behavior Processes, 5*(4), 368-378.

Bouton, M. E., & King, D. A. (1984). Conditioning history of the CS affects CS-context interactions. *Bulletin of the Psychonomic Society, 22*(4), 273-273.

Bouton, M. E., & Nelson, J. B. (1994). Context-specificity of target versus feature inhibition in a feature-negative discrimination. *Journal of Experimental Psychology-Animal Behavior Processes, 20*(1), 51-65.

Bouton, M. E., & Ricker, S. T. (1994). Renewal of extinguished responding in a 2nd context. *Animal Learning & Behavior, 22*(3), 317-324.

Buffalari, D. M., & See, R. E. (2011). Inactivation of the bed nucleus of the stria terminalis in an animal model of relapse: Effects on conditioned cue-induced

reinstatement and its enhancement by yohimbine. *Psychopharmacology, 213*(1), 19-27.

Canteras, N. S., Simerly, R. B., & Swanson, L. W. (1995). Organization of projections from the medial nucleus of the amygdala – A phal study in the rat. *Journal of Comparative Neurology, 360*(2), 213-245.

Casada, J. H., & Dafny, N. (1992). Evidence for 2 different afferent pathways carrying stress-related information (noxious and amygdala stimulation) to the bed nucleus of the stria terminalis. *Brain Research, 579*(1), 93-98.

Conway, K. P., Compton, W., Stinson, F. S., & Grant, B. F. (2006). Lifetime comorbidity of DSM-IV mood and anxiety disorders and specific drug use disorders: Results from the national epidemiologic survey on alcohol and related conditions. *Journal of Clinical Psychiatry, 67*(2), 247-257.

Corcoran, K. A., Desmond, T. J., Frey, K. A., & Maren, S. (2005). Hippocampal inactivation disrupts the acquisition and contextual encoding of fear extinction. *Journal of Neuroscience, 25*(39), 8978-8987.

Corcoran, K. A., & Maren, S. (2001). Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *Journal of Neuroscience, 21*(5), 1720-1726.

Corcoran, K. A., & Maren, S. (2004). Factors regulating the effects of hippocampal inactivation on renewal of conditional fear after extinction. *Learning & Memory, 11*(5), 598-603.

Crestani, C. C., Alves, F. H. F., Gomes, F. V., Resstel, L. B. M., Correa, F. M. A., & Herman, J. P. (2013). Mechanisms in the bed nucleus of the stria terminalis involved in control of autonomic and neuroendocrine functions: A review. *Current Neuropharmacology, 11*(2), 141-159.

Davis, M. (1998). Are different parts of the extended amygdala involved in fear versus anxiety? *Biological Psychiatry, 44*(12), 1239-1247.

Davis, M., Walker, D. L., & Lee, Y. (1997). Amygdala and bed nucleus of the stria terminalis: Differential roles in fear and anxiety measured with the acoustic startle reflex. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 352*(1362), 1675-1687.

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: Role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacology, 35*(1), 105-135.

Demyttenaere, K., Bruffaerts, R., Posada-Villa, J., Gasquet, I., Kovess, V., Lepine, J. P., . . . Conso, W. H. O. W. M. H. S. (2004). Prevalence, severity, and unmet need for treatment of mental disorders in the World Health Organization World Mental Health Surveys. *Jama-Journal of the American Medical Association, 291*(21), 2581-2590.

Erb, S., Salmaso, N., Rodaros, D., & Stewart, J. (2001). A role for the CRF-containing pathway from central nucleus of the amygdala to bed nucleus of the stria terminalis in the stress-induced reinstatement of cocaine seeking in rats. *Psychopharmacology, 158*(4), 360-365.

Falls, W. A., Miserendino, M. J. D., & Davis, M. (1992). Extinction of fear-potentiated startle – Blockade by infusion of an NMDA antagonist into the amygdala. *Journal of Neuroscience, 12*(3), 854-863.

Fanselow, M. S. (1998). Pavlovian conditioning, negative feedback, and blocking: Mechanisms that regulate association formation. *Neuron, 20*(4), 625-627.

Fanselow, M. S. (2000). Contextual fear, gestalt memories, and the hippocampus. *Behavioural Brain Research, 110*(1-2), 73-81.

Fanselow, M. S., & Poulos, A. M. (2005). The neuroscience of mammalian associative learning. *Annual Review of Psychology, 56*, 207-234.

Fendt, M., Endres, T., & Apfelbach, R. (2003). Temporary inactivation of the bed nucleus of the stria terminalis but not of the amygdala blocks freezing induced by trimethylthiazoline, a component of fox feces. *Journal of Neuroscience, 23*(1), 23-28.

Fendt, M., Siegl, S., & Steiniger-Brach, B. (2005). Noradrenaline transmission within the ventral bed nucleus of the stria terminalis is critical for fear behavior induced by trimethylthiazoline, a component of fox odor. *Journal of Neuroscience, 25*(25), 5998-6004.

Frohardt, R. J., Guarraci, F. A., & Bouton, M. E. (2000). The effects of neurotoxic hippocampal lesions on two effects of context after fear extinction. *Behavioral Neuroscience, 114*(2), 227-240.

Garcia, R., Vouimba, R. M., Baudry, M., & Thompson, R. F. (1999). The amygdala modulates prefrontal cortex activity relative to conditioned fear. *Nature, 402*(6759), 294-296.

Haufler, D., Nagy, F. Z., & Pare, D. (2013). Neuronal correlates of fear conditioning in

the bed nucleus of the stria terminalis. *Learning & Memory, 20*(11), 633-641.

Herry, C., Ciocchi, S., Senn, V., Demmou, L., Muller, C., & Luthi, A. (2008). Switching on and off fear by distinct neuronal circuits. *Nature, 454*(7204), 600-U628.

Hines, M., Allen, L. S., & Gorski, R. A. (1992). Sex-differences in subregions of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis of the rat. *Brain Research, 579*(2), 321-326.

Hobin, J. A., Goosens, K. A., & Maren, S. (2003). Context-dependent neuronal activity in the lateral amygdala represents fear memories after extinction. *Journal of Neuroscience, 23*(23), 8410-8416.

Hobin, J. A., Ji, J. Z., & Maren, S. (2006). Ventral hippocampal muscimol disrupts context-specific fear memory retrieval after extinction in rats. *Hippocampus, 16*(2), 174-182.

Holland, P. C. (1992). Occasion setting in Pavlovian conditioning. *Psychology of Learning and Motivation-Advances in Research and Theory, 28*, 69-125.

Huff, N. C., & Rudy, J. W. (2004). The amygdala modulates hippocampus-dependent context memory formation and stores cue-shock associations. *Behavioral Neuroscience, 118*(1), 53-62.

Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., Kash, T. L., & Stuber, G. D. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature, 496*(7444), 224-228.

Ji, J. Z., & Maren, S. (2005). Electrolytic lesions of the dorsal hippocampus disrupt renewal of conditional fear after extinction. *Learning & Memory, 12*(3), 270-276.

Ji, J. Z., & Maren, S. (2007). Hippocampal involvement in contextual modulation of fear extinction. *Hippocampus, 17*(9), 749-758.

Kenny, S. L., Wright, L. D., Green, A. D., Mashoodh, R., & Perrot, T. S. (2014). Expression of maternal behavior and activation of the bed nucleus of the stria terminalis during predatory threat exposure: Modulatory effects of transport stress. *Physiology & Behavior, 123*, 148-155.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions' of DSM-IV disorders in the national comorbidity survey replication. *Archives of General Psychiatry, 62*(6), 593-602.

Kim, S. Y., Adhikari, A., Lee, S. Y., Marshel, J. H., Kim, C. K., Mallory, C. S., . . .

Deisseroth, K. (2013). Diverging neural pathways assemble a behavioural state from separable features in anxiety. *Nature, 496*(7444), 219-223.

Knapska, E., & Maren, S. (2009). Reciprocal patterns of c-Fos expression in the medial prefrontal cortex and amygdala after extinction and renewal of conditioned fear. *Learning & Memory, 16*(8), 486-493.

Kocho-Schellenberg, M., Lezak, K. R., Harris, O. M., Roelke, E., Gick, N., Choi, I., . . . Hammack, S. E. (2014). PACAP in the BNST produces anorexia and weight loss in male and female rats. *Neuropsychopharmacology, 39*(7), 1614-1623.

Krasne, F. B., Fanselow, M. S., & Zelikowsky, M. (2011). Design of a neurally plausible model of fear learning. *Frontiers in Behavioral Neuroscience, 5*, 23.

Ledoux, J. E. (1993). Emotional memory-systems in the brain. *Behavioural Brain Research, 58*(1-2), 69-79.

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. *Journal of Neuroscience, 8*(7), 2517-2529.

Lezak, K. R., Roelke, E., Harris, O. M., Choi, I., Edwards, S., Gick, N., . . . Hammack, S. E. (2014). Pituitary adenylate cyclase-activating polypeptide (PACAP) in the bed nucleus of the stria terminalis (BNST) increases corticosterone in male and female rats. *Psychoneuroendocrinology, 45*, 11-20.

Maren, S. (2007). Neuroscience - The threatened brain. *Science, 317*(5841), 1043-1044.

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

Maren, S., & Hobin, J. A. (2007). Hippocampal regulation of context-dependent neuronal activity in the lateral amygdala. *Learning & Memory, 14*(4), 318-324.

Maren, S., Phan, K. L., & Liberzon, I. (2013). The contextual brain: Implications for fear conditioning, extinction and psychopathology. *Nature Reviews Neuroscience, 14*(6), 417-428.

Maren, S., & Quirk, G. J. (2004). Neuronal signaling of fear memory. *Nature Reviews Neuroscience, 5*(11), 844-852.

Myers, K. M., & Davis, M. (2002). Behavioral and neural analysis of extinction. *Neuron, 36*(4), 567-584.

Napier, R. M., Macrae, M., & Kehoe, E. J. (1992). Rapid reacquisition in conditioning of the rabbits nictitating-membrane response. *Journal of Experimental Psychology-Animal Behavior Processes, 18*(2), 182-192.

Neumann, D. L., & Longbottom, P. L. (2008). The renewal of extinguished conditioned fear with fear-relevant and fear-irrelevant stimuli by a context change after extinction. *Behaviour Research and Therapy, 46*(2), 188-206.

Orsini, C. A., Kim, J. H., Knapska, E., & Maren, S. (2011). Hippocampal and prefrontal projections to the basal amygdala mediate contextual regulation of fear after extinction. *Journal of Neuroscience, 31*(47), 17269-17277.

Orsini, C. A., Yan, C., & Maren, S. (2013). Ensemble coding of context-dependent fear memory in the amygdala. *Frontiers in Behavioral Neuroscience, 7*, 8.

Pavlov, I. (1927). *Conditioned Reflexes*. New York, NY: Oxford University Press.

Pincus, H. A., & Pettit, A. R. (2001). The societal costs of chronic major depression. *Journal of Clinical Psychiatry, 62*, 5-9.

Quirk, G. J., & Gehlert, D. R. (2003). Inhibition of the amygdala: Key to pathological states? In P. ShinnickGallagher, A. Pitkanen, A. Shekhar & L. Cahill (Eds.), *Amygdala in Brain Function: Bacic and Clinical Approaches* (pp. 263-272). New York: New York Acad Sciences.

Quirk, G. J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmacology, 33*(1), 56-72.

Quirk, G. J., Repa, J. C., & Ledoux, J. E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons – Parallel recording in the freely behaving rat. *Neuron, 15*(5), 1029-1039.

Rescorla, R. A. (1969). Conditioned inhibition of fear resulting from negative CS-US contingencies. *Journal of Comparative and Physiological Psychology, 67*(4), 504-509.

Rescorla, R. A. (1997). Spontaneous recovery after Pavlovian conditioning with multiple outcomes. *Animal Learning & Behavior, 25*(1), 99-107.

Rescorla, R. A. (2000). Extinction can be enhanced by a concurrent excitor. *Journal of Experimental Psychology-Animal Behavior Processes, 26*(3), 251-260.

Rescorla, R. A., & Heth, C. D. (1975). Reinstatement of fear to an extinguished

conditioned stimulus. *Journal of Experimental Psychology, 104*(1), 88-96.

Rescorla, R. A., & Wagner, A. R. (1972). *A theory of Pavlovian conditioning*. New York: Appelton-Centry-Crofts.

Rodriguez, B. I., Craske, M. G., Mineka, S., & Hladek, D. (1999). Context-specificity of relapse: Effects of therapist and environmental context on return of fear. *Behaviour Research and Therapy, 37*(9), 845-862.

Roman, C. W., Lezak, K. R., Hartsock, M. J., Falls, W. A., Braas, K. M., Howard, A. B., . . . May, V. (2014). PAC1 receptor antagonism in the bed nucleus of the stria terminalis (BNST) attenuates the endocrine and behavioral consequences of chronic stress. *Psychoneuroendocrinology, 47*, 151-165.

Sajdyk, T. J., Johnson, P. L., Fitz, S. D., & Shekhar, A. (2008). Chronic inhibition of GABA synthesis in the bed nucleus of the stria terminalis elicits anxiety-like behavior. *Journal of Psychopharmacology, 22*(6), 633-641.

Savander, V., LeDoux, J. E., & Pitkanen, A. (1996). Topographic projections from the periamygdaloid cortex to select subregions of the lateral nucleus of the amygdala in the rat. *Neuroscience Letters, 211*(3), 167-170.

Shammah-Lagnado, S. J., Beltramino, C. A., McDonald, A. J., Miselis, R. R., Yang, M., De Olmos, J., . . . Alheid, G. F. (2000). Supracapsular bed nucleus of the stria terminalis contains central and medial extended amygdala elements: Evidence from anterograde and retrograde tracing experiments in the rat. *Journal of Comparative Neurology, 422*(4), 533-555.

Somerville, L. H., Whalen, P. J., & Kelley, W. M. (2010). Human bed nucleus of the stria terminalis indexes hypervigilant threat monitoring. *Biological Psychiatry, 68*(5), 416-424.

Sparta, D. R., Jennings, J. H., Ung, R. L., & Stuber, G. D. (2013). Optogenetic strategies to investigate neural circuitry engaged by stress. *Behavioural Brain Research, 255*, 19-25.

Stamatakis, A. M., Sparta, D. R., Jennings, J. H., McElligott, Z. A., Decot, H., & Stuber, G. D. (2014). Amygdala and bed nucleus of the stria terminalis circuitry: Implications for addiction-related behaviors. *Neuropharmacology, 76*, 320-328.

Sullivan, G. M., Apergis, J., Bush, D. E. A., 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.

Swanson, L. W., & Cowan, W. M. (1977). Autoradiographic study of organization of efferent connections of hippocampal formation in rat. *Journal of Comparative Neurology, 172*(1), 49-84.

Treit, D., Aujla, H., & Menard, J. (1998). Does the bed nucleus of the stria terminalis mediate fear behaviors? *Behavioral Neuroscience, 112*(2), 379-386.

Vervliet, B., Craske, M. G., & Hermans, D. (2013). Fear extinction and relapse: State of the art. *Annual Review of Clinical Psychology, 9*(9), 215-248.

Waddell, J., Morris, R. W., & Bouton, M. E. (2006). Effects of bed nucleus of the stria terminalis lesions on conditioned anxiety: Aversive conditioning with long-duration conditional stimuli and reinstatement of extinguished fear. *Behavioral Neuroscience, 120*(2), 324-336.

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. *Journal of Neuroscience, 17*(23), 9375-9383.

Walker, D. L., Miles, L. A., & Davis, M. (2009). Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33*(8), 1291-1308.

Westbrook, F. R., Iordanova, M., McNally, G., Richardson, R., & Harris, J. A. (2002). Reinstatement of fear to an extinguished conditioned stimulus: Two roles for context. *Journal of Experimental Psychology-Animal Behavior Processes, 28*(1), 97-110.

Wilson, A., Brooks, D. C., & Bouton, M. E. (1995). The role of the rat hippocampal system in several effects of context extinction. *Behavioral Neuroscience, 109*(5), 828-836.

Zimmerman, J. M., & Maren, S. (2011). The bed nucleus of the stria terminalis is required for the expression of contextual but not auditory freezing in rats with basolateral amygdala lesions. *Neurobiology of Learning and Memory, 95*(2), 199-205.